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Effect of Different Water-to-powder Ratios on the Solubility and Microhardness of Calcium-Enriched Mixture Cement

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ABSTRACT

Introduction: The aim of this study was to evaluate the effect of different water-to-powder (WP) proportions on the microhardness and water solubility of calcium-enriched mixture (CEM) cement. **Methods and Materials:** One gram of CEM cement powder was mixed with 0.33 mL, 0.4 mL or 0.5 mL CEM liquid. For water solubility, a total of 60 specimens were prepared (n=20 per each ratio) in the disk-shaped stainless-steel molds with a height of 1.5±0.1 mm and internal diameter of 10.0±0.1 mm. The specimens of each WP ratio were randomly divided into two subgroups: half (n=10) were immersed for one day and the other half (n=10), were kept for 21 days in distilled water. The solubility was calculated as a percentage of the weight loss. To measure microhardness, a total of 30 samples were prepared (10 per each ratio, n=10). The mixtures were transferred to metallic cylindrical molds with internal dimensions of 6±0.1 mm height and 4±0.1 mm diameters. After 4 days the specimens were subjected to Vicker's test. The data were analyzed using two-way ANOVA and post-hoc Tukey's tests at a significance level of 0.05. **Results:** The 0.33 WP ratio showed significantly greater microhardness value (25.98±2.77) compared to 0.4 and 0.5 proportions (P=0.004 and P<0.001 respectively). Significant differences were observed between water solubility values of different WP ratios at both time intervals (P<0.001). At both time intervals, 0.33 and 0.5 WP ratios exhibited the lowest and highest solubility, respectively. **Conclusion:** According to the results of this *in vitro* study, higher WP ratios result in lower microhardness and higher water solubility of the CEM cement. Therefore, the 0.33 WP ratio would be the ideal proportion.

Keywords: Calcium-Enriched Mixture; CEM Cement; Microhardness; Solubility; Water-to-powder Ratio

Introduction

Developments in bioceramic technology have led to the emergence of a new era of endodontic materials in dentistry. In 2008, Asgary [1] introduced a new bioactive endodontic material known as calcium-enriched mixture (CEM) cement. It has been claimed that this tooth-colored water-based cement, also known as a novel endodontic cement, sets in an aqueous environment in less than 1 h [2, 3] and prompts hard tissue healing [4] . CEM cement displays favorable results regarding biocompatibility [5, 6], antibacterial effect [7], and sealing ability [8, 9]. This cement has been successfully used for pulp capping [10], repair of perforations [11] and resorptions [12], surgical root-end filling [13], and regenerative endodontic treatments [4]. In most of these clinical applications, this cement may be applied in contact with tissue fluids such as serum and blood. Being in close contact with fluids, this cement should be water-insoluble in order to avoid dissolution in the aqueous environment. Lack of solubility for an endodontic cement provides a long-term seal and prevent leakage from oral cavity and/or root canal space to the periradicular tissue [14]. Previous studies on the mineral trioxide aggregate (MTA), another endodontic cement, have shown that changes in the powder-to-liquid ratios affect the solubility of this cement. Friedland *et al.* [15], reported that increasing the ratio of liquid to powder leads to a rise in solubility and porosity of MTA. Moreover, Cavenago *et al.* [16] who examined the effect of various water-to-powder (WP) ratios on different physical properties of MTA, confirmed that increasing the amount of water results in higher solubility of the cement.

WP ratio may also influence the microhardness of hydraulic cements because this property is a reflection of hydration process and an indicator of the setting process [17, 18]. Several physical and mechanical properties of a material such as tensile strength, modulus of elasticity, the stability of its crystal structure [19], and the amount of porosity [20] affect its surface microhardness.

The CEM manufacturer has not provided precise instructions regarding the accurate proportion of liquid to powder required achieving optimal physical properties, and also there is no published research on the effect of various powder-to-liquid ratios on the water solubility and microhardness of this cement. Therefore, the purposes of this *in vitro* study were to investigate the effect of changes in the ratio of liquid to powder on the water solubility and microhardness of CEM cement.

Materials and Methods

Water solubility test

The solubility test was performed in accordance with the procedures set out in ISO 6876: 2001 [21] and by the measurement of changes in the sample weight after immersion in distilled water.

