

INFLUENCE OF 2% CHLORHEXIDINE SOLUTION ON COMPRESSIVE STRENGTH, SOLUBILITY AND CALCIUM RELEASE OF CALCIUM ENRICHED CEMENT (CEM)

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ABSTRACT

Calcium enriched mixture cement (CEM) is widely used in endodontics due to its favorable properties. The addition of chlorhexidine to (CEM) material increases its antimicrobial efficiency however; its influence on the properties of this material is unclear.

Aim: This study was conducted to evaluate the effect of mixing 2% of Chlorhexidine on the compressive strength, solubility and calcium release of calcium enriched mixture cement.

Materials and methods: Two experimental groups were included in this study. The first group, CEM was mixed with CEM cement solution and the second group, calcium enriched mixture cement was mixed with 2% Chlorhexidine solution. Thirty cylindrical samples of 4 mm diameter and of 6 mm height were constructed per each group for assessment of compressive strength at 1, 7 and 15 days intervals 10 samples per each, furthermore, 10 samples of the same dimensions per each group were constructed for assessment of solubility and calcium ion release at 1, 7 and 15 days intervals.

Statistical analysis: Data were statistically analyzed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Independent t-test was used to compare between different mixing solutions on mean compressive strength (MPa), weight loss (μg) and rate of calcium release (ppm).

Results: The compressive strength value of CEM cement solution mixed samples was significantly higher than Chlorhexidine mixed ones ($P \leq 0.001$). Solubility and calcium ion release significantly increased at 7 and 15 days ($P \leq 0.05$) by adding Chlorhexidine to mixing solution.

Conclusion: Adding Chlorhexidine to CEM had a negative influence on its compressive strength and solubility rate.

KEY WORDS: Calcium enriched mixture cement, Chlorhexidine, Compressive strength, Calcium ion level.

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INTRODUCTION

Calcium Enriched Mixture (CEM) is a favorable hydrophilic tooth colored cement⁽¹⁻⁹⁾. It is composed of major components such as calcium oxide, sulfur trioxide, phosphorous pentoxide, silicon dioxide and minor components such as aluminium trioxide, sodium oxide, magnesium oxide, chloride^(1,2). The important constituents of CEM are alkaline earth metal oxides and hydroxides such as calcium oxide and calcium hydroxide, calcium phosphate, and calcium silicate mixed with a water-based solution. It has a lot of good biological and physical properties⁽¹⁻⁹⁾. Compared to Mineral Trioxide Aggregate (MTA), it is characterized by its shorter setting time, good handling, better flowability and less film thickness⁽³⁾. It is also characterized by its sealing ability, biocompatibility, ability to form hydroxyapatite (HA), ability to induce cementum formation^(1,4-6). It is widely used as a pulp capping agent, retrograde filling and in repairing root perforation^(1,2,4,7-9).

CEM differs chemically from MTA in that phosphorous is the major component of CEM. The presence of calcium, phosphorous and oxygen ion on the surface of CEM cement was almost similar when compared to that of surrounding dentin. This finding shows that the composition of CEM cement is similar to dentin. Since HA is the main component of dentin; therefore, similarity in composition between CEM cement and dentin might help the cementogenesis in spite of the presence of high level of phosphorous in CEM cement. It seems reasonable to suspect that the presence of low concentration of phosphate ions in CEM cement media is probably due to its reaction with released calcium ion to form hydroxyapatite in the 1st h.^(3,4,6,9)

In addition, many studies reported that CEM has more potent antibacterial effect than MTA. It was found that when CEM is transferred to agar plates and makes contact with medium, $\text{Ca}(\text{OH})_2$ dissociates into calcium and hydroxyl ions which increases the pH and calcium concentration. However,

ineffectiveness of CEM has been noticed against *E. Faecalis*⁽¹⁰⁻¹³⁾. Chlorhexidine (CHX) is synthetic cationic bisguanide, comprises of two identical 4-chlorophenyl rings and two biguanide groups linked by a central hexamethylene chain. CHX is a positively charged hydrophobic and lipophilic molecule communicating with phospholipids and lipopolysaccharides on cell membrane of bacteria passing through cell by either active or passive transport mechanism. Its efficiency is due to the interaction of positive charge of the molecule and negatively charged phosphate groups on the microbial cell walls changing the cells' osmotic equilibrium. CHX has been used as an irrigant for decades due to its broad spectrum antimicrobial action⁽¹⁴⁻¹⁶⁾. A recent study showed that adding 2% of CHX to CEM mixture resulted in a significant increase in its antibacterial efficacy⁽¹⁷⁾.

