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Effects of White Chicken Eggshell Powder on Compressive Strength, Water Solubility, and Setting Time of Calcium-Enriched Mixture

Mahdi Sedigh-Shams [a], Mohammadreza Nabavizadeh [b*], Elham Jafari [c]

a Department of Endodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Fars, Iran; b Oral and Dental Disease Research Center, Department of Endodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Fars, Iran; c Student Research Committee, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

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Corresponding author: Mohammadreza Nabavizadeh, Department of Endodontics, School of Dentistry, Ghasrdasht Ave. Shiraz, Iran, postal code: 71956-15878.

E-mail: nabavizade@sums.ac.ir

Introduction: The present study aimed to evaluate the effects of adding chicken eggshell powder (CESP) to calcium-enriched mixture (CEM) cement on its compressive strength (CS), solubility, and setting time. **Materials and Methods:** In this study, CESP was added at weight percentages of 3% and 5% to the powder component of the CEM cement. To measure the CS, a total of 36 samples (height, 6 mm; diameter, 4 mm) were tested in a universal testing machine. The setting time was assessed for 18 disk-shaped samples (diameter, 10 mm; height, 1 mm). Additionally, solubility test was performed on 18 samples (diameter, 8 mm; height, 1 mm) after 24 hours, 72 hours, seven days, and 14 days under dehydration conditions by calculating the weight changes; the results were then subjected to a normality test. Next, for the comparison of different test groups, parametric ANOVA test and post-hoc Tukey's multiple comparison test were performed at a significance level of 0.05. **Results:** The addition of 5% CESP to the CEM cement significantly reduced its setting time and water solubility (*P*=0.02 and *P*=0.01, respectively). Moreover, it significantly increased the CS over a 21-day period (*P*<0.001). Additionally, the addition of 3% CESP also resulted in a significant increase in CS (*P*<0.001). While 3% CESP reduced setting time and water solubility, the difference was not statistically significant. **Conclusion:** The findings suggest that the addition of 5% CESP to CEM cement has the potential to improve its sealing ability, durability, and ability to withstand chewing forces in endodontic treatments. These results highlight the relevance of CESP as an additive for cement modifications and indicate its potential clinical implications.

Keywords: CEM Cement; Eggshell Powder; Compressive Strength; Solubility; Setting Time

Introduction

Calcium-enriched mixture (CEM) cement is a widely used biomaterial in the field of endodontics and dental applications. It is composed of various concentrations of calcium compounds, such as calcium oxide, calcium silicate, and calcium phosphate, which are mixed with a water-based solution to prepare the cement [1]. CEM cement is known for its biological activity and antimicrobial properties, making it a desirable choice in clinical practice [2, 3]. Additionally, it exhibits favorable biocompatibility [4] and sealing properties [5]. Compared to mineral trioxide aggregate (MTA), CEM cement offers advantages such as improved handling, reduced film thickness, and shorter setting time [6]. This cement sets in an aqueous environment and has been found to stimulate hard tissue healing like MTA as the gold standard [7, 8]. With its unique properties and clinical benefits, CEM cement has gained significant attention in endodontic treatments.

Clinical studies have demonstrated the effectiveness of CEM cement in various applications, such as the management of external inflammatory root resorption [9], pulp capping [10, 11], apexogenesis [12], apical plug placement [13], intentional replantation [14] or regenerative endodontic treatment [15, 16], perforation treatment [17], and surgical root end fillings [18]. However, it should be noted that CEM cement has a lower compressive strength (CS) compared to MTA and Biodentine [19]. Furthermore, its mean weight loss in an aqueous environment exceeds the American Dental Association (ADA) specification limit of 3% [20, 21].

To enhance the properties of the CEM cement, several studies have explored different methods, including those related to CS, solubility, and setting time [22-28]. One potential method involves the incorporation of crushed eggshell powder (CESP), which has a composition similar to bones and teeth, primarily consisting of approximately 98.2% calcium carbonate (CaCO3), 0.9% magnesium, and 0.9% phosphate [29]. Eggshells have been used as bone grafts without reported significant inflammation or bone necrosis [30], indicating their biocompatibility and ability to accelerate wound healing and osteoid formation [31, 32].

