

The effects of incorporation of silver-zeolite on selected properties of mineral trioxide aggregate

Çağdaş ÇINAR¹, Mesut ODABAŞ¹, Mügem Aslı GÜREL² and İlknur BALDAĞ³

¹Department of Pediatric Dentistry, Faculty of Dentistry, University of Gazi, Ankara, Turkey

²Department of Endodontics, Faculty of Dentistry, University of Gazi, Ankara, Turkey

³Department of Pediatric Dentistry, Faculty of Dentistry, University of Gazi, Ankara, Turkey

Corresponding author, Çağdaş ÇINAR; E-mail: cinarcagdas@hotmail.com

The purpose of this study was to evaluate some physical and chemical properties of MTA incorporated with antibacterial silver zeolite (SZ). 0%, 0.2% or 2% mass fraction of SZ was added to MTA powder. The amounts of calcium released from the specimen in deionized water were measured with an atomic absorption spectrophotometer. The setting times of the samples were evaluated using Gilmore-type needles. For solubility and water absorption tests, the samples were placed in a cylindrical polystyrene-sealed container. At 7 days, the samples were removed from solutions and blotted dry for solubility and water absorption test. The highest calcium release was detected in 2% SZ MTA at 24 h. The addition of 2% SZ to MTA reduced the setting time statistically ($p < 0.05$). 2% SZ MTA was significantly more soluble than MTA and 0.2 % SZ MTA ($p < 0.05$). Further research is needed prior to clinical use of MTA with incorporated SZ.

Keywords: Silver zeolite, Mineral Trioxide Aggregate, Calcium release, Setting time

INTRODUCTION

Since Mineral Trioxide Aggregate (MTA) is used in dentistry, several studies evaluated the effect of MTA on microorganisms¹⁻⁶, with conflicting results. Torabinejad *et al.*⁷ found that MTA was effective against facultative microorganism but not against other bacterial strains, including *Enterococcus faecalis*. Estrela *et al.*³ also could not detect any inhibitory effect of MTA against *E. faecalis*. In addition, a number of studies have been performed to enhance the antimicrobial features of MTA^{4,5}. In this context several investigators have experimented the use of chlorhexidine liquid or chlorhexidine gel instead of sterile water^{4,5,8,9}.

Silver zeolite (SZ) has been introduced as a crystalline aluminosilicate material with silver ions possessing antimicrobial effect against almost all microbes¹⁰⁻¹². Continuous release of small amounts of silver ions into water results in a long-term antimicrobial effect¹³. SZ also acts as an ion pump providing the controlled time release of silver ions in presence of moisture¹⁴. Thus, the controlled release provides continuous antimicrobial protection for dental products such as mouthrinse¹⁵. An *in vitro* previous study showed favourable antimicrobial effects of MTA containing SZ on several microorganisms. It also has been clarified that incorporated SZ increased the antimicrobial properties of MTA¹⁶. However it is very important that physicochemical properties of MTA containing SZ be considered in relation to their use because such filling material should present biocompatibility, adequate setting time, insolubility and antimicrobial activity¹⁷.

MTA is a Portland cement and is powder of hydrophilic particles consisting of compounds of tricalcium silicate, tricalcium aluminate, tricalcium

oxide and silicate oxide¹⁸⁻²⁰. The physicochemical characteristics of MTA depend on the particle size, the powder/liquid ratio, the temperature and presence of water²¹. The ideal dental material should have characteristics biocompatibility, adequate sealing ability and antimicrobial activity^{7,21}.

The biological properties of MTA are attributed to its calcium ion release capacity^{22,23}. In addition, an increase in calcium ion concentration promotes satisfactory biocompatibility^{19,24}. The setting time of sealer is important to allow adequate working time and proper consistency to permit complete filling of the root canal system and also a quickly setting after insertion, and low solubility under 3% of its mass, according to the International Standards Organization specification no. 6876²⁵.

The aim of this study was to evaluate the physical and chemical properties of MTA incorporated with antibacterial SZ.

MATERIAL AND METHODS

Preparation of SZ and MTA specimens

Zeolite 4A, sodium-type zeolite ($1\text{Na}_2\text{O} \cdot 1\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot \text{XH}_2\text{O}$) (X=mole fraction) (Na-Z; Sigma-Aldrich Chemie, Steinheim, Germany), was used as the host compound. Silver ions were loaded into the zeolite by the ion-exchange method. The ion exchange was carried out by the addition of Na-Z powder (10 g) into a 1 M silver nitrate (Merck KGaA, Darmstadt, Germany) solution (300 cm³). The suspension was stirred at room temperature in the dark for 24 h, then centrifuged, washed with deionized water, and air-dried. SZ was added at a 0.2% or 2% mass fraction to MTA powder (Dentsply, DeTrey, Konstanz, Germany). The control material was MTA

powder without SZ.

