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Effect of Endodontic Cement on Bone Mineral Density Using Serial Dual-energy X-ray Absorptiometry

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Abstract

Introduction—Materials with new compositions were tested in order to develop dental materials with better properties. Calcium silicate–based cements, including white mineral trioxide aggregate (WMTA), may improve osteopromotion because of their composition. Nano-modified cements may help researchers produce ideal root-end filling materials. Serial dual-energy x-ray absorptiometry measurement was used to evaluate the effects of particle size and the addition of tricalcium aluminate (C₃A) to a type of mineral trioxide aggregate–based cement on bone mineral density and the surrounding tissues in the mandible of rabbits.

Methods—Forty mature male rabbits ($N = 40$) were anesthetized, and a bone defect measuring $7 \times 1 \times 1$ mm was created on the semimandible. The rabbits were divided into 2 groups, which were subdivided into 5 subgroups with 4 animals each based on the defect filled by the following: Nano-WMTA (patent application #13/211.880), WMTA (as standard), WMTA without C₃A, Nano-WMTA + 2% Nano-C₃A (Fujindonjnan Industrial Co, Ltd, Fujindonjnan Xiamen, China), and a control group. Twenty and forty days postoperatively, the animals were sacrificed, and the semimandibles were removed for DXA measurement.

Results—The Kruskal-Wallis test followed by the Mann-Whitney U test showed significant differences between the groups at a significance level of $P < .05$. P values calculated by the Kruskal-Wallis test were .002 for bone mineral density at both intervals and $P_{20 \text{ day}} = .004$ and $P_{40 \text{ day}} = .005$ for bone mineral content.

Conclusions—This study showed that bone regeneration was enhanced by reducing the particle size (nano-modified) and C₃A mixture. This may relate to the existence of an external supply of minerals and a larger surface area of nano-modified material, which may lead to faster release rate of Ca²⁺, inducing bone formation. Adding Nano-C₃A to Nano-WMTA may improve bone regeneration properties.

Keywords

Bone healing; bone mineral density; nano–tricalcium aluminate; serial dual-energy x-ray absorptiometry; white mineral trioxide aggregate

The development and modification of dental cements has taken place for many years in order to bring about an optimal interaction between hard tissues and dental cements (1). The literature has addressed the need for osteopromotion in order for materials to become more osteogenic, osteoconductive, and osteoinductive (2). Several materials and compositions have been tested to achieve optimal bone-implant interface reaction and osseous response. Calcium silicate–based cements including mineral trioxide aggregate (MTA) would improve these characteristics because of their composition. MTA was developed as a new root-end filling material at Loma Linda University, Loma Linda, CA (3). Several studies have investigated the properties of MTA, including composition, radiopacity, setting time, and biocompatibility, and compared them with other root-end filling materials (4–6). The main constituents of MTA include Portland cement (75 wt %) with bismuth oxide (20 wt %) and gypsum (5 wt %). Portland cement itself is a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate (C_3A), and tetracalcium aluminoferrite (4). It is reported that white MTA has lower concentrations of Al_2O_3 , MgO , and FeO compared with gray MTA (7).

MTA induces cementum formation and may facilitate the regeneration of periodontal ligament and bone formation (8–10). MTA has been studied in animals and has exhibited good biocompatibility when implanted in osseous tissues (6, 11). It also presents some difficulties such as slow setting time and initial softness (12). The physicochemical properties of MTA rely on particle size, powder to liquid ratio, temperature, and incorporation of water and air bubbles during mixing. MTA stimulates the release of cytokines from bone cells, promoting the formation of hard tissues (13). Furthermore, MTA is highly bioactive and induces a strong bond with bone (14). Using Nano–white mineral trioxide aggregate (WMTA) instead results in significant improvements in some properties such as setting time, microhardness, and push bond and compressive strength (15, 16).

