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## **Basic Research - Technology**

### **Analysis of Arsenic in Gray and White Mineral Trioxide Aggregates using Atomic Absorption Spectrometry**

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## **Abstract**

**Introduction:** The aim of the present study was to investigate whether the concentration of arsenic (As) released from gray or white MTAs met the requirement of ISO for dental cements. **Methods:** Sample preparations were carried out according to the ISO methods. After centrifugation of dissolved samples, As (III) concentration in the final supernatant was analyzed by a high performance atomic absorption spectrophotometer. **Results:** As (III) concentration from both MTAs was much less than the required value (2 ppm) for dental cements regulated by the ISO. An experiment simulating pulp capping using MTA revealed that As concentration was also below the standard value of the ISO. The As concentration in white MTA was lower than the value (10 ppb) recommended for tap water and environmental standards. **Conclusions:** The present *in vitro* studies demonstrated that there is no threat to patient health in using commercially-available brands of MTA for endodontic practices.

## **Key Words**

MTA, arsenic, standard value, endodontic practice

Mineral trioxide aggregate (MTA) has been used as root-end filling material (1-3), a root or furcal perforation repair material (4, 5), and a pulp-capping material (6-10). In 2006, the clinical application of white MTA (ProRoot MTA) was officially permitted by Ministry of Health, Labour and Welfare of Japan (Recognition Number for Medical Device 21800BZY10238000). However, the indication for its use was limited to only pulp capping in Japan.

Arsenic is a poison affecting cells, blood vessels, and nerve fibers (11). The trivalent species of oxidation is the most toxic. For example, in the mouse, the oral LD<sub>50</sub> of arsenic trioxide is 36-fold lower than that of monomethylarsenoic acid (V) (12, 13). In human adult subjects, the lethal dose range of inorganic arsenic is estimated to be 1-3 mg/kg (14).

According to the ISO 9917-1:2003 standard entitled “Dental Water-based Cements. Powder/liquid acid-base cements” (15), a material which is used in dental procedures, should contain no more arsenic than 2 mg/kg (2 ppm). Four studies concerning arsenic release provided by the MTA and Portland cement have been published since 2005 (16-19). Although these four studies are now available to compare the concentration of arsenic in MTA, the methods for sample preparation and extraction from MTA are different from those described in the original ISO standard. Each report presents different data and results. This is the main reason for the controversy in regard to whether the leachable arsenic in MTA is below 2 ppm and thus is safe for patients after clinical application.

Therefore, the present study investigated the As concentration released

from several brands of commercially-available MTA cements according to the ISO 9917-1 standard methods, and used a high performance atomic absorption spectrophotometer. The test hypotheses were that there is much less than the required value for dental cements, and that the level of arsenic release under the conditions simulated for a pulp capping is below the value recommended for tap water and environmental standards.

### **Materials and Methods**

The methodology employed for the analysis of arsenic was based on the ISO 9917-1 standard (15). The standard powder/liquid ratio (MTA powder 1 g, purified water 0.33 mL) was used for this study. The mixed white and gray MTAs were maintained at 37 °C for 24 hours. After 24 hours, they were crushed to a fine powder in an agate pestle and mortar. An accurate 2 g mass of the powdered cement was mixed with 50 mL of 7% hydrochloric acid, and was allowing it to stand for 16 hours.

The simulated experiment as a pulp-capping medicament was performed for white and gray MTAs. The mixed white and gray MTAs were cut into 1 mm cubes. Each sample was placed in 100 µL of 0.1 M phosphate buffer (pH 7.4) at 37 °C for 1, 3, 24 hours, and 3, 7, 14, 28 days after immersion.

The reaction mixture was centrifuged at 1,000 rpm for 10 min. The supernatant was transferred into a sample container with a pipette.

Arsenic quantification was performed using a high performance atomic absorption spectrophotometer (limit of detection: 0.15 ppb) (Z-2000, Hitachi High-Technologies Co., Tokyo, Japan) equipped with a hydride generator

(HFS-3, Hitachi High-Technologies Co.). The analyzed conditions were the following: arsenic hollow cathode lamp and deuterium lamp for background correction, and operating at 193.7 nm wavelength with 12 mA lamp operation current. Arsenic levels (in  $\mu\text{g}/\text{kg}$ ) were obtained by means of a standard curve constructed with 0, 5, 10, 20, and 40 ppb. All measurements were performed in triplicate. The statistical differences between the white and gray MTAs were assessed using an independent *t*-test.

