



# The Effect of Different Mixing Methods on the Properties of Calcium-enriched Mixture Cement: A Systematic Review of *in Vitro* Studies

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## ABSTRACT

**Introduction:** It has been shown that the mechanical and physical properties of Calcium Enriched Mixture (CEM) cement are influenced by the mixing methods. Despite several studies conducted on different mixing methods of CEM cement, there is no systematic review to summarize the results. This systematic review was conducted to investigate the effect of different mixing techniques on mechanical and physical characteristics of CEM cement. **Methods and Materials:** A professional librarian with skills in informatics conducted a systematic search by searching electronic databases PubMed/MEDLINE, Scopus and Ovid for English language peer-reviewed articles published between 1992 and April 2019. **Results:** Initial searches from all sources identified 1175 references. Two of the authors examined the titles, abstracts of these articles and the full reports of 20 studies were obtained, and data extraction was performed. Seven studies satisfied the eligibility criteria for the review. The effect of different mixing methods was investigated on bacterial microleakage, push-out bond strength, flow rate, compressive strength, solubility, pH, film thickness, dimensional changes, working time, setting time and quality of the apical plug. **Conclusion:** Based on the results of this systematic review, some of the important properties of CEM cement were affected by different mixing methods. Although none of these mixing methods could improve all the properties, mechanical and manual methods were more effective compared to ultrasonic method.

**Keywords:** Calcium-enriched Mixture Cement; Systematic Review; Ultrasonic

## Introduction

Calcium-enriched mixture (CEM) cement includes metallic oxides and hydroxides, calcium phosphate and calcium silicate. CEM cement is commonly used for root-end filling, perforation sealing, and apexification procedures. Utilizing CEM cement does not have the disadvantages of mineral trioxide aggregate (MTA), such as long setting time and difficult handling. Also, its flow rate and film thickness are better than MTA [1, 2].

It has been shown that the mechanical and physical properties of calcium-silicate-based biomaterials like MTA and CEM cement are influenced by the mixing methods, the

ratio of the constituent components, delivery systems, chemical additives and exposure to different clinical environments [3-6]. The ideal physical properties of an endodontic biomaterial are important prerequisites for their successful clinical application. To reach these ideal properties in hydraulic cements, the powder particles should be mixed with water completely [7-10]. The technique utilized for mixing these materials provides a proper contact between the powder particles and the liquid [5]. The mixing procedure initiates hydration reactions through which calcium hydroxide is released and converted to calcium and hydroxyl ions leading to an increase in the pH value [2].

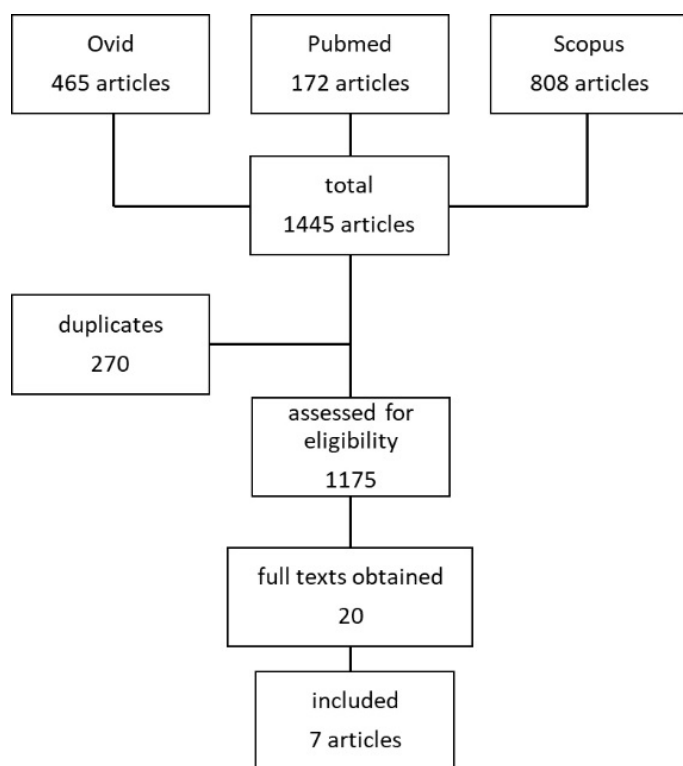


Figure 1. Flow diagram of study selection

Three major mixing techniques including conventional, mechanical and ultrasonic methods have been utilized in previous studies. Mechanical trituration method can decrease air-filled spaces between the powder particles which results in through wetting of the particles and improved uniformity of the final mixture. Ultrasonic mixing affects the dispersion of particles arranged in clusters next to each other. Therefore, it enhances the interaction of particles through increasing the surface area of the particles which take part in setting reaction [4, 5].

