# Evaluation of the Effect of Nanosilver and Bismuth oxide on the Radiopacity of a Novel Hydraulic Calcium Silicate-based Endodontic Sealer: An *In vitro* Study

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#### **Abstract**

Background and Aim: A wide range of dental materials have incorporated the concept of nanotechnology into their composition to enhance their physical and antimicrobial properties. In this pretext, silver nanoparticles (AgNPs) are among the most commonly used nanoparticles which are exceptionally noteworthy for their role in medical applications as an antibacterial agent. Another essential, desirable physical characteristic of all endodontic cements is their radiopacity, while in similar context, various radiopacifying agents such as bismuth oxide, barium sulfate, and even AgNPs have been incorporated in endodontic sealers to enhance their physical properties. The aim of the present study was to assess whether the incorporation of AgNPs and 10% bismuth oxide imparted the required radiopacity to the novel cement material (Nano CS) as per the requirement and standards laid by the International Organization for Standardization (ISO) guidelines and whether it complied with the ISO 6876:2001 specifications to achieve the necessary norms. Materials and Methods: The structural characteristics of the novel cement material (Nano CS) were observed using energy-dispersive X-ray analysis under a Zeiss Gemini 500 Field Emission Scanning Electron Microscope, while radiopacity of the test material (Nano CS) was assessed with the help of an aluminum (Al) step-wedge using a nondestructive testing method following ISO guidelines. The optical density of the test material (Nano CS) was tested with the specimens of mineral trioxide aggregate (MTA) as the standard cement material along with the specimens of enamel and dentin that were 1 mm thick, and Al of appropriate thickness with the desired and equivalent radiopacity. Results: The findings of the present study suggested MTA to have higher radiopacity index equivalent to 4.56 ± 0.00 mm thickness of Al when compared to the test material (Nano CS) (2.78 ± 0.01 mm thickness of Al) and enamel (4.09 ± 0.01 mm thickness of Al) and dentin (2.01 ± 0.01 mm thickness of Al) specimens. Furthermore, the radiopacity index of test material (Nano CS) was found to be more when compared to dentin, though, less when compared to the enamel specimens with the results being statistically highly significant (P < 0.001). Conclusion: The addition of nanosilver and bismuth oxide to the test material (Nano CS) imparted characteristic radiopacity, though the required specifications laid down by the ISO standards were not achieved. Increasing the concentration of the additives used might be considered to bring in the required radiopacity without having a significant impact on the physical and biological properties of the test material (Nano CS) intended to be used for endodontic applications.

Keywords: Bismuth oxide, calcium silicate, endodontic sealers, in vitro study, nanosilver, radiopacity

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## INTRODUCTION

For many years, calcium silicate-based bioceramics have been used in endodontics. The reign of such hydraulic cement began in the mid-1990s with the launch of mineral trioxide aggregate (MTA), and since then, a wide range of alternative cement have been tested and tried. Endodontic applications