Sixty disk-shaped stainless steel molds with a height of 1.5±0.1 mm and internal diameter of 10.0±0.1 mm were used for sample preparation. Acetone was used to clean all molds in an ultrasound bath for 15 min. All molds were then weighed three times (with an accuracy of 0.0001 g) before use and the average weights were calculated. One gram of CEM powder was mixed with either ratios of 0.33, 0.4 or 0.5 mL CEM liquid (20 samples per each ratio). Then the specimens were transferred to the molds and the excess material was removed. The specimens were left to set for 24 h on a grating in an incubator at 37°C and 95% humidity. After this period, the CEM samples in their molds were weighed three times and average reading was recorded up to four decimal digits in grams. The specimens of each WP ratio were randomly divided into two subgroups: half of the samples (*n*=10) were immersed for one day and another half were kept for 21 days in 160 mL of distilled water at 37 °C (±1°C). The samples were placed in a closed container with a relative humidity of 95-100%. After the immersion period, the specimens were removed from the dish and washed with 3 mL of distilled water. Afterwards, they were dried in the incubator at a temperature of 37 °C for 24 h. The weight of the specimens was then measured three times and average reading was recorded. The difference between the initial weight of the material and its final weight was recorded at 0.0001 g. The CEM solubility was calculated as a percentage of the difference in the sample weight to the initial weight of the sample with a precision of 0.001%.

Vickers microhardness

Six custom-made two-part split metal molds were used in the experiment. Each mold had five holes with internal diameter of 4±0.1 mm and thickness of 6±0.1 mm. Thus, finally a total of 30 samples were prepared. Before the molds were filled with CEM, they were randomly divided into three groups. One g of CEM powder (BioniqueDent, Tehran, Iran) was weighed to an accuracy of 0.001 gr using a digital scale (Precisa 180A, PAG Oerlikon AG, Zurich, Switzerland) and was mixed with either ratios of 0.33 , 0.4 or 0.5 mL CEM liquid (*n*=10). Light force was applied to insert the mixtures into the molds and the excess material was removed with moist cotton pellets. The molds were then wrapped in gauze soaked with saline on the top and bottom of the molds and incubated at 37°C in 100% humidity. After 4 days, the samples were polished using silicon carbide paper then subjected to the microhardness test using a Vickers Testing Machine (Bareiss Prufgeratebau GmbH, Oberdischingen, Germany). Samples were loaded with a diamond indenter of 50 g load for 10 sec. This force was applied to the polished surface of each specimen at three separate points in accordance with the ASTM E384 standard for the Vickers microhardness test. Then the Vickers microhardness was calculated using the following formula: $HV=0.102\ F/A\approx 0.1891*F/d^2$ and $A=d^2/(2*\sin 136^{\circ}/2)$ Where *F* is load in Newton's; *0.1891* is Vickers constant; *d* is arithmetic mean of the two diagonals, *A* is impression surface in mm^2, and *HV* is Vickers hardness.

Data analysis

Data were analyzed using SPSS software (SPSS, version 16.0, SPSS, Chicago, IL, USA). Two-way ANOVA was used to compare different variables between three WP ratios, and post-hoc Tukey's test was performed to show significant differences in subgroup comparisons. The level of significance was set at 0.05.

Results

The means and standard deviations of the solubility and microhardness of different experimental groups in both designated time intervals are presented in Tables 1 and 2 respectively.

Solubility

Significant differences were observed between water solubility values of different WP ratios at both time intervals ($P<0.001$) (Figure 1). At both time intervals 0.33 and 0.5 WP ratios exhibited the lowest and highest solubility, respectively (Table 1). While after 1 day all ratios fulfilled the requirements of the international standard 6876, showing a weight loss of less than 3%, none of them were within this standard range after 21 days.

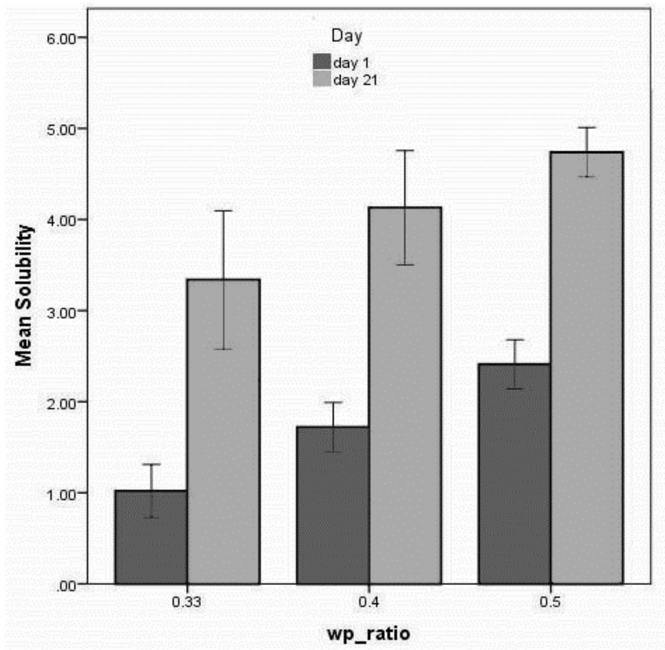

Figure1. Water solubility of different water-to-powder ratios at both time intervals

Vickers microhardness

A statistically significant difference was found between groups (*P*<0.001). The highest and lowest microhardness values were observed in the 0.33 and 0.5 WP ratios, respectively. All three groups had statistically significant differences (*P*<0.001) (Table 2).