Despite several studies conducted on different mixing methods of CEM cement, there is no systematic review to summarize the results of studies and demonstrate if different mixing methods could significantly affect CEM cement properties. Therefore, this systematic review was conducted to investigate the effect of different mixing techniques on mechanical and physical properties and indicate the method with positive effects on more of these characteristics of CEM cement.

## Materials and Methods

### Search strategy

A systematic search was conducted by a professional librarian with skills in informatics by searching electronic databases Pubmed/MEDLINE, Scopus and Ovid for English language

peer-reviewed articles published between 1992 and April 2019 using the following search terms:

(((((calcium enriched mixture) OR calcium enriched cement) OR (((CEM[Title/Abstract] OR CEM[MeSH Terms]) OR CEM[Other Term]))) AND (((("Materials Testing"[MeSH]) OR ((mixing) OR (((hand mix\*) OR manual mix\*) OR mechanical mix\*) OR ultrasonic mix\*)))) OR ("Biocompatible Materials"[MeSH]))) OR ((calcium enriched cement) OR calcium enriched mixture).

A database of the first search results was created and subsequent search results were entered and duplicate entries were removed. After searching the databases, some recognized journals in this field including the International Endodontic Journal and Journal of Endodontics were also hand searched. In addition, the reference lists of selected articles were manually searched in order to complement the search database.

### Study selection

The initial selection was based on the titles and abstracts of the obtained studies. Two reviewers independently screened and identified studies against the below inclusion and exclusion criteria. Whenever fulfillment of these criteria was not clear from the abstract, the full text of the study was obtained for verification. Any disagreement between the authors was resolved through discussion and a third reviewer conducted a random check of approximately 10% of titles and abstracts to check the reliability of initial screening. All papers that passed the abstract screening were retrieved in their complete forms, and data extraction was conducted.

### Inclusion criteria

- Abstract available in English.
- In vitro studies which used at least two techniques for mixing of CEM cement

### Exclusion criteria

- Incomplete data which were not accessible by contacting with authors
- Letters to editor, presentations in conferences, case reports, and unpublished papers.

### Risk of bias assessment

Due to *in vitro* nature of this systematic review, risk of bias assessment was performed by using modified previous tools [11-13]. The quality of assessment and risk of bias were evaluated based on standardization of specimens, randomization, explanation of sample size calculation, study protocols, statistical analysis, blinding of the operator and reporting of data.

Two reviewers scored the methodological quality as low, moderate and high risk of bias. If the authors reported the all mentioned parameters in the manuscript without ambiguity, the risk of bias was low. Moderate risk of bias was used when one of the parameters was eliminated or indicated ambiguously. The lack of two or more than two parameters, also indicate a high risk of bias.

#### Data extraction

A standardized data extraction form was developed, pilot tested and employed by two independent reviewers. Independent data extraction by two reviewers was performed for all eligible studies. Study authors were contacted for additional information when needed. Disagreements were resolved through discussion. If disagreement persisted, the judgment of a third reviewer was decisive.

The following data were then extracted from the articles using

the data extraction form: first author's name, year of publication, country, investigated properties, mixing method, and sample size.

## Results

#### Search results

Initial searches from all sources identified 1445 references of which 270 were duplicates. Then, two of the authors examined the titles and abstracts of these articles and the full reports of 20 studies were obtained and data extraction was performed. Seven studies satisfied the eligibility criteria of the review. There was 90% agreement for inclusion of papers when complete papers were reviewed.

The 7 articles were reviewed independently by two of the authors to ensure that they met all of the review criteria. The data extraction table for included studies is shown in Figure 1.