### **Results**

The concentration of As (III) in white and gray MTAs was  $9.365 \pm 1.101$  and  $17.523 \pm 1.303$  ppb, respectively. There was a statistically significant difference between these two MTAs ( $p < 0.01$ ).

The concentration of As (III) in the phosphate-buffer immersion study of white and gray MTAs varied from 0.3 to 0.5 ppb, and from 0.5 to 1.1 ppb at each sampling time, respectively (Table 1). All concentrations in white MTA at each period were statistically lower compared to gray MTA ( $p < 0.05$  for 1 and 3 hours;  $p < 0.01$  for after 24 hours).

### **Discussion**

The present high performance analyses of As (III) clearly demonstrated that the present hypotheses were validated, because the As (III) concentration in commercially-available MTAs was much lower than the cutoff value (2 ppm) for dental cements regulated by the ISO. Furthermore, it is clinically interesting that all data in white MTA were lower than 10 ppb,

which is the level recommended for tap water and environmental standards.

The methods for sample preparation and extraction from MTAs in published studies are different from those described in the original ISO standard. They include differences in preparation in cement-mixing, powdering, the concentration of hydrochloride, reaction volume, and reaction time. The present materials and methods are consistent with the original ISO standard, which is important and meaningful for this type of experiment (20, 21). Furthermore, the ISO 2590 standard (22) dates from 1973. The present atomic absorption or inductive coupled plasma chemical determination methods are thought to be appropriate for arsenic determination compared to the original wet chemical and photometric methods (ISO 2590 standard). Therefore, a high performance atomic absorption spectrophotometer was selected and used for the present analysis with a precise standard curve. The present determination of As (III) released from MTAs in the ISO standard methods revealed extremely low As concentrations, suggesting that commercially-available MTA materials met the ISO criterion for As.

MTA has been used as a biocompatible medicament for pulp capping (6-10). The pulp volume must be calculated to investigate the release of As after MTA medication. The simulated pulp volume of molar was estimated according to the CT data of the premolar. The pulp volume of the premolar (approximately  $30 \text{ mm}^3$ ) was about 6% (23) of the tooth volume (about  $500 \text{ mm}^3$ ) (24). The size of the molar was evaluated to be  $1.5^3$  (= about 3.4) in volume according to the anatomical data (25). Thereafter, the pulp volume of

the molar was hypothetically estimated to be  $102 \text{ mm}^3$  ( $30 \times 3.4$ ). We finally used 100  $\mu\text{l}$  of phosphate buffer as the liquid for immersion experiment.

The concentration of As (III) in the present phosphate-buffer immersion study of white and gray MTAs was 10- to 13-fold lower than that employed using the ISO method, even though the volume of MTA was calculated one-half. This discrepancy was influenced by the solvent volume and the fact that the sample was immersed in hydrochloric acid. Furthermore, the concentration of As (III) in phosphate-buffer immersion study was decreased at each sampling time after 14 days. This may indicate the possibility of saturation in the immersion liquid against As. However, there were no statistical differences among the sampling after 7 days. These findings indicate that the pulp volume simulated in the present study is consistent with the immersion experiment for the 1 mm-cube MTA sample mimic to pulp-capping medicament. The present determination of AS (III) released from MTAs, especially white MTA, indicated that this value was extremely low at all sampling times. The concentration definitively meets the criterion for tap water {standards in WHO (2004), USA (40 CFR, 2001), and Japan (2003)} and environmental standards {Japan (1993, 1999, 2001)}. White-colored MTA lacks tetracalcium aluminoferrite, which is responsible for the gray color (26). The total iron concentration is reported to be extremely high in gray MTA (19). The possibility of tooth discoloration and the slightly high level for tap water and environmental standards in gray MTA indicates that white MTA is more biocompatible for clinical applications than gray MTA.



In conclusion, the present *in vitro* studies demonstrated that there is no problem regarding patient health concerning the released level of As (III) after the application of commercially-available brands of MTAs for endodontic treatments.

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TABLE 1. Determination of As (III) released from MTAs in phosphate buffer

Sampling time	White MTA (ppb)	Gray MTA (ppb)
1 hour	$0.291 \pm 0.009$	$0.5 \pm 0.102$
3 hours	$0.378 \pm 0.058$	$0.682 \pm 0.154$
24 hours	$0.447 \pm 0.017$	$0.682 \pm 0.063$
3 days	$0.413 \pm 0.005$	$0.992 \pm 0.113$
7 days	$0.5 \pm 0.137$	$1.104 \pm 0.064$
14 days	$0.396 \pm 0.161$	$1 \pm 0.085$
28 days	$0.39 \pm 0.086$	$0.969 \pm 0.136$