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Nanomodification of mineral trioxide aggregate for enhanced physiochemical properties

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Abstract

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Aim To analyse the physicochemical properties of a Nano white mineral trioxide aggregate (NWMTA) and compare it with white mineral trioxide aggregate (WMTA).

Methodology White mineral trioxide aggregate and NWMTA were prepared and mixed according to the manufacturer's instructions. Surface area of powder before hydration, setting time, X-ray diffraction and microhardness at pH values of 4.4 and 7.4 were evaluated by Brunauer–Emmett–Teller, ISO Specification no.6876, Vickers microhardness, and energy-dispersive X-ray spectroscopy equipped with X-ray colour (dot) map for both cements. ANOVA and Mann–Whitney were used for statistical analysis at a significance level of 0.5.

Results The mean ± SD of surface area and setting time were 1.8 ± 0.2 $m^2 g^{-1}$ and 43 ± 2 min for WMTA and 7.8 ± 1.2 $m^2 g^{-1}$ and 6 ± 1 min for NWMTA, respectively. Mean ± SD of Microhardness were 16 ± 2, 51 ± 1, 69 ± 1 and 81 ± 2 for WMTA at pH values of 4.4 and 7.4 and for NWMTA correspondingly. Numbers of open porosity over the surface were 88 ± 24 and 44 ± 13 for WMTA and NWMTA, respectively. Statistical tests revealed significant differences between the groups ($P < 0.001$) in surface area, setting time and surface hardness for both cements. Uniform distribution of strontium was only observed in NWMTA. However, other compounds were not significantly different.

Conclusion Increasing surface area of powder can reduce setting time and increase microhardness even at lower pH values after hydration.

Keywords: Energy-dispersive X-ray spectroscopy, setting time, surface area, WMTA, X-ray colour (dot) map, XRD.

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Introduction

White mineral trioxide aggregate (WMTA) (ProRoot, Dentsply Tulsa Dental, Tulsa, OK, USA) has several advantages over other materials used in endodontics, including biocompatibility, good sealing ability and antibacterial properties (Torabinejad *et al.* 1995). Despite these advantages, it has a long setting time (Kogan *et al.* 2006, Ber *et al.* 2007) and low resistance to acid (Saghiri *et al.* 2008), which may prevent MTA setting (Namazikhah *et al.* 2008) as well as increase its porosity (Saghiri *et al.* 2008). Mixing MTA with an acidic solution such a 2% lidocaine HCl with an epinephrine concentration of 1 : 100 000 reduced the compressive strength of MTA in an acidic environment (Watts *et al.* 2007).

Attempts have been made to improve the properties of WMTA by incorporating materials into its structure. However, the physical and chemical properties are often affected adversely (Torabinejad & Parirokh 2010). Strontium salts improve the bioactivity of bone substitute materials (Peng *et al.* 2010). Moreover,

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bioactivity is highly desirable for root-end filling materials (Gandolfi *et al.* 2010). Studies have shown that the smaller particle size and increased surface area of Portland cement (Tennis & Jennings 2000) and WMTA (Komabayashi & Spångberg 2008) play an important role in physical and chemical properties, partly because of better and more rapid hydration with lower porosity (Tennis & Jennings 2000). Final setting time of WMTA is more than 3 h (Torabinejad *et al.* 1995). Initial setting time has been reported to be approximately 40 min (Islam *et al.* 2006a), which is not desirable when WMTA is used as a root-end filling material (Torabinejad & Parirokh 2010).

Microhardness has an inverse relationship with porosity (Saghiri *et al.* 2010a). Therefore, WMTA with a higher microhardness value has less porosity (Namazikhah *et al.* 2008, Saghiri *et al.* 2008). Lower porosity is highly desirable for an impermeable root-end filling material (Torabinejad & Parirokh 2010). Studies have shown that environments with low pH values can adversely affect WMTA by reducing microhardness and increasing microleakage (Namazikhah *et al.* 2008, Saghiri *et al.* 2008).