Compressive strength is one of the important criteria for the setting and strength of a material.⁽¹⁸⁾ None of the previous studies evaluated the effect of CHX on the compressive strength of CEM. The aim of this in vitro study was to investigate the effect of 2% CHX on the compressive strength of CEM as well as assessment of solubility and calcium ion release.

MATERIAL AND METHODS

The material investigated was CEM (Bionique Dent; Tehran, Iran). Two custom-made two-part split Teflon mold held together by 2 tightening screws were used in this experiment. Each mold had five holes with internal diameter of 4 mm and height of 6 mm (**Fig 1**). The molds were randomly allocated, prior to being filled with CEM. Samples were constructed per each group as following:

Group 1 : CEM mixed with CEM cement solution.

Group 2: CEM mixed with 2% Chlorhexidine gluconate solution (Consepsis V, Ultradent Products, USA)

CEM was prepared according to the manufacturer's instructions and was then homogenized and positioned incrementally into the molds by amalgam carrier. After gentle packing and compacting with condensers, excess material was removed with wet cotton pellets. Wet pieces of gauze were then placed above the molds but without coming into close contact with the CEM surface to produce fully saturated humidity. The plates were sealed and then placed in an incubator at 37°C. After 1 day, the samples were removed from the incubator and the molds were split. The set CEM blocks were removed carefully by applying light force, taking care not to damage the CEM samples, all samples were evaluated for voids or cracks.

Compressive strength test

Thirty samples per each group were prepared for assessment of compressive strength at three intervals 1, 7 and 15 days. All samples were kept in an incubator at 37°C until the test was applied. Compressive strength was assessed by placing the samples length wise between the platens of a universal testing machine (Instron model 3345 England) (Fig 2). Data was recorded using computer software program Bluehill 3 version 3.3.

The samples were compressed at cross head speed of 1 mm/min, and maximum compressive strength, at fracture, was recorded in Mega Pascals (MPa).

Determination of solubility rate and calcium ion release

Solubility test was performed according to ISO standard 6876 and ADA protocol, 10 samples of the same dimensions as those used for compressive strength per each group were used. After setting each sample separately placed in a glass bottle containing 50 mL of distilled water at 37°C for 1 h. Subsequently, all the samples were left to dry for 1 h and were then weighed. After weighing, the samples were returned to the same bottles without changing their water content. The drying and weighing steps of the samples were reported at 1, 7 and 15 day intervals by subtracting W2 (the weight of sample at the end of related time interval) from W1 (the initial weight) indicating the weight loss. The amount of weight loss in µg was interpreted as solubility. After each period 5 mL was taken from each tube for chemical analysis and measurement of calcium ion content using (Integrated Chemistry System, Siemens, Dimension RxL Max, Germany) (Fig 3).

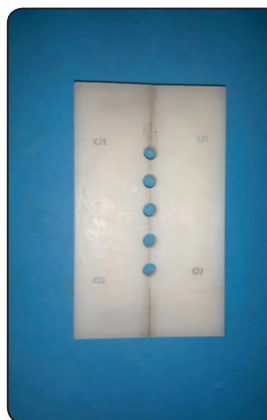


Fig. (1) Teflon mold



Fig. (2) Instron testing machine



Fig. (3) Siemens integrated chemical system

Data were analyzed as mean, standard deviation (SD), Minimum and Maximum. Data explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Independent t-test used to compare between different groups regarding mean Compressive strength (MPa), loss of weight and calcium ion level. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 22 for Windows.

RESULTS

a. Compressive strength results

Mean and standard deviation (SD) of compressive strength (MPa) for Group 1 (CEM + CEM cement solution) and Group 2 (CEM + 2% CHX solution). Group 1 showed higher significant compressive strength than group 2 at all time intervals and time of assessment had significant effect within each group.