Discussion

In this research, tooth-colored CEM cement was investigated. Our results demonstrated that higher amount of liquid in the mixture caused higher solubility of CEM cement. The manufacturer does not provide meticulous instructions with regard to the exact ratio of powder to liquid; merely stating that the ratio is accurate once the paste reaches a 'creamy'consistency. In the present study, three different WP ratios of 0.33, 0.40, and 0.50 were evaluated. A previous study by the same group of authors had demonstrated that WP ratios lower than 0.33 and higher than 0.50 are not suitable for practical uses [22].

Solubility of a cement can affect its structural integrity and dimensional stability and therefore its clinical success and durability in the oral cavity [23, 24]. This property is determined by amount of weight loss due to the elution of the released residual particles by a solution or solvent [25]. Factors such as the immersion time, amount of unreacted substrate, the chemical composition and size of elutable material ,as well as the chemistry of the solvent affect solubility [26].

It should be mentioned that similar to findings of the current study, previous studies on MTA [15, 16] also reported higher amounts of water caused higher solubility of this cement. It seems that increasing the water causes ease of breakup of the molecules from the cements [27].

A recent study demonstrated that increasing the water content of CEM mixture decreases the sealing ability of this material when used as an apical plug [28]. This finding is in accordance with the results of the present study because it is logical to presume that increasing the solubility of an endodontic cement results in loss of adaptation and increasing its microleakage.

In the present study, the weight loss was measured after 24 h or 21 days. All ratios of CEM cement showed some degrees of weight loss at both time intervals especially after 21 days. The percentage of weight loss of all ratios after 24 h was below 3% which was within the acceptable range (≤3%) according to modified ADA guidelines [18]. However after 21 days of immersion in water, the weight loss of all ratios increased and exceeded this limit. Similar to this finding, a previous study reported that the weight loss of hydrated and dehydrated specimens of CEM cement was more than 3% [29]. On the other hand, in the study by Shahi *et al.* [30] the percentage of weight loss of this cement was reported to be below 3%. However in their results, they only reported the weight loss in micrograms not in percentages [30].

Table 1. Mean (SD) values of percentage of solubility recorded for different water to powder ratios at both time intervals

WP ratio	Solubility (1 day)	Solubility (21 days)
0.33	1.02 (0.29) [A]	3.34 (0.76) [A]
0.40	1.72 (0.27) [B]	4.13 (0.63) [B]
0.50	2.41 (0.27) [C]	4.74 (0.27)[B]
***P*-Value**	<0.001	<0.001

**Different upper case letters show significant difference between values in a column*

Table 2. Mean (SD) values of microhardness values of different water to powder ratios

WP ratio	Microhardness
0.33	25.98 (2.77) [A]
0.40	22.54 (1.71) [B]
0.50	20.07 (1.89) [C]
***P*-Value**	<0.001

**Different upper case letters show significant difference between values in a column*

It should be mentioned that a previous study reported no weight loss, but increase in the weight of CEM cement and MTA after 7 and 27 days [31]. This inconsistency can be explained by the different media in which the cements were immersed. While in the present research distilled water was used, in the mentioned study phosphate buffered saline (PBS) was used. This explanation is supported by the finding of Kaup *et al.* [14] who reported weigh loss in distilled water but weight gain in PBS for Biodentine and MTA when comparing the solubility of these materials.

In the present study the effect of different water to powder ratio was also evaluated on the microhardness of CEM cement. Microhardness testing is based on evaluating the resistance of materials to deformation [32]. Therefore, it can be supposed that when microhardness of cements decrease they can be removed more easily [33].

Our results showed the surface microhardness of CEM cement increases with reducing WP ratio. These outcomes reflect the adverse effect of an increased volume of liquid on the hydration process of this hydraulic cement. This finding could be attributed to the increase in porosity that occurs subsequent to a higher amount of liquid. It should be mentioned that microhardness has an inverse relationship with porosity [20] and a previous study reported that higher amount of water caused increased in the porosity of MTA [15]. However, so far there is no study on the effect of different WP ratio on the porosity of CEM cement. Therefore further studies are recommended on this subject.

Conclusion

Within the limitations of this study, when the volume of water in the mixture of CEM cement was increased, higher solubility and lower microhardness were observed. Therefore, the 0.33 WP ratio would be the ideal proportion.

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Conflict of Interest: 'None declared'.

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