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for these materials include retrograde filling, perforation repair, pulp capping, and obturation, apexogenesis, and apexification procedures.[1] Following the success of MTA, a new generation of calcium silicate cement has emerged for dental applications. These newer cements incorporate tricalcium silicate and dicalcium silicate as key components. Furthermore, like MTA, they offer significant advantages in the form of a rapid setting of the cement when exposed to moisture due to their hydraulic properties, and promoting the survival of vital pulp tissue, along with promoting blood vessel formation, potentially aiding the process of regeneration. Their bioinductive properties may even stimulate the growth of new dentin offering a more comprehensive approach to the endodontic treatments done. [2] However, despite the exceptional characteristics of these calcium silicate-based cements, deterrents that include an extended setting time of around 3 h, a bounded antimicrobial action owing to their alkalinity, and their being expensive substitutes to the routine cement render these an undesirable option for endodontic applications.[3] Numerous efforts have, also, been made to overcome these limitations with the addition of certain additives such as calcium chloride, sodium hypochlorite, KY jelly, and chlorhexidine gluconate which have been used to substitute distilled water in MTA. It has been observed that 10% calcium chloride when added to MTA, speeds up the setting reaction of cement, as well as enhances its compressive strength.<sup>[4,5]</sup> In a similar context, silver nanoparticles (AgNPs) are among the most commonly used nanoparticles (NPs) which are exceptionally noteworthy for their role in medical applications as an antibacterial agent, while it has been demonstrated that adding AgNPs to MTA potentially enhances its antibacterial efficacy. [6,7] With this background, a novel dicalcium silicate-based hydraulic endodontic cement material (Nano CS), incorporated with AgNPs, was introduced by Dsouza et al. [8,9] has been found to be biocompatible, dimensionally stable, maintain alkalinity, and has a shorter setting time. In addition, the newly introduced cement material (Nano CS) exhibited an increased calcium ion release that contributed to the bioactivity of the material. Another essential, desirable physical characteristic of all endodontic cements is their radiopacity. According to the International Organization for Standardization (ISO) 6876:2001 specifications, all dental root canal sealing materials, which set with or, without assistance of moisture and are used for the permanent obturation of the root canals with or, without obturating points or, cones during orthograde use, must show radiopacity higher than 3mm thickness of aluminum (Al) (reference value). Likewise, all endodontic sealing materials must have a value of radiopacity equivalent to at least 2mm thickness of aluminum (Al) more than the radiopacity exhibited by dentin or, bone as per the American National Standards Institute (ANSI)/American Dental Association (ADA) specification no. 57.<sup>[10]</sup> In a similar context, various radiopacifying agents have been incorporated in the endodontic sealers to enhance their physical properties which include a wide range of radiopacifiers such as bismuth oxide, barium sulfate, and even more recent additions like the AgNPs.<sup>[11]</sup> Previously, this novel cement material (Nano CS) has been tested for evaluation of its physical and biological properties.<sup>[8,9]</sup> However, radiopacity of the test material (Nano CS) has not been evaluated yet. The present study was conducted with a similar intent wherein the aim of the present study was to assess whether the incorporation of AgNPs and 10% bismuth oxide imparted the required radiopacity to the novel cement material (Nano CS) as per the requirement and standards laid by the ISO guidelines, and whether it complied with the ISO 6876:2001 specifications to achieve the necessary norms.

# MATERIALS AND METHODS

According to the method originally presented by Dsouza et al., [8,9] the novel cement material (Nano CS) was made. For at least 2 h, 98% ethanol was manually mixed with the calcium oxides (60%) and silicon oxides (20%) as well as minor components including aluminum oxide, 10% bismuth oxide, and 0.5% of 20 nm-sized AgNPs. The powdered combination was further subjected to sintering for 24 h at 100°C in a hot air oven. The liquid component added was 10% calcium chloride solution which sped up the setting reaction.

#### **Preliminary investigations**

Preliminary work was done to optimize the test material (Nano CS) by subjecting it to different temperatures of 100°C, 250°C, 400°C, and 600°C. A further temperature increase was not attempted as silver has a melting point of 961.8°C. Setting time was determined at each temperature set, which showed that the setting time was favorable at temperatures of 100°C and 600°C. A pilot study on the antibacterial activity against *Enterococcus faecalis* at these temperatures showed a zone of inhibition of 3–4 mm only for the test material (Nano CS) which was heated to 100°C. Therefore, this test material (Nano CS) was taken up for further evaluation of the properties.

#### Characterization of the test material (Nano CS)

Energy dispersive X-ray analysis (EDXA), also, known as energy dispersive X-ray spectroscopy or, EDXA was performed using a Zeiss Gemini 500 Field Emission Scanning Electron Microscope (FE-SEM) to identify the components of the material (Nano CS).

#### **Exposure conditions**

An X-ray tube with a focus-film distance of 30 cm, and with an applied tube voltage of 54 kV with 4 mA tube current and 18.7 s exposure period was used for exposure.