TABLE (1) Compressive strength (mean± standard deviation) expressed as MPa

Group Day	Group 1	Group 2	
1	12.831± 1.53 MPa (a)	7.012± 2.1 MPa (b)	p≤0.05
7	13.155± 2.3 MPa (a)	7.324± 2.5 MPa (b)	
15	13.532± 2.1 MPa (a)	7.823± 2.3 MPa (b)	

Different letters indicate significant differences between groups. ($p \leq 0.05$)

b. Calcium ion release results

TABLE (2) Calcium released (mean± standard deviation) expressed as ppm

Group Day	Group 1	Group 2	
1	7.83± 1.53(a)	9.32± 2.1(a)	p≤0.05
7	12.5± 2.3(ab)	19.24± 3.5(b)	
15	14.2± 2.1(b)	27.8± 4.9(c)	

Different letters indicate significant differences between groups. ($p \leq 0.05$)

Solubility rate results

TABLE (3) Weight difference (mean± standard deviation) expressed as µg

Group Day	Group 1	Group 2	
1	12.3± 2.34(a)	17.9± 3.7(a)	p≤0.05
7	27.3± 3.8(b)	43.4± 5.5(c)	
15	38.3±5.1(c)	57.8± 6.9(d)	

Different letters indicate significant differences between groups. ($p \leq 0.05$)

DISCUSSION

In this laboratory experimental study, the compressive strength, solubility rate and calcium ion level of CEM were evaluated after CEM with 2% CHX solution. We investigated the compressive strength, which is a property related to complete setting of a material as well as its strength⁽¹⁹⁾. Despite the mechanical test is unable to represent the clinical situation, still it can display the effects of various mixing liquids and setting conditions on various types of cement⁽¹⁹⁻²³⁾. CEM is widely used in pulp capping and perforation repair. As a result, it is subjected to occlusal and masticatory loads^(18,20). It has been shown that mixing with CHX increases the antimicrobial effects of CEM cement^(17,21). The literature regarding the effect of such mix on the physical properties of CEM is minimal. The results of the present study showed that there was a statistically significant difference between the compressive strength of groups 1 and 2 (i.e., CEM+ CEM cement solution and CEM+ 2% CHX solution) at all time intervals investigated. The results revealed an adverse influence of this addition. This is partly in accordance with the results reported by Sobhnamayan et al⁽²²⁾. They found that push out bond strength of CEM cement samples mixed with 2% CHX was significantly reduced compared to that of conventionally mixed samples, and they

concluded that CHX may affect the mechanical and physical properties of CEM and interfere with hardening of the cement ⁽²²⁾.

The results of the present study also revealed that compressive strength insignificantly increased in both tested groups by increasing time intervals which may be due to slight maturation and completed setting of the cement this is supported by the findings of Sobhnamayan et al ⁽²²⁾ evaluated the effect of time on the push out bond strength. It was affirmed in many studies that the push out bond strength increased with time ⁽²²⁾.

It is well documented that CEM has dental applications similar to those of MTA^(3,4). Comparing the two materials, it was shown that they have comparable sealing ability⁽⁴⁾; although, the antibacterial activity of CEM is higher than that of MTA⁽¹²⁾. In a previous study, it was observed that by using CHX as an irrigant, remarkable crystalline structures on the surface of both accelerated and non-accelerated MTA samples were not obtained⁽²³⁾. They stated that “the surface crystals had thin plate structures, and their size was reduced almost to one-tenth of those of the conventional group (with CEM cement solution)”. In addition they did energy dispersive spectroscopy and they found that silicon was found along with calcium, oxygen, and carbon, which ascertained that they were not the typical calcium hydroxide crystals ⁽²³⁾. These findings might justify why the compressive bond strength of the CEM was significantly decreased and solubility rate and calcium release were significantly increased in our samples exposed to 2% CHX. Future studies are needed to prove the correlation.

Furthermore, addition of chlorhexidine to the mixing solution may lead to incomplete or delayed setting of CEM altering its mechanical and physical properties leading to decreased compressive strength and increased solubility and calcium ion release. This is in agreement with Jacinto RC et al ⁽²⁴⁾ as they concluded that the addition of 2% chlorhexidine to

MTA affected the setting of the material and that extended setting resulted in increased its solubility rate and calcium ion release. Our study finding is supported by Kogan et al ⁽²⁵⁾ as they found that MTA mixed with chlorhexidine gel did not set at the end of a 4 hours observation period. One more possible explanation affecting the solubility of the cement could be related to the change in its powder: liquid ratio. ⁽²⁶⁾

CONCLUSION

Within the limitations of the current study, adding 2% CHX to the CEM cement had a deleterious influence on its compressive strength and solubility.

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