CESP is a natural, low-cost, readily available waste product of the food industry, making it a cost-effective option. Previous studies have investigated the integration of CESP to enhance the strength of restorative dental materials. Positive effects have been observed when CESP is used as a filler in glass ionomer (GI) [33] and biocomposite acrylic resin as the denture base [34]. Additionally, CESP has been used as a rich source of $CaCO_3$ for the mechanical reinforcement of polyethylene/polypropylene composites in various industrial applications [35]. However, the the impact of adding CESP on the CS, solubility, and setting time of the CEM cement has not yet been investigated. Therefore, the present study aims to evaluate these parameters by incorporating CESP at weight percentages of 3% and 5% to the cement powder.

Materials and Methods

This *in vitro* study was approved by the Ethics Committee of Shiraz University of Medical Sciences (Shiraz, Iran) (IR.SUMS.DENTAL.REC.1400.108).

Preparation of eggshell powder

Ten chicken eggs were cleaned with distilled water and boiled for 10 minu at 100° C to facilitate the removal of inner and outer membranes. The eggshells were crushed to small particles with a mixer mill (SPEX950; Nano Tadbir, Alborz, Iran). The crushed particles were kept in a muffle furnace at 1200° C for two h to obtain a pathogen-free powder [36]. The powder was then filtered through a filter paper to achieve a particle size less than 2.5 µm. In all the tests, the CEM cement (Bionique Dent, Yektazdandan, Tehran, Iran) and the crushed eggshell powder were used to prepare the samples in three groups: group A, CEM cement without CESP; group B: CEM cement with 3% CESP (CEM+3% CESP); and group C: CEM cement with 5% CESP (CEM+5% CESP).

The CESP was added to the CEM cement powder at weight ratios of 3% and 5% and then mixed using an amalgamator (Farazmehr, Isfahan, Iran) at 4500 rpm for 45 sec. It should be noted that in the CEM cement, the exact liquid-to-powder ratio is not specified, and the ratios are only accurate when the paste has a creamy consistency. To achieve an equal consistency for all the samples, 1 g of powder was mixed with 0.33 g of the CEM cement liquid and mixed thoroughly. The prepared paste was placed in custom-made molds for each test, and the excess material was removed with a damp cotton ball. All the specimens were prepared by one operator and packed vigorously. Only specimens without any cracks or air bubbles were used in the tests.

Compressive strength (CS)

Twelve specimens were prepared for each group using cylindrical Teflon molds, with an internal diameter of 4 mm and a height of 6 mm [37, 38]. The samples were stored in deionized water and placed in an incubator at 37° C with 100% humidity and then removed from the mold after 21 days [39]. For the CS test, the samples were placed lengthwise between the platens of a universal testing machine (Z020, ZwickRoell GmbH & Co., Germany) [20, 40]. The CS values were measured using the universal testing machine at a speed of 1 mm per minute until fracture. The results of the CS test were calculated automatically using the machine operating software, which divided the maximum force applied to the surface of the specimen before fracture, based on the following formula:

$$CS=4p/\pi d^2$$

where *CS* is the compressive strength, *p* is the loading failure in Newton (N), and *d* is the diameter of the sample in mm [38].

Setting time

The setting time test was performed following the ADA Specification No. 57 and C266-03 [41]. Six samples in each test group were prepared using custom-made ring molds with an inner diameter of 10 mm and a height of 1 mm. At 150±1 sec after mixing, the samples were placed in a Gilmore machine, with a 300-g metal piece attached to its cylindrical tip with a flat end, measuring 1.0±0.02 mm. The tip freely touched the samples perpendicular to the surface at the following intervals: 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, and 180 minutes. The time when the metal tip did not cause depression in three independent areas of the cement was considered as the final setting time of the mixture [20].

Water solubility

The water solubility test was carried out according to ISO-6876:2001. For each group, six specimens were prepared using custom-made Teflon O-ring molds, with an inner diameter of

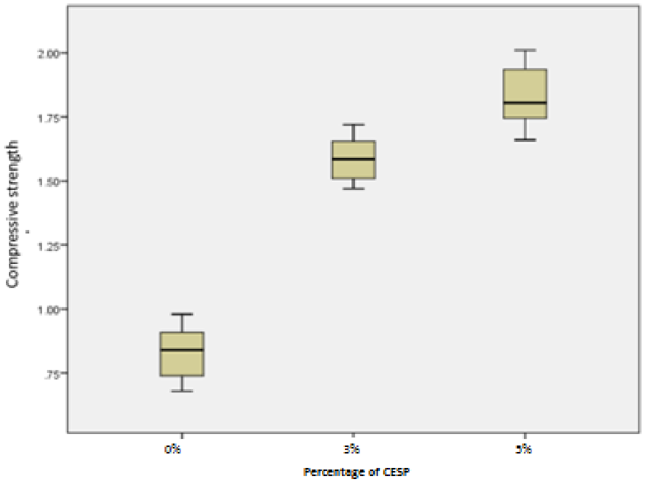

Figure 1. The mean compressive strength (CS) and standard deviation (SD) of the CEM cement mixed with different CESP percentages