#### **Evaluation of radiopacity**

The radiopacity of the test material (Nano CS) was assessed with the help of an Al step-wedge using a nondestructive testing method (Kirti NDT and Engineering Services, Maharashtra, India) following the ISO guidelines. The optical density of the test material (Nano CS) was tested with the

specimens of MTA as the standard cement material along with the specimens of enamel and dentin that were 1 mm thick, and Al of appropriate thickness with the desired and equivalent radiopacity. After generating and extracting the digital radiographs, ImageJ software, which is a Java-based image processing program (software) has been developed at the National Institutes of Health, and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, and that allows to visualize, inspect, quantify, and validate the scientific image data obtained, was used to determine the radiopacity of the test material (Nano CS). By using the software cursor to define a region in the center of an image, the program gives an output expressed in the form of mean gray value (MGV). The final radiopacity of the sample was determined by taking the mean of the obtained readings. The following formula which was modified from Vivan et al.[12,13] was used to convert these values into the equivalent of millimeters of the thickness of Al:

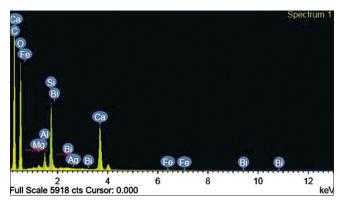
Millimeters (Al) =  $A - B/C - B \times \text{sample thickness} + 1 \text{ mm}$  Al below the test material's (Nano CS) MGV wherein A stands for the test material's (Nano CS) MGV, B stands for the MGV of the Al step-wedge increment immediately below the test material's (Nano CS) MGV, and C stands for the MGV of the Al step-wedge increment immediately above the test material's (Nano CS) MGV.

#### Statistical analysis used

Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) (also, known as one-factor ANOVA) was used to compare the means of independent groups to determine whether the associated means were significantly different. Furthermore, post hoc analysis was done with the help of Tukey's honest significant difference test which was used to assess the significance of differences between pairs of group means, while  $P \leq 0.05$  were contemplated as being statistically significant.

### RESULTS AND ANALYSIS

When EDXA was performed using a Zeiss Gemini 500 FE-SEM, pronounced peaks were observed for calcium, silica, oxygen, and bismuth in the test material (Nano CS) with smaller peaks observed for silver and iron [Figure 1]. Furthermore, the surface characteristics observed under FE-SEM revealed a mostly uneven and coarse surface for the test material (Nano CS) [Figure 2]. The evaluation of radiopacity of the test material (Nano CS) (in equivalent of millimeters of thickness of Al) revealed the radiopacity value of MTA as 4.56 equivalent of millimeters of thickness of aluminum as against 2.78 equivalent of millimeters of thickness of aluminum for the test material (Nano CS) [Figure 3 and Table 1 and Graph 1]. The findings of the present study, therefore, suggested MTA to have a higher radiopacity index equivalent to 4.56 ± 0.00 mm thickness of Al when



**Figure 1:** Energy dispersive X-ray analysis spectrum of test material (Nano CS) revealing pronounced peaks for calcium, silica, oxygen, and bismuth with smaller peaks for silver and iron

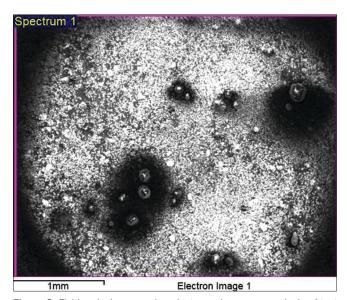
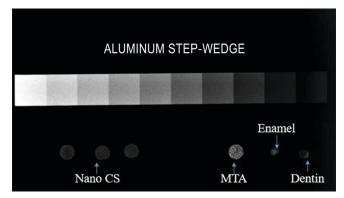


Figure 2: Field emission scanning electron microscope analysis of test material (Nano CS) revealing uneven and coarse surface topography



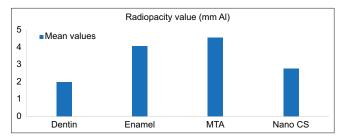
**Figure 3:** Evaluation of radiopacity of test material (Nano CS) (in equivalent of millimeters of thickness of aluminum) using ImageJ software. MTA: Mineral trioxide aggregate

compared to the test material (Nano CS) ( $2.78 \pm 0.01$  mm thickness of Al) and enamel ( $4.09 \pm 0.01$  mm thickness of Al) and dentin ( $2.01 \pm 0.01$  mm thickness of Al) specimens. Furthermore, the radiopacity index of the test material

(Nano CS)  $(2.78 \pm 0.01 \text{ mm}$  thickness of Al) was found to be more when compared to dentin  $(2.01 \pm 0.01 \text{ mm}$  thickness of Al), though, less when compared against the enamel specimens  $(4.09 \pm 0.01 \text{ mm}$  thickness of Al) with the results being statistically highly significant (P < 0.001) [Table 2].