The effect of surface area of powder on properties of WMTA has not been well documented. Incorporating various trace elements such as strontium on the physical and chemical properties of MTA has not been elucidated. A new version of MTA (Nano) has been patented in the USA and claimed to set faster with acceptable resistance to acidic environments by adding a small amount of strontium and reducing its particle size (US Patent application No. 13/211.880). Therefore, the aim of this study was to compare the specific surface area, initial setting time, surface porosity, crystallography and hardness of Nano white mineral trioxide aggregate (NWMTA) with those of ProRoot WMTA.

Materials and methods

White ProRoot MTA (Dentsply Tulsa Dental) Batch number (083006) and Nano Endodontic Cement (Patent application #13/211.880) were used. All experiments were conducted at 37 °C.

Surface area

The Brunauer–Emmett–Teller (BET) technique was used to measure the surface area of the powder of the cements by the physical adsorption of a nitrogen gas (N_2). This part of the study was similar to those carried out by Rößler *et al.* (2008). Ten specimens of each cement, weighing 0.5 g, were out-gassed under vacuum overnight. The specific surface area was determined by measuring isothermal N2 adsorption with a Micromeritics (ASAP-2010, Norcross, GA, USA). This machine works by an area procedure of adsorption–desorption cycles of nitrogen. The samples were cooled down in liquid nitrogen under a flow of N_2 and were then heated up to room temperature. The amount of desorbed nitrogen was measured by a thermoconductive detector and allowed to determine specific surface area for each sample under standard temperature and pressure with the following specifications: Specimen weight: 0.5 ± 0.1 g; saturation pressure: 789.57 mmHg; evacuation time: 2.0 min; and analysis mode: equilibration, equilibration time: 5 s. Then, BET multipoint surface area was reported for each specimen.

Setting time

Five 1-g sachets of WMTA and five 1-g samples of NWMTA were used to measure setting time. This part of the study was similar to those carried out by Islam *et al.* (2006a) and according to ISO Specification No. 6876:2001. In brief, after mixing each sample with 0.3 g of distilled water, the samples were placed in cylindrical moulds (height, 1 mm; diameter, 10 mm), covered with water-moistened gauze (Saghiri *et al.* 2010b) and incubated at 37 °C. Sample testing started just before their anticipated initial setting time and at 1- to 5-min intervals until the initial set was reached. A Gilmore needle (Humboldt, Schiller Park, IL, USA) 2.0-mm flat-ended indenter with 100-g mass was applied at right angles to the surface of the sample for 5 s. The initial setting time was defined as the quantity of time during which the indenter failed to leave a definite mark on the surface of the sample. ANOVA was used for statistical analysis at a significance level of 0.5.

Microhardness

The methodology was similar to Saghiri *et al.* (2010a,b). Briefly, 8 WMTA powder sachets were mixed with distilled water at 0.3 mL g^{-1} liquid-to-powder ratio; the same procedure was repeated for NWMTA. The cements were packed in 40 cylindrical stainless steel moulds with a diameter of 5 mm and a height of 1.5 mm using a nonsurgical manual MTA carrier (Dentsply Tulsa Dental) and hand pressure (Watts *et al.* 2007, Saghiri *et al.* 2010a) to obtain 10 specimens for each group. Two groups (n = 20) containing both cements were placed in a vial with a piece

of gauze soaked in butyric acid buffer solution with a pH value of 4.4 for 3 days. The remaining groups (n = 20) were stored in sterile deionized water at a pH of 7.4. The gauze pieces were replaced daily to ensure a constant pH value (Saghiri *et al.* 2009). The pH of the gauze pieces was check twice before placement (Lotfi *et al.* 2011). A two-way ANOVA was conducted that examined the effect of material and pH on microhardness. Our dependent variable, microhardness, was normally distributed for the groups formed by the combination of the material and pH as assessed by the Kolmogorov–Smirnov test.