Bone mass is an important factor in measuring bone quality (17). The most common method of measuring bone mass is dual-energy x-ray absorptiometry (DXA), and it is a reliable and noninvasive method (18–20). Surgeons can predict bone health and fracture risk and can measure bone response to osteoporosis treatment by using DXA evaluation. DXA has been modified for application in small mammals, facilitating the use of animals in longitudinal studies to quantify bone mass changes.

In fact, it is a precise tool for determining bone mineral content (BMC) and bone mineral density (BMD) in animal models. A study by Kastl et al (19) on rat humeri showed that DXA is a simple, accurate, and precise technique for measuring BMC and BMD in isolated small animal bones and is therefore an excellent tool for assessing osteopathies. Their study revealed that water would be a good substitute for determining BMC and BMD using DXA. In their study, BMC measured by DXA was compared with bone dry weight, ash weight,

and bone calcium content. Furthermore, DXA, BMD, and BMC evaluation precision was determined.

The hard tissue response and osteopromotion of WMTA cements may be improved by adding nano-additives to their composition, partly because of a better and more rapid hydration rate. Although C₃A constitutes a small amount of WMTA composition, it has an undeniable role in the hydration process (21, 22). The addition of C₃A to C₃S, (Ca₃SiO₅)/C₃A mixtures, which are derived from MTA, has been shown to accelerate the hydration process. As a result, the setting time significantly reduces, compressive strength increases, and the mixtures assumes similar bioactivity and biocompatibility when compared with pure C₃S (23). The aim of this study was to evaluate the bone response to the implantation of Nano-WMTA (patent application #13/211.880), WMTA, WMTA without C₃A, and Nano-WMTA + 2% Nano-C₃A in the mandible of rabbits by using serial DXA measurement.

Materials and Methods

Forty healthy 6-month-old mature male Dutch rabbits ($N = 40$) weighing 2000 ± 200 g were selected. This study was conducted in accordance with the guidelines and approval of the Animal Ethics Committee of Shiraz University of Medical Sciences #4253 and the Helsinki Declaration in care and use of laboratory animals.

This part of the study was similar to that of a study by Gallas Torreira et al (24). Briefly, the rabbits were divided into 2 equal groups. Each group was subdivided into the following 5 subgroups with 4 animals in each subgroup based on the defect filled by the following: group A: Nano-WMTA (patent application #13/211.880), group B: tooth-colored MTA (Dentsply Tulsa Dental, Tulsa, OK) as a standard group, group C: calcium silicate cement similar to WMTA ingredients without C₃A (Kamal Asgar Research Center, Encino, CA), and group D: Nano-WMTA + 2% Nano-C₃A (15–80 nm; Fujindonjnan Industrial Co, Ltd, China), whereas the last group received no treatment and served as the control group.

The rabbits were anesthetized with an intramuscular injection of 10% ketamine (Alfasan, Woerden, Netherlands) at a dose of 44 mg/kg and 2% xylazine (Bayer, Munich, Germany) at a dose of 8 mL/kg. Local anesthesia was administered by the infiltration of approximately 0.25 mL 3% lidocaine. The hair on the skin around the ventral surface of the mandible and neck regions was shaved, and the skin was prepared followed by aseptic surgery. A 3-cm incision was made on the ventral surface of the mandible to expose mandibular symphysis. A bone defect was drilled into the mandible of each animal using a round carbide bur in a high-speed handpiece with continuous application of sterile saline solution. A bone defect with a dimension of $7 \times 1 \times 1$ mm was created on the right semi-mandible. Figure 1A and B show the defect and its position.

After irrigation with normal saline, control of bleeding, and drying the site, the materials were hand mixed with water using a 3:1 powder to liquid ratio to achieve a putty-like consistency according to manufacturers' instructions and directly placed in the osseous cavity. In the control group the bone defects were left to heal naturally without the

application of any external material. All the other parameters of placement, including mixing, length, and packing were kept consistent. The incisions were then closed with 3-0 silk sutures; Flunixin (Norbrook Laboratories, Newry, UK) (0.15 mg/kg) as an analgesic drug and penicillin (22,000 IU/kg) were injected for 3 days. The animals were subjected to the same diet and environmental conditions.