**Table 1.** Basic characteristics of the included studies

Author	N	Assessed properties	Mean (SD) of Each Mixing Technique			P-value
			Hand	Mechanical	Ultrasound	
Shahi <i>et al.</i> [14]	15	Bacterial microleakage	62.13 (12.44)	68.87(12.79)	77.53 (12.52)	P>0.05
Shojaee <i>et al.</i> [15]	30	Push out bond strength (MPa) after 3 days	4.86 (1.41)	4.01(1.32)	4.84 (2.12)	P>0.05
	30	Push out bond strength (MPa) after 21 days	7.59 (5.06)	4.54(4.48)	5.10 (3.87)	P>0.05
Sahebi <i>et al.</i> [3]	10	Compressive strength (MPa) after 6 days	2.93 (3.94)	12.52(13.44)		P<0.05
Shahi <i>et al.</i> [4]	6	Flow rate (mm)	12.27 (0.52)	11.45 (0.19)	12.48 (0.56)	P<0.05
	6	Compressive strength (MPa) after 21 hours	257.33 (20.53)	211.50 (12.82)	221.67 (28.43)	P<0.05
	6	Compressive strength (MPa) after 21 days	267.67 (21.96)	238.33 (10.41)	248.33 (24.19)	P<0.05
Shahi <i>et al.</i> [2]	6	Solubility in µg after 1 day	0.09 (0.09)	0.12 (0.01)	0.09 (0.03)	P<0.05
	6	Solubility in µg after 7 day	0.09 (0.01)	0.11 (0.08)	0.08 (0.07)	P>0.05
	6	Solubility in µg after 21 day	0.09 (0.01)	0.11 (0.03)	0.08 (0.07)	P<0.05
	6	pH	10.86 (0.12)	10.76 (0.05)	10.57 (0.10)	P>0.05
Shahi <i>et al.</i> [5]	6	Film thickness (mm)	1.3 (0.05)	0.8 (0.03)	1 (0.02)	P>0.05
	6	Dimensional changes (mm)	-0.34 (0.03)	-0.28 (0.05)	0.1 (0.13)	P<0.05
	6	Working time (hours)	5:38:58 (0:2:8)	5:56:34 (0:17:13)	12:8:53 (0:37:3)	P<0.05
	6	Setting time (hours)	0:52:50 (0:0:36)	0:0:30 (0:0:50)	0:36:45 (0:0:31)	P<0.05
Rahimi <i>et al.</i> [16]	40	Void count	50%		50%	P>0.05
	40	Void dimension	Score1: 39% Score2: 39%		Score1: 11.25% Score2: 10.75%	P>0.05

**Study characteristics**

This final sample of studies was published in 2015, 2016 and 2017. The study characteristics are shown in Table 1. All of the studies were conducted in Iran. The effect of different mixing methods was investigated on bacterial microleakage, push-out bond strength, flow rate, compressive strength, solubility, pH, film thickness, dimensional changes, working time, setting time and quality of apical plug.

**Bacterial microleakage**

Shahi et al. [14] compared the different mixing methods on the bacterial microleakage of CEM cement. Based on the results of the study it can be concluded that different mixing methods had no significant effect on the bacterial microleakage of CEM cement.

**Push out bond strength**

Shojaee et al. [15] demonstrated the effect of different mixing methods on the push-out bond strength of CEM cement in 3 and 21 days. According to the results, various mixing techniques did not affect the push-out bond strength of CEM cement.

**Flow rate of CEM cement with different techniques**

Shahi et al. [4] Investigated the effect of conventional, mechanical and ultrasonic mixing methods on the flow rate of CEM cement. They reported that the flow rate was significantly different from the mixing methods. Accordingly, the flow rate was significantly lower with mechanical mixing technique. However, there was no significant difference between the hand and ultrasonic mixing techniques.

**Compressive strength of CEM cement with different techniques**

Shahi et al. [4] investigated the effect of conventional, mechanical and ultrasonic mixing methods on the compressive strength of CEM cement after 21 h and 21 days. The compressive strength with different mixing techniques was statistically significant at both time intervals with the highest values belonging to the hand technique at both 21-h and 21-day intervals. The two other techniques were not significantly different from each other at both assessment times.

Sahebi et al. [3] compared conventional and mixing methods and reported that compressive strength after 6 days was significantly higher in mechanically mixed samples.

**Solubility of CEM cement with different techniques**

Shahi et al. [2] compared the effect of conventional, mechanical and ultrasonic mixing methods on the solubility of CEM cement after 1, 7 and 21 days. The solubility of samples in a mechanical mixing group was significantly higher after 1 and 21 days. No significant difference was observed between methods on day 7.

**pH of CEM cement with different techniques**

Shahi et al. [2] reported that no significant difference was observed in pH of CEM cement mixed with conventional, mechanical and ultrasonic mixing methods.