Scanning electron microscopy

After microhardness testing, one specimen from each group was evaluated under the scanning electron microscope EDS mode to analyse elemental distribution. Specimen surfaces were polished and sputter-coated with 10 nm of gold and observed under a scanning electron microscope (VEGA; TESCAN, Brno, Czech Republic) and Leo 440i (Oxford Microscopy, Cambridge, UK). Secondary electron (SE) and back-scattered electron (BSE) detectors at ×1000 and ×5000 magnifications were selected. EDS colour dot map analysis was performed for each sample at ×1000. The ImageJ program (Rasband WS, ImageJ; US National Institute of Health, Bethesda, MD, USA) was used to calculate open pores over the surface of specimens after hydration. Digital images were recorded using a Microsoft picture manager (Redmond, WA, USA) to standardize each picture at 640 × 480 pixels. Then, surface porosity was calculated by the ImageJ program (Fig. 1). Each figure was inverted (Fig. 1a) by this program, and brightness was adjusted to select the orifices of each one (Fig. 1b). Binary images were made considering the orifices of the pores as outlined, and the total number of outlines in each micrograph was calculated (Fig. 2e,f). The data were analysed by two-sample Kolmogorov–Smirnov test to check a normal distribution and the Mann–Whitney test to detect any significant difference between the groups ($P < 0.05$).

X-ray diffraction

After the setting time measurement, one specimen of each group was randomly selected and crystalline phases of the cements were determined by XRD analysis. Specimens were milled into powder with a mortar and pestle. The XRD patterns were recorded with an XRD device (Seifert XRD 3000, Ahrensburg, Germany). Samples were scanned at a range of 5–70,

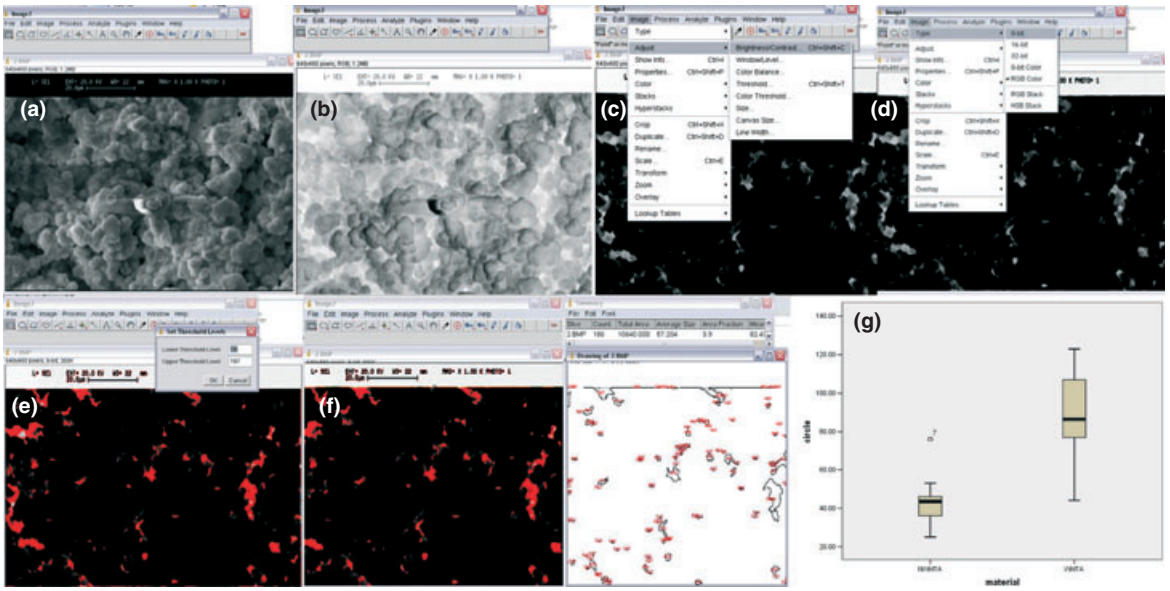

Figure 1 Sequence of image analysis by Image J. (a) Micrograph of white mineral trioxide aggregate (WMTA) polished perpendicularly; (b) inverted image for feasible calculating. (c) Enhanced contrast. (d) Conversion to 8-bit type for better calculation. (e) Threshold adjustment for better calculation. (f) Calculation of the amount of circles or ellipses in the micrograph in regards to the scale. (g) Means ± SD of the number of outlines were 44 ± 13 and 88 ± 24 for Nano white mineral trioxide aggregate and WMTA, respectively. Mann–Whitney test revealed significant differences ($P < 0.001$).