The animals in each group were sacrificed 20 and 40 days after surgery by putting them in a 70% carbon dioxide chamber for 5–10 minutes. Subsequently, the animals' semimandibles were removed and embedded in 10% buffered formalin.

DXA scans were performed with a Hologic WI (Bedford, MA) bone densitometer with the serial number of #84107, which was calibrated daily in accordance with the manufacturer's recommendations. To measure BMC and BMD using DXA, the specimens were positioned centrally at the bottom of a cubic thin-walled plastic container filled with water up to a height of 8 cm. After a semimandible was sunk in the container, the device was set for starting the test. The regional high-resolution mode of the small animal scan protocol (scan field 1.0 [width] $\times$ 1.2 [height] cm², a scan time of 3 minutes) was used. The mandibular bone density and bone content of the rabbits were measured and compared in each group. All the DXA measurements and analyses were performed by the same operator, who was kept blinded during the analysis.

Statistical Analysis

Because the data were shown to be parametric, the results could be compared between groups. However, considering the small sample size, a nonparametric Kruskal-Wallis test followed by the Mann-Whitney *U* test was run to analyze the data. Statistical analysis was performed using SPSS software (SPSS Inc, Chicago, IL). Differences were considered significant at $P < .05$.

Results

BMD

Figure 1 shows the bone defect positioning and the region of interest on the semimandibles of rabbits. The mean BMD data $\pm$ standard deviation for 20- and 40-day intervals in different groups and their significances are listed in Figure 2. In both the 20-day and 40-day groups, BMD was higher than that in the control group, showing more mineral content in the region of interest. Statistical analysis using the Kruskal-Wallis test showed a significant difference between the groups at both time intervals ($P_{20 \text{ day}} = .002$ and $P_{40 \text{ day}} = .002$).

BMC

The BMC data showed the same characteristics as BMD data (Fig. 1). At the 20-day interval, the BMC of test groups was higher than that of the control group. At the 40-day interval, the BMC increased in all the test groups as expected. The mean BMC data $\pm$ standard deviation for the 20-day and 40-day intervals and their significances are listed in Figure 2. The Kruskal-Wallis test showed a significant difference between the groups at both time intervals ($P_{20 \text{ day}} = .004$ and $P_{40 \text{ day}} = .005$).

Discussion

Rabbits are one of the most commonly used subjects for animal studies; they are used in approximately 35% of musculoskeletal research studies (25). Although there are differences between human and rabbit bone compositions and densities, Wang et al (26) reported some similarities in BMD and fracture resistance of mid-diaphyseal bone between rabbits and humans. In another study, DXA with small animal software was used to measure BMD in rabbit vertebrae, confirming that QDR-4500 (Hologic, Waltham, MA) can be used to yield precise and accurate measurements of rabbit spine BMD (18).

Lu et al (20) studied porous ceramic (hydroxyapatite and β -tricalcium phosphate with the same porosity measured by mercury intrusion porosimetry) implanted in rabbit femurs and used DXA to evaluate BMD and BMC in the region of interest. DXA allows for the analysis of the implant inside the bone and material modification according to information provided by histomorphometry. Their results confirmed the potential for DXA in bone substitution evaluation and also excellent new bone contact at the bone-implant interface for both hydroxyapatite and β -tricalcium phosphate. Consistent with these studies, serial DXA testing was selected for bone healing measurement. In this research, Teflon tubes or silicone tubes were not used as a material carrier for WMTA into bone defects because the aim was to evaluate WMTA response in direct contact with osseous tissue.