**Film thickness of CEM cement with different techniques**

Shahi et al. [5] reported that film thickness was not affected by the use of conventional, mechanical and ultrasonic mixing methods.

**Dimensional changes of CEM cement with different techniques**

Shahi et al. [5] reported that there was some shrinkage in samples mixed with conventional and mechanical mixing method while ultrasonic mixing method caused an expansion in the samples. However, the differences were not statistically significant.

**Working time and setting time of CEM cement with different techniques**

Shahi et al. [5] reported that the use of ultrasonic mixing technique significantly decreased the working time. Setting time decreased significantly with the use of mechanical and ultrasonic mixing techniques compared to the hand mixing method.

**Quality of apical plug of CEM cement with different techniques**

Rahimi et al. [16] compared manual and ultrasonic mixing techniques on the void count and the void dimension of apical plugs. The quality of the apical plug did not show significant differences with different techniques.

**Risk of bias assessment**

Quality assessment was taking into consideration of seven parameters in the implementation of the study including standardization of specimens, randomization, explanation of sample size calculation, standardized protocol, statistical analysis, blinding of the operators to study objectives and reporting of data (Table 2).

All of the papers standardized the specimens but none of them reported the randomization assignment tool. The experimental protocols were clearly demonstrated in all the papers except one of them [16], which not reported the ultrasonic device type. Only two out of seven studies not mentioned the statistical analysis software [15, 16]. None of the studies mentioned the sample size calculation and blinding of the operators to study objectives. The authors of the current systematic review strongly suggest that these details should be considered to decrease the risk of bias. However, the authors speculate that studies may have considered these points, but not reported them

## Discussion

Calcium-enriched mixture (CEM) cement has similar clinical applications as MTA. The chemical composition of CEM cement is more similar to dentine than that of MTA and Portland cement and comprises different concentrations of calcium salt, calcium oxide, calcium silicate, and calcium phosphate, mixed with a water-based solution to obtain an enriched mixture cement [1, 17, 18]. The major reported advantages of CEM cement over MTA are better handling, lower film thickness and shorter setting time. It has been noted that CEM cement sets in an aqueous environment and can stimulate hard tissue healing [17, 19].

CEM cement has favorable sealing ability [20, 21]. Bacterial contamination is the main reason of endodontic failures. The periodontal tissue of an endodontically treated tooth should be free of bacteria and their by-products to ensure regeneration. The results showed that different mixing techniques have no effect on bacterial microleakage of CEM cement [14]. The bond strength of CEM cement is always considered as one of excellent characteristics related to sealing ability of this biomaterial.

Previously it has been demonstrated that various mixing methods had no effect on push-out bond strength of white MTA [7]. It seems that different mixing methods affect the compressive strength, not the push-out strength of hydraulic cements like MTA and CEM which can be attributed to the fact that push-out test and compressive strength have different natures [15].

The most favorable properties of CEM cement are related to its ability to enhance the pH of its environment [17]. An alkaline environment is a key factor, which assists in the healing of inflamed dental pulp and mineralization [17, 22-24]. CEM pulpotomies provided successful outcomes in management of the inflamed pulp of primary and permanent teeth [25, 26]. Our results revealed that pH was not affected by different mixing methods. Researchers suggest that for stimulating mineralization in the process of hard tissue healing high pH and released calcium and phosphorus ions are required. The excellent biocompatibility of CEM cement may contribute to its ability to release calcium ions which react with phosphate ions of body tissue fluid, resulting in hard tissue formation [27]. Furthermore, high pH levels contribute to antibacterial activity that is a critical factor in the formation of a mineralized tissue barrier [28]. While a pH