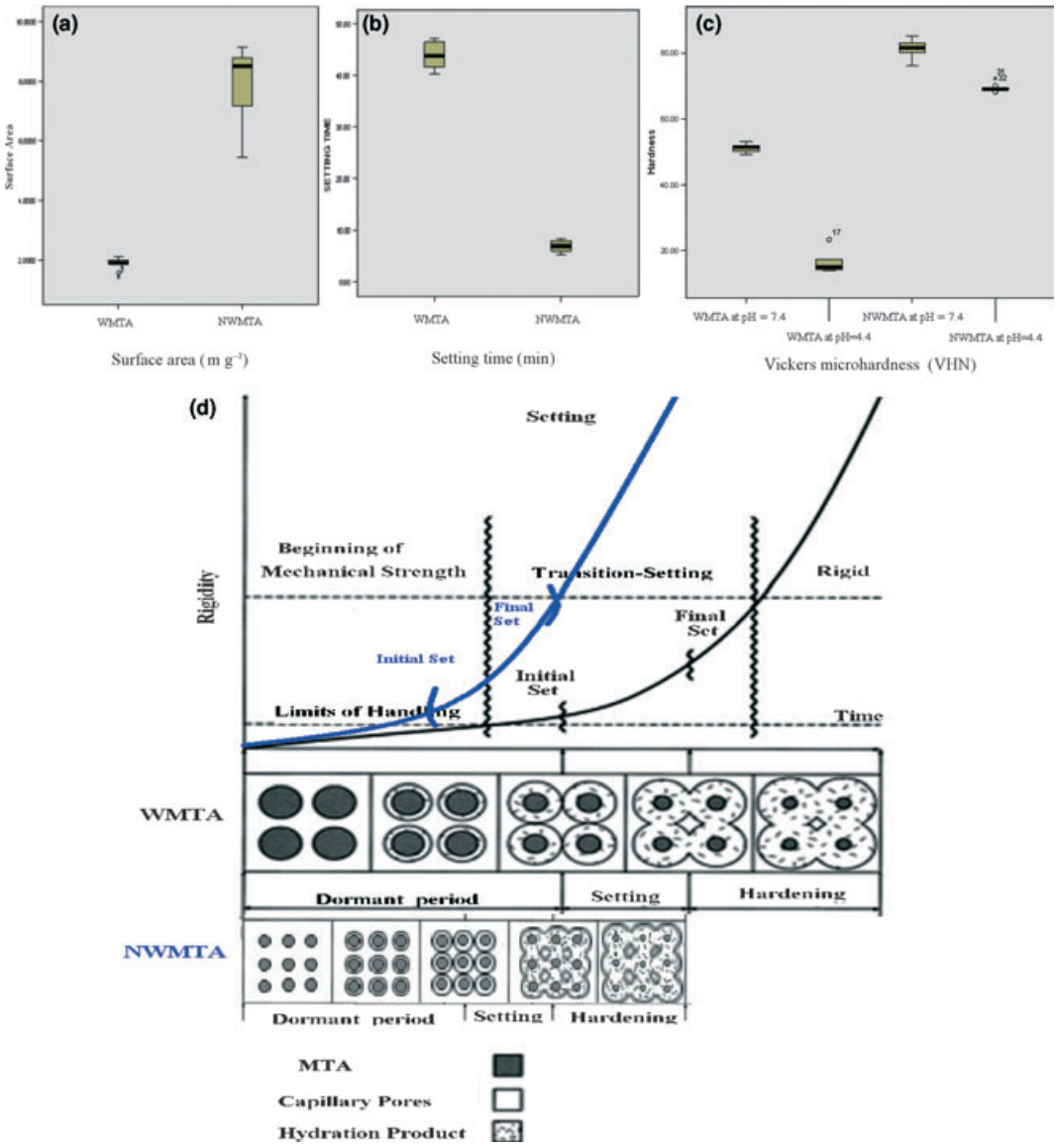

Figure 2 Box plots of surface area (m^2/g) (a), setting time (min) (b), surface microhardness (VHN) (c) values in both groups, which illustrate the means ± SD, minimum and maximum amount of time for specific surface area, initial setting and surface microhardness, as well as the variance in each experimental group. Schematic representation of white mineral trioxide aggregate and Nano white mineral trioxide aggregate hydration process (d).

and all data were collected in a continuous scan mode at a scanning rate of 2°/min.

