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Effect of Silver Nanoparticles of Herbal Origin on the Compressive and Push-out Bond Strengths of Mineral Trioxide Aggregate

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Introduction: The purpose of this *in vitro* study was to investigate the effect of incorporating silver nanoparticles (AgNPs) of herbal origin into mineral trioxide aggregate (MTA) on the push-out bond strength (PBS) and compressive strength (CS) in simulated furcal area perforations. **Materials and Methods**: In this *in vitro* study, simulated furcal area perforations (1.3 mm in diameter and 2 mm in depth) were created in 40 extracted human lower molar teeth, which were divided into two groups (n=20): MTA alone and MTA combined with AgNPs (2% wt). Using a universal testing machine, PBS was evaluated by performing push-out tests, while CS was assessed using cylindrical specimens. The normal distribution of data was checked using the Kolmogorov-Smirnov test, and statistical analysis was performed using two-way ANOVA. **Results**: The CS results showed no significant difference between the MTA group at 4 and 21 days (P=0.297), but a significant difference was observed in the nanosilver/MTA group (P=0.013). However, there was no significant difference in the push-out bond strength among the study groups (P>0.05). **Conclusion**: The incorporation of herbal origin silver nanoparticles did not significantly affect the PBS or CS of MTA.

Keywords: Bond Strength; Compressive Strength; MTA; Nano Particle; Push-out

Introduction

Mineral trioxide aggregate (MTA) is hydrophilic calcium silicate-based cement used for various purposes in endodontic treatment procedures. It has several desirable features, such as high sealing ability, biocompatibility, promoting tissue regeneration, osteogenesis, cementogenesis, dentinogenesis, antibacterial properties, ability to set in the presence of human blood as well as bond well to dentin walls [1-3]. In spite of mentioned advantages, there are some drawbacks, though, such as extended setting time and difficult manipulation [4-7]. In this regard, various techniques have been proposed in order to shorten the setting time, including the incorporation of widely used additives in dentistry, such as $CaCl_2$ and Na_2HPO_4 [8, 9]. Moreover, silver nanoparticles (AgNPs), Zn and TiO_2 have been exploited to promote the physical and antibacterial properties of MTA [4, 10-13].

The applicability of Ag in oral care has been thoroughly understood for decades, acquired worldwide acclaim in the 19th century as one of the primary elements in dental amalgam used for tooth restoration. Since 1930, the use of Ag in amalgams has decreased due to the increased replacement of silver with aesthetic polymeric resins [14]. With the advancements in the realm of nanoscience and the recognition of remarkable antimicrobial characteristics of nanostructured Ag-based components against various microorganisms, including fungi, viruses and bacteria [15-18], attention to Ag has been restored. Given the circumstances, AgNPs have been considered as practical antimicrobial elements in implants [19], adhesives [20] and prosthetic materials [21] in order to foster osteogenic induction [22], hinder biofilm establishment [23] and the arrest of caries [24]. Regarding the studies on silver NPs, AgNPs have been incorporated into composite resins and MTA to improve the physical and antimicrobial properties of these materials [19, 20].

Furthermore, it has been suggested that their usage, decreases the risk of oral bacterial infections and accelerates the wound healing process [25, 26]. In a similar study, Vazquez-Garcia *et al.* Investigated the effects of AgNPs on physiochemical and antimicrobial properties of various types of calcium silicate cements such as MTA, concluding that the incorporation of AgNPs into WMTA would effectively enhance the antimicrobial properties and decrease the solubility as well as initial and final setting times in these kinds of composites. Also, no significant difference could be observed between the CS of WMTA and WMTA/AgNPs experimented groups [27]. Considering the clinical application of MTA and the importance of compressive strength as well as push-out bond strength to dentin as major characteristics of dental materials, various studies have been conducted to improve these two properties.

The synthesis of metal nanoparticles is an immense and advancing area because of their capacity to be applied in numerous fields, including chemistry, energy, electronics and medicine. Metal NPs indicate novel and enhanced characteristics based on their morphology, distribution and size [28]. To accomplish the increasing demand for eco-friendly NPs, investigators are using microorganisms to synthesize different metal NPs [29, 30]. However, currently, plant extracts are used as reducing and capping agents in the synthesis of these NPs. For instance, plant leaf extracts of *Syngonium podophyllum* [31], *Elephantopus scaber* [32], *Geranium* [33], *Shikakai* and *Reetha* [34] have been applied to AgNPs synthesis, resulting in the formation of pure metallic AgNPs with direct utility. These NPs have proper antibacterial qualities and small amounts that are needed to achieve antimicrobial action that would have been generated by a large quantity of bulk counterparts [35, 36].