**Table 2.** Risk of bias assessment

Author	Standardization of specimens	Randomization	Sample size calculation	Standardized protocol	Statistical analysis	Blinding	Reporting of data
Shahi <i>et al.</i> [14]	Low All specimens standardized	Moderate Not mentioned	High Not mentioned	Low Spacemen preparation & mixing methods reported clearly	Low Statistical analysis and tests reported	High Not mentioned	Moderate Control groups data not mentioned
Shojaee <i>et al.</i> [15]	Low All specimens standardized	Moderate Not mentioned	High Not mentioned	Low Spacemen preparation & mixing methods reported clearly	Moderate The statistical analysis software was not mentioned	High Not mentioned	Low All outcomes reported
Sahebi <i>et al.</i> [3]	Low All specimens standardized	Moderate Not mentioned	High Not mentioned	Low Spacemen preparation & mixing methods reported clearly	Low Statistical analysis and tests reported	High Not mentioned	Low All outcomes reported
Shahi <i>et al.</i> [4]	Low All specimens standardized	High Not mentioned	High Not mentioned	Low Spacemen preparation & mixing methods reported clearly	Low Statistical analysis and tests reported	High Not mentioned	Low All outcomes reported
Shahi <i>et al.</i> [2]	Low All specimens standardized	High Not mentioned	High Not mentioned	Low Spacemen preparation & mixing methods reported clearly	Low Statistical analysis and tests reported	High Not mentioned	Low All outcomes reported
Shahi <i>et al.</i> [5]	Low All specimens standardized	High Not mentioned	High Not mentioned	Low Spacemen preparation & mixing methods reported clearly	Low Statistical analysis and tests reported	High Not mentioned	Low All outcomes reported
Rahimi <i>et al.</i> [16]	Low All specimens standardized	Moderate Not mentioned	High Not mentioned	Moderate The ultrasonic device type not reported	Moderate The statistical analysis software was not mentioned	High Not mentioned	Low All outcomes reported

greater than 9 may reversibly or irreversibly inactivate bacterial cellular membrane enzymes resulting in a loss of biological activity, a pH greater than 11.5 is inhibitory for majority of bacteria specially *Enterococcus faecalis* [29, 30].

Mechanical mixing significantly increased the solubility of CEM cement after 1 and 21 days due to decreases in voids between material particles and improvement of particles wetting. Because root filling materials are usually in contact with tissue fluids, they should be practically insoluble in water and chemically inert to be able to resist solubility and disintegration in the aqueous environment [14, 17, 31]. In addition, the clinical success and durability of cements in the oral cavity depend on properties such as structural integrity and dimensional stability which are functions of water sorption and solubility [17, 27, 32]. Solubility indicates released residual particles which are eluted by a solution or solvent resulting in the loss of weight. Factors contributing to this include the chemistry of the solvent, temperature, immersion time, amount of unreacted substrate and the size and the chemical composition of materials. Another possible factor affecting the solubility of the cement could be related to its powder- liquid ratio [17, 28, 33].

Our results demonstrated that setting time decreased significantly with the use of mechanical and ultrasonic mixing techniques compared to the hand mixing method. Setting time is considered as one of the most important physical properties of materials and is defined as the duration of time a material needs to become rigid [5]. Long setting time has always been considered as one of the disadvantages of CEM and it is still impossible to carry out one-visit treatment procedures. Clinically, a setting time of 25-30 min is considered favorable [5, 34]. In fact, when a material sets fast there is a short time for its contamination in the oral cavity; on the other hand, the increase in initial strength, decreases the probability of its being washed-out. As a result, the restorative material can safely be placed over it in the same session. More specifically, any change in the setting process of bioactive materials, including the time and production of reaction products, which are mainly calcium and hydroxyl ions, might affect the production of hydroxyapatite layer and the bioactivity of these materials. The mixing technique, the amount of liquid used, the force used for packing and the environmental moisture affect the setting process [5, 35].

The dimensional change is influenced by setting time, as the setting time increases dimensional stability reduces consequently. Slow expansion during the setting reaction can increase the adaptation of the material with the cavity walls, improving the seal; however, the rapid expansion of the root-end filling material might result in the formation of cracks in the

thin walls at the apical end. On the other hand, shrinkage results in the loss of marginal adaptation, leading to leakage [1, 5, 34, 36]. Generally, slight expansion and reasonable flow and film thickness have been associated with effective seal after setting and reduction of the subsequent leakage [37].

The compressive strength is another important property that needs to be considered when using different mixing techniques. The results showed that hand mixing significantly increases the compressive strength of CEM cement comparing to other techniques. Mechanical mixing leads to uniform and adequate wetting of powder particles and facilitates hydration process and improves the mechanical properties of the cement. Reduced compressive strength will not be a major drawback since minimal forces are applied to the retro-filling material [4, 17].

#### **Implications for current practice and conclusion**

Based on the results of this systematic review, some of the important properties of CEM cement were affected by different mixing methods. Although none of these mixing methods could improve all the properties, mechanical and manual methods were more effective compared to ultrasonic method. However, it seems that long-standing clinical evaluations are required in future studies.

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