Results

Surface area

The means ± SD of the surface areas were 1.8 ± 0.2 and 7.8 ± 1.2 $m^2\ g^{-1}$ for WMTA and NWMTA, respectively. The Mann–Whitney *U* test demonstrated significant differences between the two groups ($P < 0.001$) (Fig 2, left).

Setting time

The means ± SD of initial setting time were 43 ± 2 and 6 ± 1 min for WMTA and NWMTA, respectively. The Mann–Whitney *U* test demonstrated significant differ-

ences between the two groups ($P < 0.001$) (Fig 2-centre).

Microhardness

The means ± SD of microhardness of WMTA at pH 7.4, WMTA in pH 4.4, NWMTA at pH 7.4 and NWMTA at pH 4.4 were 51.31 ± 1.14, 16.10 ± 2.84, 81.53 ± 2.62 and 54.59 ± 1.08, respectively. There was homogeneity of variance between groups as assessed by Levene's test for equality of error variances. There was a significant interaction between the materials and pH on microhardness, F (1, 36) = 305.481, $P < 0.001$. In other words, NWMTA had significantly greater microhardness than WMTA at both pH. The same test showed that the effect of pH on reducing microhardness was significant for both WMTA and NWMTA ($P < 0.001$) (Fig 2-right).

Scanning electron microscopy

Micrographs from the BSE analysis revealed the qualitative microstructure of set WMTA and provided a means of directly examining the relative densities of different phases of the microstructure in terms of the presence of unhydrated cement grains. Surface topography of both specimens revealed many needle-shaped crystals predominantly covering the surface. Some irregular crystal structures were observed on the surface of WMTA. At high magnification, nonporous grey images were observed by BSE in NWMTA. Separate unhydrated flat particles with hydrated cores surrounded by shallow pores were seen in the WMTA specimens (Fig. 3).

EDS dot map (SEM)

A spectrum was obtained, and the elements were identified to derive atomic percentage concentrations of the elements. Four main elements (calcium, silicon, bismuth and strontium) were analysed from each sample. The elements of NWMTA were the same as that of WMTA, but strontium as a trace element was absent in WMTA (Fig. 3g,h).

Surface porosity (SEM)

Means ± SD of the number of open porosities over the surface were 88 ± 24 and 44 ± 13 for WMTA and NWMTA, respectively. The two-sample Kolmogorov–Smirnov test revealed that the distribution of samples was not normal ($P = 0.003$). Therefore, the Mann–Whitney test was used and revealed significant difference ($P < 0.001$) (Fig. 1).

Phase composition (XRD)

X-ray diffraction results of cements and bismuth oxide (Purum, Fluka, Germany) are presented in Fig. 4. The same main constituent phases were observed in both cements; however, for NWMTA the intensity of the peak at $2\theta = 25.9°$ decreased but increased at $2\theta = 30.8°$, which may suggest the presence of a small amount of carbonated strontium.

Discussion

Short-setting time prevents washout or dislodgement of MTA cement plugs (Watts *et al.* 2007). Many efforts have been made to improve setting time by incorporating some additives such as $CaCl_2$ (Bortoluzzi *et al.* 2006), polymers (Chng *et al.* 2005), plasticizers (Oliveira *et al.* 2010) or other materials. However, additives increase toxicity and compromise physical properties or thwart bioactivity phenomenon (Torabinejad & Parirokh 2010). MTA cement is a complex material, and its hydration provides additional complexity. Indeed, as yet no single method exists that completely determines all chemical reactions taking place in a WMTA structure from the start of mixing. Therefore, several complementary techniques must be used.

Allen (1997) confirmed that surface area of powder is related directly to the setting time of a cement base material, which was confirmed by the results of the present study. The present study revealed that an acidic environment had an adverse effect on the microhardness of WMTA cement, which is consistent with previous studies (Lee *et al.* 2004, Namazikhah *et al.* 2008, Saghiri *et al.* 2008). However, WMTA was affected to a large extent than NWMTA, which might be attributed to the greater porosity of WMTA compared to NWMTA. The greater porosity might accelerate acid penetration into the surface texture and decrease surface microhardness. Greater porosity can also increase crack propagation.