# **D**ISCUSSION

Since the introduction of MTA, a plethora of new calcium silicate-based cements have been introduced for endodontic applications, however, none of these novel cement materials



**Graph 1:** Radiopacity means and standard deviation of the materials. MTA: Mineral trioxide aggregate

Table 1: Radiopacity means and standard deviation of the materials

Specimen	Radiopacity (equivalent of mm of thickness of aluminum), mean±SD		
Dentin	2.01±0.01 <sup>a</sup>		
Enamel	$4.09{\pm}0.01^{a}$		
MTA	$4.56\pm0.00^{\rm b}$		
Test material (Nano CS)	2.78±0.01°		

Different superscripts (small letters) represent statistically highly significant differences (P<0.001). SD: Standard deviation, MTA: Mineral trioxide aggregate

Table 2: Comparison of the mean radiopacity (in equivalent of millimeters of thickness of aluminum (AI)) of the materials by One-way analysis of variance (ANOVA), and Tukey's Honest Significant Difference (HSD) test

Source of variation	df	SS	MS	F-statistics	P
Variation among samples	3	25	MSG=8.26	83,249.7899	<0.001*
Variation within samples	20	0	MSE/ MSD=0.00		
Total	23	25			

\*P<0.001- Statistically highly significant. Tests used: a) One-way analysis of variance (ANOVA), and b) Tukey's Honest Significant Difference (HSD) test. a) One-way analysis of variance (ANOVA) is a statistical method that compares the means of two or, more independent groups to determine if there are significant differences between them; b) Tukey's Honest Significant Difference (HSD) test is a post-hoc test commonly used to assess the significance of differences between pairs of group means. ANOVA: Analysis of variance, HSD: Honest significant difference, df- Degrees of freedom, SS- Sum of squares, MS- Mean square, MSG- Mean square group value, MSE- Mean squared error MSD- Mean squared deviation, Mean square (MS) is calculated by dividing the sum of squares (SS) by its degrees of freedom (df)