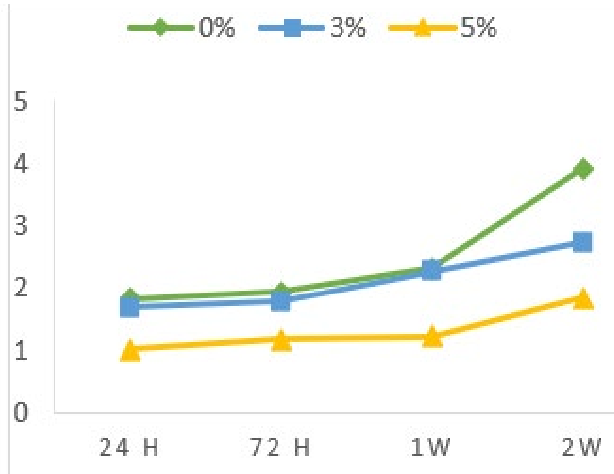

Figure 2. Comparison of water solubility percentages between the groups in different intervals

8 mm and a height of 1 mm. Next, a 10-cm nylon string was inserted into the specimens while they were placed in the molds for suspension inside a plastic vial, containing Milli-Q water (DACell, Tehran, Iran). Subsequently, the rings were filled with cement and pressed between two plastic matrix strips and glass slabs to remove excess materials. The mold assemblies were then placed in an incubator at 37° C with 95% humidity and removed after three h. After the samples were placed in a desiccator for 24 h and weighed (original dry weight, DW0), each ring was suspended with a nylon string in a plastic vial, containing 25 mL of Milli-Q water for 24 h and then washed with Milli-Q water. The surface water was removed with an absorbent paper and returned to the desiccator for 24 h to be weighed (24-h dry weight, 24h DW).

The same procedure was repeated in intervals of 72 h, seven days, and 14 days by weighing the specimens after immersion in a new plastic flask filled with fresh Milli-Q water and returning them to a desiccator for 24 h (DW72h, DW7d, and DW14d, respectively). The mass difference of each specimen was recorded and expressed as percentage. The amount of weight loss (μg) was interpreted as solubility. The percentage of solubility was calculated using the following formula:

$$\text{Solubility percentage}=(\text{Weight loss} \times 100)/DW0$$

Table 1. Mean (SD) of descriptive results of compressive strength (CS) and setting time *(*different letters represent significant differences)*

Percentage of CESP	Compressive strength	Setting time
0	0.833 (0.099) [a]	63.33(5.164) [a]
3	1.585 (0.0839) [b]	56.67(5.164) [ab]
5	1.828 (0.116) [c]	51.67(4.082) [b]

Data analysis

The collected data were entered in SPSS Version 17 (IBM Co., Chicago, IL, USA). In all the experiments, the results were subjected to a normality test to investigate their normal distribution. The homogeneity of variance was examined using Levene's test. If the *P*-values were greater than 0.05, ANOVA test was performed. Parametric ANOVA test and post-hoc Tukey's multiple comparison test were also performed at a significance level of 0.05.

Results

The results of Levene's test showed equal variances regarding the CS (P=0.384), setting time (P=0.07), and water solubility (P=0.283).

Compressive strength (CS)

Table 1 compares the CS between the three experimental groups. The results showed that the addition of 5% CESP to the CEM powder could significantly increase the CS of the samples compared to the control group and the CEM+3% CESP group (P<0.001 and P<0.001, respectively). Additionally, the CEM+3% CESP group showed a significantly higher CS compared to the group without CESP (P<0.001) (Figure 1).