Previous studies (Islam *et al.*2006b Saghiri *et al.* 2010a) have shown the feasibility and reliability of XRD test for evaluating hydration of MTA. XRD structural analysis revealed that specimens in both groups were crystalline and showed similar patterns. The results of XRD analysis of WMTA cement were

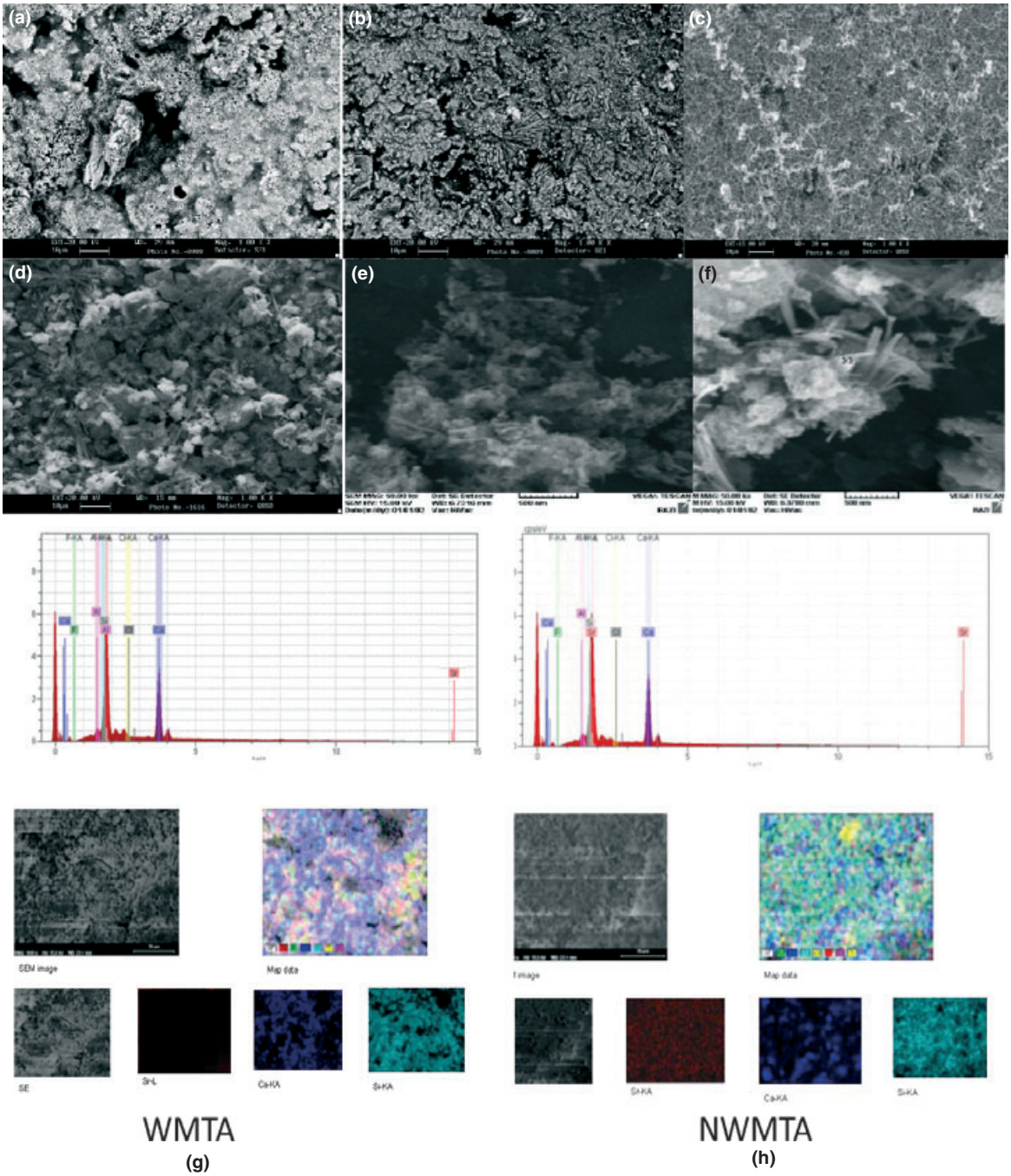

Figure 3 Scanning electron micrographs of white mineral trioxide aggregate (WMTA) specimens and Nano white mineral trioxide aggregate (NWMTA) by using secondary and back-scattered electron (BSE) detector. (a), WMTA exposed to an acidic environment showed wide and heterogeneous dispersion of porosity (») than NWMTA exposed to an acidic environment (b). (c) BSE mode illustrated hydrated products and a better interlocking solid (») in NWMTA than that observed in WMTA (d). High magnification showed better nucleation of calcium-silicate-hydrated needle (») of NWMTA (e) compared to WMTA (f). Energy-dispersive spectroscopy dot map of the specimens in both groups (g, WMTA and h, NWMAT). Normal dispersion of strontium was only observed in NWMTA. However, other compounds were not significantly different.

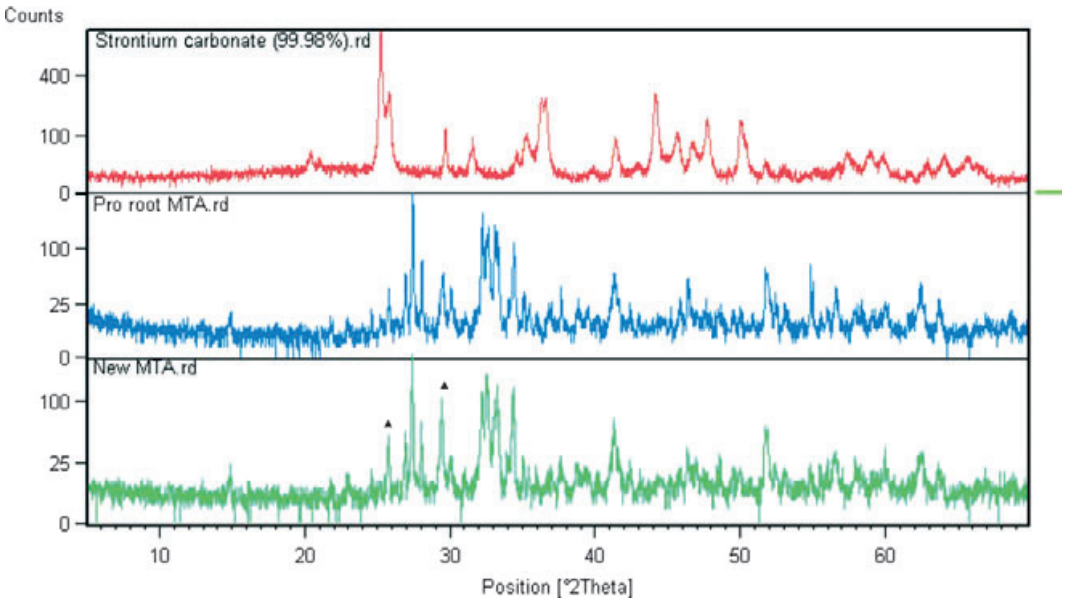

Figure 4 X-ray diffraction patterns of white mineral trioxide aggregate, NWMAT and strontium carbonate, indicating the presence of the same main constituents (same phases in the materials composition hydration); however, the