Considering the benefits of MTA as well as silver NPs of herbal origin, this study investigated the effects of AgNPs incorporation into MTA on compressive strength and push-out bond strength.

Materials and Methods

Synthesis of silver nanoparticles

Silver nanoparticles were synthesized by the Center for Pharmaceutical Nanotechnology of Tabriz University of Medical Sciences (Tabriz, Iran) benefiting green chemistry method [37]. Briefly, 10 mL of *Prunus domestica* (*P. domestica*) (commonly known as European plum which is a hexaploid fruit tree species cultivated around the world) extract solution was added drop-wisely to 100 mL of $AgNO_3$ (Merck) (5 mM) solution with continuous agitation at 85°C for 20 min. During the procedure pH was adjusted at 7 by 0.1 M solution of NaOH. The *P. domestica* extract was combined with $AgNO_3$ aqueous solution (*i.e.* silver ions complex) and altered the solution's color to yellowish brown due to the surface plasmon resonance (SPR) excitation that denotes the AgNPs formation [30, 37]. Subsequently, the synthesized AgNPs were centrifuged at 13000 RPM for 15 min and re-dispersed in deionized water. This procedure was repeated three times to eliminate any unorganized biological molecules and purify the synthesized NPs.

Push-out test

Sample preparation

Forty extracted lower human molar teeth were used to evaluate the push-out bond strength. The outcome of a similar study was used to determine the sample size [5]. The extracted teeth were removed from soft tissue, and then stored in 0.5% chloramine-T solution until the test was performed. The crowns of the cleaned teeth were cut off from the CEJ, using a diamond disk (SP 7600 Microtome, Leica, NuBlock, Germany). Afterwards, the teeth were placed in acrylic molds, with the furcal floor of about 3 mm out of the acrylic resin. This was done to make some space below the furcal area to put a cotton ball as a matrix to pack the experimental materials in order to repair the perforated furcal area [38].

Creating perforations and experimental materials

Each perforation was created using a #1/2 round bur, placed perpendicular to the furcation floor and parallel to the longitudinal axis of the tooth, so that the perforation diameter reached 1.3 mm. The height of the dentinal walls of the perforated area was measured using a periodontal probe and it was standardized at 2 mm for all of them. The samples in which the thickness of dentin at the perforated area was less than 2 mm were excluded. Following to this, all the samples were thoroughly washed with normal saline in order to remove the remaining debris. Based on the materials in the perforation area, the experimental samples were divided into two groups (n=20). In group 1, MTA powder alone was mixed with MTA liquid at a ratio of 3:1; according to the manufacturer's instructions. In group 2, however, MTA and 2%wt of herbal-derived silver nanoparticles were mixed with MTA liquid. Subsequently, the test materials were carried to the perforation site with the MTA carrier and condenser. The normal saline-moistened cotton pellet was used for the elimination of excessive materials. Eventually, the samples were stored in closed containers at 37°C and up to 100% relative humidity [38].

The push-out test was conducted using the universal testing machine. The materials in the perforated area were driven by a cylindrical rod 1/1 mm in diameter, at a rotational velocity of 0.5 mm/min, and in an apical direction toward and parallel to the

longitudinal axis of the tooth. Prior to dislodgment, the universal testing machine recorded the maximal forces exerted to the substance in Newton (N). The push-out bond strength was calculated in mega-Pascal (mPa) using the equations I and II [39]:

(II)

$$\text{bond strength (debond stress)} = \frac{\text{debonding [dislodging] force (N)}}{\text{[bond] surface area (mm}^2\text{)}}$$

(II)

$$\text{(bond)surface area} = \text{height of perforation (mm)} \times 3.14 \times \text{radius of the perforated area (mm)}$$

Compressive test

Based on the materials and evaluation time of compressive test, four separated groups were considered in this study. For each of the groups, 5 study-based similar specimens were allocated depending on the type of specimens that were placed in the cylinders as well as the setting time for evaluation of CS standard test. The study groups were as illustrated in Table 1.