Setting time

The calculation of setting time was based on mean±standard deviation (SD) (Table 1). The addition of 3% CESP decreased the mean setting time of the CEM cement from 63.33±5.2 to 56.67±5.2 min; however, the difference was not significant (P=0.074). Based on the results, the addition of 5% CESP significantly decreased the mean setting time of the CEM cement from 63.33±5.2 to 51.67±4.1 min (*P*=0.02). There was no significant difference between the CEM+3% CESP and CEM+5% CESP groups (*P*=0.2).

Water solubility

The median and mean values of water solubility in all intervals are presented in Table 2. The addition of 5% CESP significantly decreased the water solubility of the CEM cement on day 1, day 3, day 7, and day 14 (*P*=0.01, *P*=0.012, *P*=0.036, and *P*=0.01, respectively). The addition of 3% CESP to the CEM cement caused no significant difference in the solubility of the cement. The pairwise comparison of the subgroups showed that the solubility was significantly higher on days 1, 7, and 14 in the CEM cement without CESP compared to the other two groups (Figure 2).

Discussion

The CESP has been used to improve the mechanical and biological properties of different materials. It has been added to many dental materials, including glass ionomer [33], acrylic-resin bases [34], Portland cements [42, 43], and bone graft materials [44, 45], as a biocompatible, strengthening, and water-resistant agent to enhance their physical and chemical properties. In many previous studies, the addition of CESP to dental materials exerted favorable effects on the mentioned properties [33, 34, 42-45].

Although the CEM cement can be used as an endodontic root filling material, it has a lower CS compared to ProRoot MTA and Biodentine. The comparison of ProRoot MTA, Biodentine, and CEM cement in different incubation time intervals and exposure environments revealed that the CS of the CEM cement was lower than that of ProRoot MTA and Biodentine [46]. The lower CS of the CEM cement compared to ProRoot MTA, MTA Angelus, and Biodentine is related to the larger amount of sulfate in the CEM cement compared to both ProRoot MTA and MTA Angelus [19]. According to a study by Sohelipour *et al.*, the greatest distribution of the CEM particle size was in the range of 0.5-2.5 µm [47]. To match the mean particle size of the CEM cement, the powder was filtered through a filter paper to achieve particle sizes smaller than 2.5 µm, which allowed for a better distribution of eggshell powder in the cement.

The CS is considered as one of the main physical properties of hydrophilic cements, which is related to the hydration stage. The CS of cement is important in the repair of furcal perforations, pulp capping, and apexogenesis. Generally, cements should withstand the direct force of restorative material placement and indirect chewing forces [48]. Special compounds, such as lidocaine, calcium chloride, propylene glycol, silver nanoparticles, nano-hydroxyapatite, sodium hypochlorite, chlorhexidine, and alkaline salts, have been added to the CEM cement to improve its physicochemical properties (12, 21, 30-36).

In the present study, the CS was measured according to the ISO-6876:2012 after 21 days [49]. The highest and lowest CS values were attributed to CEM+5% CESP and CEM without CESP, respectively. Based on the present results, the addition of 3% or 5% CESP to the CEM cement could significantly increase the CS of the samples as compared to the control group. Since the CS of hydraulic cements is an indicator of hydration reactions, this finding may be related to changes in the hydration process of powder particles when the CEM cement is mixed with CESP.

In this regard, Jaber *et al.* reported that the addition of CESP to Portland cement enhanced its physical properties compared to the control group at all weight ratios (5%, 10%, 15%, and 20%). Based on the results, 15% CESP could increase the CS values by about 29% compared to the control group; this finding might be related to the major component of CESP, which is $CaCO_3$. This addition accelerates the hydration process of Portland cement by reacting with C_3S and forming C-S-H, which leads to an increase in strength in an early stage [42]. Since CEM has a similar hydration process, the increased CS values can be justified. Similarly, in a study by Allam *et al.* [33], the addition of CESP to conventional glass ionomer cement (GIC) at 3% and 5% enhanced the mechanical properties of GIC.

In another study by Rahmi *et al.*, by adding CESP to chitosan led to the improved distribution of eggshell particles in the microstructure of composites and improved their tensile strength [50]. As its name suggests, the CEM cement contains high amounts of calcium cations [6]; therefore, the mentioned reason may explain the increased CS of the CEM cement.