intensity of the peak at $2\theta = 25.7$ and 29.8 for Nano white mineral trioxide aggregate is strengthened group increased, which maybe imply the disperse of this material without any reaction with ingredients of cement over the texture.

consistent with previous results reported by Camilleri *et al.* (2005) and Islam *et al.* (2006b).

X-ray diffraction revealed a diffraction peak at $2\theta = 29.3°$, which is ascribed to C-S-H phases, the main binding phases in MTA-based systems (Saghiri *et al.* 2010a). In the WMTA group, the intensity of the peak at $2\theta = 29.3°$ decreased, which might be attributed to lower C-S-H content in the final hydrated product leading to detrimental effects on the hydration reaction.

One disadvantage of XRD is that it may not be accurate in some compounds with ingredients in quantities of <5% (Islam *et al.* 2006b). Therefore, after examining the scanning rate in a pilot study the scanning rate was decreased to $2° \text{ min}^{-1}$ for better detection of trace compounds such as strontium carbonate.

A previous study (Saghiri *et al.* 2010a) showed that storage at higher or lower than room temperature affected some physicochemical properties of calcium silicate cements, such as MTA, and may have altered its phase formation. Therefore, to exclude any interfering parameters, all specimens were stored in an incubator (EN 025, Nuve San, Turkey) for 48 h before testing.