appear to have all the desired properties. In a similar context, the novel dicalcium silicate-based hydraulic endodontic cement material (Nano CS) was introduced by Dsouza et al.[8,9] has been found to be biocompatible, dimensionally stable, maintain alkalinity, and have a shorter setting time. Furthermore, the newly introduced cement material (Nano CS) was made using pure starting materials to address the high-cost limitation of most of the endodontic cement available apart from numerous other limitations. This new calcium silicate-based cement material (Nano CS), also, offers reduced cytotoxicity risks, and improved antibacterial properties aiming to fulfill many of the key requirements for an ideal retrograde root canal sealing material.<sup>[8,9]</sup> With the inclusion of silver and carbon, the constitution of the novel cement material (Nano CS) is, also, comparable to that of MTA wherein as per the SEM analysis, this novel cement material (Nano CS) has been found to be made up of irregularly shaped, coarse particles in a range of sizes. One of the desired properties of all restorative and root canal sealing materials is the ability to block X-rays or, the property of their being radiopaque which enables to differentiate these materials from the surrounding tissues and the tooth itself. This property allows identifying issues like cement dissolving or, discrepancies in the marginal adaptation of the cement/sealer material based on variations in radiopacity from the surrounding tissues. Research has shown that the ideal radiopacity of any dental material depends on its intended use. For restorative applications, the desired radiopacity of the material intended to be used should closely match the natural tooth structure it is replacing whether that is dentin or, enamel.[14] All hydraulic endodontic cement/sealer materials have been advised to include a radiopacifier to meet the current requirements.<sup>[15]</sup> Furthermore, the ISO 6876:2001 guidelines, and the ANSI/ADA specification no. 57 specify a minimal radiopacity equivalent to 3 mm thickness of Al (reference value), and a baseline differential measurement of at least 2 mm thickness of Al for all endodontic sealing materials more than the radiopacity exhibited by dentin or, bone as the desired radiopacity index to be achieved by all materials to be used for endodontic applications.<sup>[10]</sup> Again, as per the guidelines laid down by the ANSI/ADA specifications, commercial calcium silicate-based cement must be dry-blended with nonreactive metal oxide powders (such as bismuth oxide [monoclinic α-Bi2O3], and zirconium oxide [monoclinic ZrO2]) which have been used as the most popular radiopacifiers to achieve the desired radiopacity index acceptable to meet these specifications. In this context, various other radiopacifiers such as barium sulfate and titanium tetrafluoride have, also, been incorporated into the newer cement materials, though, they have not been studied extensively, while bismuth oxide (monoclinic α-Bi2O3), and zirconium oxide (monoclinic ZrO2) only have so far been the most widely used radiopacifying agents. Furthermore, since none of the components of the white Portland cement, wherein these additives have commonly been used, engage in hydration reactions, both bismuth and zirconium oxides serve basically as inactive filler elements in the cement system.<sup>[16]</sup> It was due to these reasons that bismuth oxide (monoclinic α-Bi2O3) was used as an additive to the novel test material (Nano CS) to satisfy the criterion put forth by the existing standards. Again, nanotechnology offers exciting possibilities for numerous medical applications including disease prevention and treatment using materials engineered at the nanoscale. Studies suggest that NPs can enhance the handling, and flow of dental materials by influencing their hydration process, and filling their microscopic gaps. Notably, these improvements can be achieved with minimal NPs incorporation, while in addition to their antibacterial action, the radiopacifying ability of nanosilver (AgNPs) has, also, been reported. [17,18] Addition of AgNPs and 10% bismuth oxide was, therefore, attempted and studied for the test material (Nano CS), though, the radiopacifying ability of AgNPs did not confirm the ISO 6876:2001 specifications which set a minimal radiopacity equivalent to 3 mm thickness of Al as the reference value for all endodontic sealing materials for practical applications. Numerous other researchers have, also, investigated the effects of AgNPs on addition to MTA to understand how AgNPs affect the radiopacity of the material. In one such study conducted by Mendes et al.[11], the authors concluded that AgNPs emerge as a promising alternative due to their excellent radiopacifying properties, while this is coupled with their well-established antimicrobial activity, and relatively low cytotoxicity making them an attractive candidate for appropriate dental applications. Similarly, Bueno, et al., [19] also, suggested that the addition of 10%-15% of AgNPs to Portland cement obtained the desired radiopacity equivalent to 3 mm thickness of Al as per the requirements laid down by ISO 6876:2001 specifications, and maintained better physical properties. The findings of the present study, though, on the contrary, observed that the addition of 0.5% of AgNPs with 10% bismuth oxide did not achieve the requirement of the desired radiopacity equivalent to 3 mm thickness of Al according to the ISO 6876:2001 specifications. Simultaneously, MTA was found to have an increased radiopacity when compared to the test material (Nano CS), as well as the enamel and dentin specimens, while, also, the test material (Nano CS) was found to be more radiopaque than dentin, though, less radiopaque than the enamel specimens. The possible explanation behind the observed differences in the radiopacity of different specimens might be substantiated on the basis of the relatively lower concentrations of the AgNPs and bismuth oxide added to the test material (Nano CS) wherein increasing the concentrations of both the radiopacifiers could have achieved the desired radiopacity as specified by the international norms.

#### CONCLUSION

Addition of nanosilver and bismuth oxide to the test material (Nano CS) imparted characteristic radiopacity, though, additives in the form of 0.5% of AgNPs with 10%

bismuth oxide did not achieve the minimal requirement of radiopacity equivalent to 3 mm thickness of Al according to the ISO 6876:2001 specifications. The study, therefore, concludes in favor of nanosilver and bismuth oxide as suitable radiopacifiers to be added to calcium silicate-based bioceramics to attain radiopacity, however, the addition of 0.5% of AgNPs with 10% bismuth oxide did not achieve the minimal requirement of radiopacity equivalent to 3 mm thickness of Al according to the ISO 6876:2001 specifications which call for further studies wherein increasing concentration of the additives used in the present study or, incorporation of alternative radiopacifiers might be considered to bring in the required radiopacity without having a significant impact on the physical and biological properties of the novel hydraulic calcium silicate-based cement material (Nano CS) and other materials intended to be used for endodontic applications.

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#### **Conflicts of interest**

There are no conflicts of interest.

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