Calcium release test

Each composition of the MTA powder was mixed with the recommended liquid according to manufacturer's instruction. Five teflon molds per group with a thickness of 2 mm and a diameter of 4 mm were filled with the freshly mixed MTA. Each specimen was immersed in vials containing 15 mL of deionized water at 37°C. The amounts of calcium released from the specimen in deionized water were measured with an atomic absorption spectrophotometer (AA-6300; Shimadzu Scientific Instruments, Columbia, MD, USA). The instrument was calibrated using standards containing 1, 2, 3, and 4 ppm calcium ion in deionized water. The specimens were removed from the vials, rinsed with deionized water, and reimmersed in equal amount of fresh deionized water. Calcium release was measured at 10 min, 3-, 24-, 48-, 72- and 168-h periods. The results were statistically analyzed using one-way ANOVA and Scheffe's test at a significance level of 0.05.

Setting time

The setting time of test materials was performed according to the American Dental Association (ADA) #57 and American Society of Testing Materials Specification (ASTM) C266-03^{26,27}. Each composition of the MTA powder was mixed with the recommended liquid according to manufacturer's instruction. Three stainless steel rings per group with an internal diameter of 10 mm and thickness of 2 mm were filled with the freshly mixed MTA. The assembly was placed in an incubator at 37°C and relative humidity of 95%. At 150±10 s each specimens was indented with the 113.5 g Gilmore type needle until the initial setting time was determined. After initial setting time, a 456.5 g Gilmore type needle was used to determine the final setting time. The needles were vertically placed on the cement surface. This procedure was repeated at 60-s intervals for each setting time. The setting times were defined as the time elapsed between the ends of mixing and the time when the needles fails to make a complete circular indentation on the surface of the test material. The initial and final setting times of each cement were obtained by arithmetic mean of three repetitions of the test for each cement. The results were statistically analyzed using one-way ANOVA and Tukey test at a significance level of 0.05.

Solubility

The solubility test was performed as described by Gandolfi *et al.*^{28,29}. The materials ($n=3$) were weighed (*i.e.*, the initial weight) and immersed in 20 mL deionized water at 37°C. At 7 day (168-h periods), the materials were removed from the solutions, rinsed with deionized water, blotted dry and placed in an incubator at 37°C for 48 h, and then reweighed until they reached a constant weight (*i.e.*, the dry weight). The solubility (percentage weight variation) was calculated according to the following equation: solubility= $[(\text{dry weight at}$

each time point-initial weight)/initial weight] $\times 100$. Any specimen that showed evidence of disintegration was discarded, and the test was repeated. The results were statistically analyzed using one-way ANOVA and Tukey's test at a significance level of 0.05.

Water absorption

The procedure was the same as that used in the solubility test. The materials ($n=3$) were weighed (*i.e.*, the initial weight) and immersed in 20 mL deionized water at 37°C. At 7 days (168-h periods), the materials were removed and weighed. Each weight measurement was repeated 3 times, and the mean was recorded as the wet weight. Then, the materials were blotted dry at 37°C for 48 h until weight stabilization (*i.e.*, the dry weight). The water absorption at each time point was calculated as follows: water absorption= $[(\text{wet weight at each time point-dry weight})/\text{dry weight}]\times 100$. The results were statistically analyzed using one-way ANOVA and Tukey's test at a significance level of 0.05.

RESULTS

Calcium release test

Changes in the calcium ion release (mg/L) from the MTA specimens are shown in Table 1. In a comparison of calcium ion released from the specimens, the highest calcium release was detected in 2% SZ MTA at 24 h. There were no statistically significant differences between the 2% SZ, 0.2% SZ, and control specimens at 10 min, 24-, 48-, 72- and 168-h periods ($p>0.05$).

Setting time

The mean initial and final setting times for MTA specimens are shown in Table 2. The setting time of specimens decreased with the increase in the ratios of incorporated SZ. Two percentage of SZ MTA exhibited the lowest initial and final setting time ($p<0.05$).

Table 1 Calcium release from 2% SZ MTA, 0.2% SZ MTA and MTA. Mean values expressed in mg/L (mean±SD)

	2% SZ MTA	0.2% SZ MTA	MTA
10 min	7.77±1.26 ^a	8.54±0.40 ^a	7.66±1.73 ^a
3 h	13.66±1.16 ^a	13.69±1.80 ^a	15.23±2.45 ^a
24 h	30.78±2.79 ^a	27.88±0.48 ^a	25.23±1.78 ^a
48 h	12.87±1.08 ^a	14.67±1.97 ^a	15.23±0.89 ^a
72 h	11.86±2.17 ^a	14.48±3.70 ^a	16.22±1.50 ^a
168 h	10.17±0.80 ^a	10.72±0.75 ^a	9.50±1.03 ^a

Different letters within the same period of time (row) indicate statistically difference ($p<0.05$).

MTA, mineral trioxide aggregate; SZ, silver zeolite.

Table 2 Initial and final setting time of the 2% SZ MTA, 0.2% SZ MTA and MTA. Mean values expressed in minutes.

