Original Article

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The effect of different concentrations of radiopacifier agents on the radiopacity level of nano-calcium hydroxide intracanal medication

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Objective: To assess radiopacity among nano-calcium hydroxide without and with the addition of various concentrations of barium sulfate as a radiopacifier.

Materials and Methods: The radiopacity of six materials: nano-calcium hydroxide, nano-calcium hydroxide with 25%, 30%, 35%, and 40% by weight of barium sulfate, and Ultracal XS® were evaluated according to ISO 6876:2012, with 5 specimens per group. Nano-calcium hydroxide and the various percentages by weight of barium sulfate were combined using a blending machine, then mixed with distilled water with a ratio of 1 g per ml. All samples were placed in the acrylic mold which was positioned alongside 0.5-mm increment aluminium step wedge and irradiated with x-ray 60 kV, 8 mA, for 0.2 seconds on a digital receptor. The radiopacity of the materials was compared with the aluminium step wedge using the ImageJ program and transformed into mm of Al.

Results: The radiopacity of nano-calcium hydroxide ranged from 0.675 to 2.642 mmAl. The group without a radiopacifier exhibited the lowest radiopacity, followed by the groups with 25%, 30%, and 35% by weight of barium sulfate, while the highest radiopacity was observed in the group with 40% by weight of barium sulfate. Increasing the concentration of barium sulfate in nano-calcium hydroxide resulted in a proportional increase in radiopacity. Ultracal XS® showed lower radiopacity compared to nano-calcium hydroxide with 30% by weight of barium sulfate. Statistically significant differences in radiopacity were observed among all examined materials.

Conclusion: The radiopacity of nano-calcium hydroxide increases with the addition of barium sulfate. Approximately 30% by weight of $BaSO_4$ should be added to achieve a radiopacity greater than 2 mmAl, based on an adaptation of the recommendation from the American National Standards Institute and the American Dental Association.

Keywords: Nano-calcium hydroxide, Calcium hydroxide, Barium sulfate, radiopacifiers, radiopacity

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Introduction

The aim of root canal treatment is to control and reduce bacteria within the root canal, crucial for treatment success [1]. Thorough mechanical instrumentation and irrigation help reduce the microbes, but cannot entirely eliminate them due to complexities of the root canal system [2]. Therefore, medication, typically calcium hydroxide, is used between visits to further reduce bacteria in the canal.

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Calcium hydroxide has a pH of approximately 12.5-12.8, indicating high alkalinity. When mixed with a liquid solvent, it dissolves into calcium ion and hydroxyl ion, resulting in several beneficial properties including antimicrobial properties, dissolution of organic tissues, inhibition of root resorption, stimulate formation of hardened tissues and creates a suitable environment for the healing of periapical tissue. However, to achieve these favourable effects, it needs to penetrate the dentinal tubules, reaching microorganisms directly [1, 3].

Nanotechnology has been developed due to the distinct and more favorable physical, chemical, and biological characteristics exhibited by materials at the nano-scale, which are less than 100 nm in size [4]. Nano-calcium hydroxide (NCH) particles demonstrate greater antimicrobial activity compared to conventional calcium hydroxide [5]. Moreover, they exhibit significantly deeper penetration into dentinal tubules [5, 6]. With the emergence of resistant bacterial strains in root canals and the proven antimicrobial efficacy of nanoparticles, the utilization of nanoparticles in endodontics seems promising.

Currently, various forms of calcium hydroxide are used in dentistry, including powder form mixed with distilled water and pre-mixed form commercially available in tubes for ease of use. Calcium hydroxide mixed with any vehicle—aqueous, viscous, or oily—lacks radiopacity and is not easily visible on radiographs. Thus, radiopacifiers such as barium sulfate ($BaSO_4$), zinc oxide (ZnO), and other compounds containing iodine (I) and bromine (Br) [7] are added to enhance visibility, aiding in the identification of lateral and accessory canals, resorptive defects, fractures, and other structures, as well as in determining the location of the medication in the root canal system from the radiograph. Barium sulfate is a white-powder ionic compound with a molecular weight of 233.43. The insoluble form, which is inexpensive and nontoxic, is preferred in clinical practice for use as a radiocontrast agent in x-rays [8, 9]. In endodontics, $BaSO_4$ is used as a radiopacifier in ready-mixed calcium hydroxide pastes such as UltraCal XS® (Ultradent, South Jordan, UT, USA), Hypocal (Ellinan Co., Hewlatt, NY, USA), and DiaPaste (Diadent, Seoul, South Korea). Moreover, iodoform (CHI_3) is another radiopacifier which is used in ready-mixed calcium hydroxy paste Vitapex® (Neo-Dental, Federal Way, WA, USA).

Several studies have noted that dentin and a 1-mm thick enamel layer have radiopacity levels equivalent to 1 and 2 mm of aluminum, respectively [10, 11]. According to the American National Standards Institute and American Dental Association (ANSI/ADA) specification No. 57, endodontic filling materials should demonstrate a radiopacity difference of at least 2 mmAl compared to bone or dentin. However, no standard radiopacity for medication has been established yet. Therefore, the criterion of 2 mmAl radiopacity should also be applied to intracanal medicaments.