Studies have evaluated the particle size and shape of MTA by using methods, such as SEM (Lee *et al.* 2004), flow particle size analysis (Komabayashi & Spångberg 2008) and X-ray diffractometry (Kozul 2010). However, these methods are unreliable because of the following disadvantages: transmission electron microscope (TEM) and SEM only focus on a small part of the specimen and do not demonstrate the exact particle sizes of the samples; they depend on how the image's area analysed, which yield different results (Sarkar 1985, Mitiche & Bouthemy 1996). Particle size analysers cannot calculate irregular shapes (Bowen 2002). However, previous studies have shown the feasibility and practicality of using BET for measuring a specific surface area (Sang-Jin *et al.* 1999, Odler 2003).

Recent studies have confirmed that incorporation of a small amount of strontium to bone cements can create or increase bioactivity and bioconductivity properties (Peng *et al.* 2010, Yang *et al.* 2011). This study confirmed that adding small amounts of strontium to WMTA did not adversely affect its physical properties.

Many studies evaluated the potential for variation in porosity resulting from manual condensation of the mixed MTA slurry (Aminoshariae & Moon 2003, Nekoofar *et al.* 2007) but all of them agreed that manual hand pressure may produce the least effects on surface porosity; besides, in the clinic, this material is condensed by hand pressure. The present study used hand pressure to simulate its clinical application.

For SEM, specimens were prepared to evaluate the crystal size that occurred and the interstitial porosity (Saghiri *et al.* 2012a,b). Energy-dispersive X-ray spectroscopy EDS is an analytical technique that qualitatively and quantitatively identifies the elemental composition of materials analysed in an SEM. EDS generally analyses the top two microns of the sample with a spatial resolution of one micron.

Energy-dispersive spectroscopy analysis was performed twice for each gold-coated sample at ×1000 magnification. Low magnification was selected to observe the type of elemental distribution over the texture and to evaluate the homogeneities distribution of strontium. The results were consistent with the results of studies carried out (Torabinejad *et al.* 1995, Camilleri *et al.* 2005), which revealed that bismuth is one of the sub-ingredients of WMTA and other elements are the same as those found in Portland cement.

The difference between the constituent elements of NWMTA and WMTA was related to the presence of strontium with a uniform distribution on the surface. The high magnification (×5000) was selected to view porosity and crystal formations within a sample only for the SE mode of SEM. In NWMTA, a constant and uniform nonporous grey image was observed, which might be ascribed to a more proper hydration of products and good interlocking of the crystal compounds of WMTA (Fig. 3).

The results of setting time in this study were similar to those of Islam *et al.* (2006a), who reported that the initial setting time of WMTA was approximately 40 min. However, despite the lack of significant differences in chemical composition of WMTA and NWMTA, the initial setting time of NWMTA was approximately 6 min. The difference in initial setting time might be attributed to the total surface area of NWMTA that was greater than WMTA specimens, which means that NWMTA may react more rapidly with water and thus prevent washout of the cement plug before final setting.

Conclusion

- Within the limitations of the present experiments, the following could be concluded.
- Increasing the surface area of WMTA decreased setting time ($P < 0.001$).
- Incorporation of a small amount of strontium (<5%) to WMTA does not have a deleterious effect on its physical properties.
- Despite the lack of significant differences in XRD patterns (crystallography change), the numerical microhardness and initial setting time and surface porosity comparison of both groups revealed significant differences ($P < 0.001$). These findings are probably due to the influence of specific surface area of powders on the exothermic reaction of the cement during hydration, which may affect physical properties of calcium silicate cements to some extent.
- NWMTA revealed low surface porosity and acid resistance. However, the faster hydration reaction influenced volumetric changes and may lead to dislodgement of cement plug in a clinical scenario. Therefore, further investigation is recommended to evaluate other physiochemical properties such as comprehensive strength, volumetric change and push-out bond strength.

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Conflict of interest

The authors affirm that they have no financial affiliation or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript and deny any conflicts of interest related to this study. M Ali Saghiri and Mehrdad Lotfi hold a US patent for this new endodontic cement.

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