Calcium aluminate plays an important role because it facilitates the application of the material in bone defect, allows for dynamic degradation, promotes the formation of new tissue, and thus accelerates healing of the surrounding bone and further increases bone density (27). The addition of Nano- C_3A to Nano-WMTA may influence both physicochemical and biological properties of Nano-WMTA by enhancement of its hydration process as a result of an increase in its surface area. C_3A is known to have the fastest hydration rate among the main components of Portland cement so that it may accelerate the hydration process in WMTA (28).

Saghiri et al (15) compared the physicochemical properties of Nano-WMTA and conventional WMTA, reporting that increasing the surface area of powder could shorten the setting time and increase the microhardness even at lower pH values after hydration. The present study revealed that bone regeneration was enhanced by reducing the particle size (nano-modified) and C_3A mixture as well.

Some researchers claim that MTA promotes apatite decomposition when exposed to simulated body fluid (SBF) (29). Liu et al (23) studied the formation of hydroxyapatite (HA) on C_3S/C_3A mixtures derived from MTA. Their results verified that the C_3S/C_3A mixtures could induce the formation of an HA layer on their surface in SBF, contributing to *in vitro* bioactivity. Although the addition of C_3A partially affected the apatite induction rate, the C_3S/C_3A composites with 10% C_3A or less were still bioactive in SBF. The current study showed that C_3A might have a great impact on the bone response of WMTA *in vivo* when comparing the test results of groups C and B at both time intervals. At the 20-day interval, there were no significant differences between these 2 groups, but at the 40-day interval, the difference was significant for the results of BMD.

The hydration product of WMTA has some amorphous phase in its constitution (30, 31), and MTA has been shown to promote the release of calcium into hard tissues (32). A faster hydration process resulting from the addition of Nano-C₃A to Nano-WMTA causes faster setting time. A shorter time for hydration products to become crystalline also results in the development of amorphous phases. The small particle size in group D might have resulted in the material having a more amorphous phase in its hydration product.

Short-term studies have shown that MTA can accelerate osteoblastic function, improving the bone-healing response (33, 34). In fact, the existence of an external supply of minerals, the larger surface area of the nano-material, and the faster release rate of Ca²⁺ induced bone formation, resulting in an increase in BMD and BMC at 20- and 40-day intervals in groups in which the material had been implanted in the osseous defect compared with the control group.

In all the 40-day samples, there was a complete bone bridge on the implanted material, indicating bone generation around the test materials. There was a significant difference between groups in relation to BMD and BMC data at both time intervals. It was evident from BMC and BMD data that nano-modified WMTA exhibited a better response in bone healing compared with commercial WMTA. Statistical analysis of BMD and BMC data showed significant differences at both the 20- and 40-day intervals. There was faster bone generation in the presence of Nano-WMTA mixed with Nano-C₃A followed by Nano-WMTA. This might be because of the enhancement of simulating cytokine release and interleukin production from bone cells, indicating that nano-modification of the material may actively promote hard tissue formation as reported by other researchers (13).

As other researchers have reported, MTA can induce bone generation, as confirmed by the results of the current study (10, 23, 33–35). Nano-WMTA was supposed to accelerate the bone-healing process, and it exhibited a better response in the bone-healing process when nano-tricalcium aluminate was added to it. In fact, tricalcium aluminate in both nano and regular forms exerted a positive effect on the WMTA osseous response as expected.

Conclusions

Within the limitations of the current experiment, it can be concluded that nano-modified WMTA has a positive effect on the bone-healing process. However, the addition of Nano-C₃A to Nano-WMTA may also improve its bone-regenerating properties.

Acknowledgments

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The authors deny any conflicts of interest related to this study.

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Figure 1.
(A) The creation of a defect on the right semimandible. (B) The region of interest (position of local analysis) in BMD and BMC.

Figure 2.

The mean $\pm$ standard deviations of BMD and BMC data 20 days and 40 days after implantation. Significant differences between groups at time intervals are specified by symbols. (A) Nano-WMTA, (B) WMTA, (C) calcium silicate cement similar to WMTA ingredients without C3A, and (D) Nano-WMTA + 2% Nano-C3A, and the control group.