Table 2. The water solubility percentages (*different letters represent significant differences)

Time	% of CESP	Mean (SD)	Time	% of CESP	Mean (SD)
24 h	0	1.83 (0.25) ^{a}	1 week	0	2.32(0.30) ^{a}
	3	1.68 (0.30) ^{a}		3	2.27 (0.71) ^{ab}
	5	1.01(0.31) ^{b}		5	1.22(0.53) ^{b}
72 h	0	1.94(0.44) ^{a}	2 weeks	0	3.92 (0.66)^{a}
	3	1.78 (0.40) ^{a}		3	2.74(0.42) ^{ab}
	5	1.17 (0.18) ^{b}		5	1.84 (0.17) ^{b}

Moreover, in studies by Lubis *et al.* [34], by adding eggshell as a filler to the acrylic resin as the denture base, the mechanical properties, including modulus of elasticity and modulus of rupture (MOR) of the denture base, increased. The addition of CESP to acrylic resin base increased the modulus of rupture because of the load transfer between the filler and the matrix as a result of adhesion. Since fillers absorb some of the load received by the denture base, the modulus of elasticity can be increased in the denture base. The best MOR was obtained when the fillers were spread evenly and made a homogeneous mixture with a wide interphase, which reduced the incidence of empty cavities on the biocomposite. Since CS has different entities and tests from tensile strength to MOR, the direct comparison of studies is not accurate; however, the findings are not entirely irrelevant [34].

The results of the abovementioned studies are consistent with the present study. The CS of the CEM cement increased by adding CESP, which could be due to increased hydration over time, higher density of interfaces, larger specific surface area, higher crystallinity, lower porosity, and the size and chemical composition of cement compounds [42, 51, 52]. The setting strength represents the minimum strength at which the cement can tolerate its weight. One of the drawbacks of MTA is its long setting time, which is approximately four h [53]. A long setting time increases the number of treatment sessions and the risk of contamination, making it time-consuming and costly [54, 55]. On the other hand, the setting time of the CEM cement is almost one-fourth of MTA; therefore, it is considered superior to MTA. In the present study, the mean setting time was 63.33 min in the CEM group, 56.67 min in the CEM+3% CESP group, and 51.67 minin the CEM+5% CESP group.

A decline in the setting time of other cements after adding CESP has been reported in previous studies [20, 43]. In this regard, Abbaszadegan *et al.* [20] evaluated the effects of adding calcium chloride to CEM. They found that the mean setting time significantly reduced by adding calcium chloride. They proposed that the significant effect of 10% $CaCl_2$ on the setting time might be related to the penetration of $CaCl_2$ into the pores of the CEM cement, which could partly lead to the acceleration of hydration in silicates and facilitate the crystallization process. Similarly, in the present study, the addition of CESP to the CEM cement caused a significant reduction in the setting time, as the small particles of CESP could fill most of the pores in the cement samples.

It is known that $CaCO_3$ is the main component of the eggshell powder, which changes to calcium oxide by sintering at 1200° C [56]. Several studies have reported that calcium oxide in calcium silicate-based cements probably reacts with water in tissue fluids and produces calcium hydroxide. Besides, calcium hydroxide dissociates into hydroxide and calcium ions; the hydroxide ions in turn reinforce the pH environment [57, 58]. The alkaline environment can significantly enhance the CS of calcium silicate-based cements, including the CEM cement [23, 58, 59].

Generally, solubility indicates the released residual particles that are eluted by a solution or a solvent, resulting in weight loss. The clinical success and durability of cements in tissues depend on properties, such as structural integrity and dimensional stability. High solubility can be explained by the incomplete setting reaction of cement owing to its specific chemical composition. A prolonged setting time is the reason why a cement shows cracks and porosities after a solubility test [60]. In the present study, the solubility of the CEM cement after three weeks exceeded the standard ISO-6876, which is consistent with the findings of some other studies on CEM (12, 13). The addition of 5% CESP dramatically reduced the cement solubility at the same immersion time, which fulfilled the ADA recommendation (<3%). The lower solubility was attributed to the shorter setting time, as it provided a shorter contact time between the unset material and water. The advantages of this cement include the reduced possibility of contamination in the oral environment and facilitation of the placement of a second restorative material on the cement [61].

Conclusion

Based on the present results, the addition of 5% CESP to the CEM cement reduced its setting time. It also decreased the water solubility of CEM, which could potentially improve the sealing ability and durability of the CEM cement. Moreover, the addition of 5% CESP to the CEM cement significantly increased the CS over 21 days, which could be useful in withstanding chewing forces after the application of cement in endodontic treatments.

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Conflict of Interest: 'None declared'.

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