Procedural steps

The process of measuring CS was based on the No. 9971 Protocol [40]. All the materials and tools were set at a temperature of 23±1°C for one h prior to use. Following to this, the MTA powder of all the four groups was prepared with a powder/liquid ratio of 3:1; in accordance with the manufacturer's instructions (Angelus, Londrina, PR, Brazil). While in groups 1 and 2, MTA alone was mixed with MTA liquid, in groups 3 and 4, MTA and 2%wt AgNPs were added to MTA liquid. The entire specimens were mixed for 10 sec each. Before the insertion of samples, the interiorsurfaces of the holes were filled with paraffin wax. Two minutes after the placement, samples were rolled in wet sterile gauze pieces and stored in a container at 37°C until CS was measured. Compressive strengths of samples were measured in accordance with ISO 6876, using a universal testing machine (Hounsfield test equipment, Model: H5K-S, Surrey, UK).

Table 1. The study groups and their characteristics

Group no.	Characteristics of the experimented samples
Group 1	MTA compressive strength evaluation period: 4 days after placement of the materials into the cylinder
Group 2	MTA compressive strength evaluation period: 21 days placement of the materials into the cylinder
Group 3	MTA+2% herbal-derived silver nanoparticles compressive strength evaluation period: 4 days after placement of the materials into the cylinder
Group 4	MTA+2% herbal-derived silver nanoparticles compressive strength evaluation period: 21 days after placement of the materials into the cylinder

On the 4th day, the first and third groups' samples, and on the 21st day, the second and fourth groups 'samples were evaluated. In this regard, the samples were removed from the generator and introduced to a force of the horizontal round bur, running at a velocity of 1mm/min along with the longitudinal axis of the generator so that the materials would be crushed or broken. This force was described by Newton's laws of motion, and converted to mega-Pascal using the equation III [41]: $CS = 4P/\pi D^2$, where P is the maximum applied load in Newton, D is the measured diameter of sample in mm, and π is the constant 3.14.

Statistical analysis

The Kolmogorov-Smirnov test was used to check the normal distribution of the data. After calculating the mean ± standard deviation of push-out and CS test results, the two ways ANOVA was conducted to assess the significance of the effect of time and type of material (with and without AgNPs). $P<0.05$ was considered statistically significant.

Results

AgNPs were synthesized with an average diameter of 27.85±9.54 nm. Figure 1 shows the purity of synthesized AgNPs in the image of field emission scanning electron microscopy (FE-SEM) with energy dispersive X-ray spectroscopy (EDS) which illustrates the peaks indicating the presence of Ag and O. The peaks indexed in Ag label are related to AgNPs and the heterocyclic compounds of *P. domestica* extract can be the origin of O peak. Because of the SPR, metallic Ag nano-crystals have an absorption peak of around 3 keV [42, 43].

As shown in Table 2, the highest mean of push-out bond strength in the MTA group was 93.10±30.77. There was a difference of 8.63 units between the means of push-out bond strength values between the two experimented groups, while that of MTA group was higher. This difference is considered insignificant (P=0.457).

The CS value of nano-silver/MTA group was higher than the MTA group in the period of 4 days (134.78 units of mean

Table 2. The comparison of push- out bond strength between the two experimented groups

Study groups	Bond strength	*P*-Value
MTA	93.10±30.77	0.457
MTA+AgNPS	84.47±42.23	

Table 3. Comparison of compressive strength in two evaluated groups within 4 and 21 days [Mean (SD)]

Study groups	4 days	21 days	*P*-Value
MTA	818.94 (116.38)	666.30 (195.96)	0.297
MTA+AgNPS	953.72 (91.34)	600.98 (120.91)	0.013