	Initial	Final
2% SZ MTA	9.00 ^a	19.50 ^b
0.2% SZ MTA	11.00 ^a	50.50 ^a
MTA	15.50 ^a	56.00 ^a

Different letters within column indicate statistically difference ($p < 0.05$).

MTA, mineral trioxide aggregate; SZ, silver zeolite.

Table 3 Means of water absorption and solubility (%) for 2% SZ MTA, 0.2% SZ MTA and MTA

	2% SZ MTA	0.2% SZ MTA	MTA
<i>Water Absorption</i>			
168 h	6.79 ^a	8.59 ^a	8.98 ^a
<i>Solubility</i>			
168 h	-7.09 ^b	1.38 ^a	1.01 ^a

Different letters within row indicate statistically difference ($p < 0.05$).

MTA, mineral trioxide aggregate; SZ, silver zeolite.

Water absorption and solubility

The results for the water absorption and solubility of MTA specimens are showed in Table 3. The highest values of water absorption were found at MTA and the lowest values at 2% SZ MTA ($p > 0.05$). The highest solubility values were found at 2% SZ MTA and the lowest values at MTA. 2% SZ MTA was significantly more soluble than MTA and 0.2 % SZ MTA ($p < 0.05$).

DISCUSSION

It has been showed that incorporating SZ into MTA was sufficient for enhancing its antimicrobial activity¹⁶. However, there was no study to evaluate the influence of SZ on chemical and physical properties of MTA. This study evaluated the influence of SZ on calcium ion release, setting time and water absorption of MTA.

In this study, the highest calcium release was detected in SZ MTA at 24 h. However, no statistically significant differences were found between the experimental, and control specimens at 10 min, 24-, 48-, 72- and 168-h periods according to the amounts of calcium released ($p > 0.05$). The amounts of the calcium released were assessed by using an atomic absorption spectrophotometer with a calcium-specific cathode lamp. This is an extensively described method^{20,30}. MTA stimulated hard tissue deposition and it has been suggested that this important formation mechanism

started with calcium ions release from MTA³¹. Similar to present study, Santos *et al.*²⁰, stated that the main component of MTA is calcium oxide, which in contact with water is converted to calcium hydroxide during setting. The calcium hydroxide dissociated into calcium and hydroxyl ions, increasing the pH and calcium concentrations. Therefore, calcium release increased in the first 24-h. In our study, adding SZ into MTA did not adversely affect the calcium release of MTA.

Regarding the setting time property, the setting time of MTA incorporated with 2% SZ was significantly shorter than that of MTA with 0.2 % SZ and without SZ. MTA mixture is affected by many factors such as quantity of water used during mixing, environment humidity, the mixing procedure itself, and temperature³². Therefore, it is difficult to standardize methods used to determine the properties of MTA³³. These factors are not in the present study, control group (MTA without SZ) presented initial and final setting times of 15.50 and 56.00 min, respectively, which are different from observed for MTA by other studies^{8,18,19,34}. Although adding small amounts of SZ (0.2%) was sufficient for enhancing antibacterial properties of MTA¹⁶, it did not improve the setting time of MTA. However, adding 2% amounts of SZ into MTA shortened the setting time. This property of SZ could be explained the structure of zeolite. Zeolites are aluminum silicate crystalline structures that present void spaces within the frameworks, 3–10 angstroms in a diameter that are capable of hosting cations, water, or organic molecules. It has been found that the use of lead zeolite and zinc zeolite leads to acceleration of setting time of Portland cement³⁵.

Regarding the water absorption, in this study, experimental and control group absorbed water at 7 days period. This is an agreement with previous studies^{36,37} that reported Portland cement was shown to absorb water. The other study, Bortoluzzi *et al.*¹⁸ denoted that MTA mixed with CaCl_2 presented weight gain. Camilleri stated³⁶ that water absorption is important to reduce leakage between material and tooth structure. However, an increased water level within the material will increase the porosity of material, thus the potential risk of leakage increase within the material itself.

Regarding the solubility, 2% SZ MTA was significantly more soluble than other groups. Despite the fact that ISO 6786: 2001 suggests evaluating the solubility at 24 h, in the present study, the solubility test was performed at 7 days following a modification previously proposed by Gandolfi *et al.*^{28,29}. However, it is important to explain that the solubility testing standards recommend immersion of the materials only after complete setting, this situation is impossible to be achieved clinically because the materials are immediately in contact with fluid and blood. Therefore, the solubility values are probably even higher under clinical conditions¹⁸.

CONCLUSION

In spite of several limitations, adding SZ into MTA did not adversely effect the physico-chemical properties of MTA. The results of this study suggest that the addition of 2% SZ to MTA reduced the setting time. Although the ratio of 0.2% is recommended, further research is necessary to establish whether incorporation of SZ in MTA would effect the dimensial stability. And laboratory research on cell cultures are needed before clinical use of SZ MTA can be recommended.

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