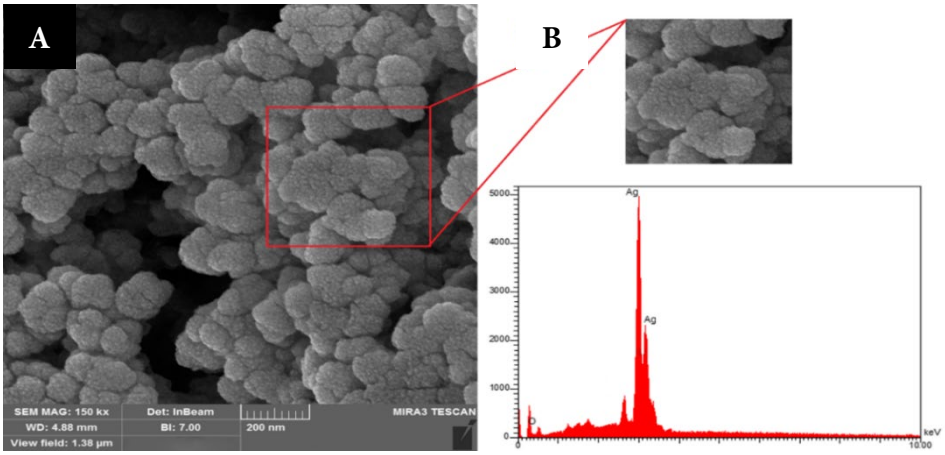

Figure. 1. *A*) Field emission scanning electron microscopy image of the synthesized silver nanoparticles (AgNPs); *B*) Energy-dispersive X-ray spectrum of the synthesized AgNPs

difference), whereas the same value was higher in the MTA group (65.32 units of mean difference) in comparison with nano-silver/MTA group in 21 days (Table 3).

The CS in both groups was higher in 4 days compared to 21 days. Consequently, the results showed no statistically significant difference between 4 and 21 days period in the MTA group (P=0.297); however, the difference was meaningful in the nanosilver/MTA group (P=0.013).

Discussion

The present study was designed to assess the effect of incorporation of herbal silver nanoparticles to MTA on the push out bond and compressive strength. The results manifested no negative effect on mentioned properties.

Microbial control in periodontal and endodontic tissues is essential in order to achieve success in endodontic treatments. In order to prevent post-treatment infections, there is a need for a biocompatible obturation with high antimicrobial properties in the treatment of perforations and external and internal root resorption [44, 45]. Therefore, the antimicrobial properties of materials and cements used in root canal treatments are of great importance [36, 46, 47]. With this aim in mind, in order to investigate the antimicrobial effects of silver NPs mixed with MTA, several studies have been carried out in which NPs have been synthesized in different ways. For instance, several studies have reported on the NPs synthesized as silver zeolite mixed with MTA, and most studies have indicated that mixing these particles result in an improvement of MTA antimicrobial properties [7, 48]. Other similar studies on mixtures of NPs have also demonstrated an improvement in antimicrobial properties of MTA. Furthermore, to achieve this goal, other studies have also been conducted to examine the effect of NPs on other physical properties of MTA, including compressive strength and push-out bond strength. In addition, the incorporation of silver NPs into MTA resulted in increased CS [7], or similar to the results of our study, no significant difference in its magnitude was noticed [27].

Similar to these studies in our study the 2% weight nanoparticles with MTA was used; due to the best result of evaluated properties [12, 13, 49]

Due to the new properties and applications that nanoscale materials have exhibited in the industry, there is currently a strong interest in their processing and application. Basically, the characteristic properties of surface area and the volume of materials in the nanometer scale demonstrate significant changes. In other words, in a nanometer scale, the surface and volume properties of materials properly correlate with each other; surface molecules can cause a significant increase in hardness of metals and thus are more efficient to produce electronic devices and pharmaceuticals with much better performance. Biotechnology is one of the most promising approaches for science and technology in the new era. This technology is emerging in various fields of science, including chemistry, biology and material science [50].

Nanotechnology has experienced significant advances in nanomaterials and major trends toward new methods and materials. With the development of new materials and techniques, environmental concerns have been raised in relation to the NPs produced through chemical methods and the subsequent dangerous side effects have doubled. Hazard-free biological methods can substitute conventional chemical methods to prepare NPs [51, 52]. Silver NPs have various biological applications due to their biocompatibility. Chemical methods usually leave some toxic chemicals on NPs. Therefore, the use of herbs and herbal derivatives

as a sustainable and available source for production of biocompatible NPs has drawn the attention of many researchers in recent years [35, 53]. Among the advantages of this method are non-toxicity, biocompatibility, low cost and production of high-purity NPs. Given these benefits, the NPs synthesized in these methods have biological applications. Many plants are able to produce NPs for use in such a valuable and treasured industry that is still unknown. Many of these plants have not yet been tested, and thus, their intrinsic nanoscale features have not yet been understood [54]. Considering many properties and applications of silver NPs in biomedical and pharmaceutical industries, this study took into account the unique properties of green chemistry and used it to synthesize silver NPs.

Conclusion

Based on the findings of the present study, the incorporation of herbal origin silver NPs did not result in a significant difference in the push-out bond strength as well as compressive strength of the MTA.

Conflict of Interest: 'None declared'.

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