Based on previous studies [12], it was found that when calcium hydroxide was mixed with different types of radiopacifiers for example; CHI_3, ZnO, and $BaSO_4$, the radiopacity of the mixing paste of all three radiopacifiers was significantly higher than calcium hydroxide paste without a radiopacifier and 1-mm thickness of dentin blocks. Although the antimicrobial activity between calcium hydroxide mixed with $BaSO_4$ and CHI_3 remains controversial, both still exhibit good antimicrobial properties [12, 13] and are more effective than ZnO [12]. However, when CHI_3 is mixed with calcium hydroxide and a liquid solvent, the resulting paste appears thick and yellowish, which may lead to slow dissociation of calcium hydroxide into calcium ion and hydroxyl ion [14], making it difficult to be eliminated from the root canal [15] and unsuitable for use as medication between visits in root canal therapy. Therefore, $BaSO_4$ is commonly used as radiopacifier in calcium hydroxide intracanal medication.

However, it has not yet been added to NCH, and its radiopacity has not been tested. Therefore, this study aims to assess the radiopacity of NCH with and without the addition of various amounts of $BaSO_4$ as a radiopacifier.

Materials and Methods

Ethical approval

The study protocols were ethically approved by the Institutional Review Board of the Faculty of Dentistry and Faculty of Pharmacy, Mahidol University (MU-DT/PY-IRB 2023/051.2011).

Material preparation

Preparation of nano-calcium hydroxide and nano-calcium hydroxide with radiopacifier

The NCH particles were prepared by grinding calcium hydroxide (Merck, Darmstadt, Germany) powder with a Planetary Ball Mill PM400 (Retsch, Haan, Germany) at a speed of 100 rpm for 45 minutes. After that, the obtained powder was mixed with 70% ethanol in a ratio of 1:1 and then dispersed using an ultrasonic device (40 KHz, Crest Ultrasonic, Ewing, NJ, USA) for 20 minutes. The resulting solution was dropped onto a formvar carbon coated surface (Polysciences, Warrington, PA, USA) and dried at room temperature. Subsequently, the dried powder was analyzed under a JEM-1400 transmission electron microscopy (100 KV, Jeol, Tokyo, Japan), and the particle size was measured using the ImageJ program (National Institutes of Health, Bethesda, MD, USA). The measured NCH powder was then blended with varying percentages by weight of $BaSO_4$ as a radiopacifier using the Planetary Ball Mill PM400 at a speed of 100 rpm for 1 hour, as done in the pilot study, resulting in a medication with radiographic homogeneity and was kept separately in closed containers with a desiccant at room temperature.

Radiopacity investigation

According to ISO 6876:2012, the radiopacity of the materials is evaluated using an aluminum step wedge which is made of 98% aluminum and contains 18 steps of different radiopacity. At each step, the step's height increases 0.5 mm from bottom to top. Before placing the material into the mold, a clear acrylic box, aligned parallel to the horizontal plane, creating a plane for an x-ray, with a RVG 6200 digital x-ray phosphor plate size 2 (Carestream, Atlanta, GA, USA) was prepared. The samples in the mold were then laid next to an aluminum step wedge and both of these components were placed on top of the acrylic box as in Figure 1.

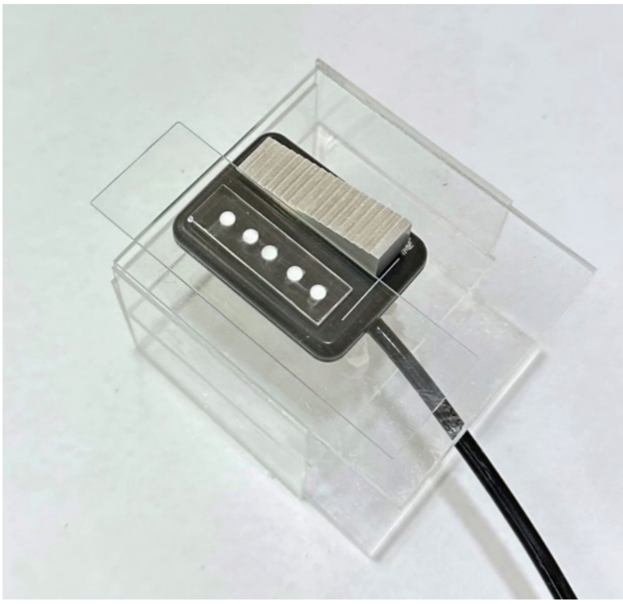

Figure 1 Placement of specimens in an acrylic mold and an aluminum step wedge on an clear acrylic box with an RVG 6200 digital x-ray phosphor plate size 2.

The samples were divided into six groups, each containing 5 specimens.

Group 1: NCH without radiopacifier

Group 2: NCH with 25% by weight of $BaSO_4$

Group 3: NCH with 30% by weight of $BaSO_4$

Group 4: NCH with 35% by weight of $BaSO_4$

Group 5: NCH with 40% by weight of $BaSO_4$

Group 6: Ultracal XS®

In this experiment, 25-40% by weight of $BaSO_4$ was used, based on the pilot study that demonstrated the clear radiographic visibility of the medication in the root canals of extracted human teeth. Additionally, pH values were found to be greater than or equal to 12.5 at a concentration of 1 g/ml, consistent with clinical applications and material concentrations from previous studies [16]. Therefore, in groups 1-5, different materials were mixed with distilled water to achieve this concentration. Each mixed material was loaded to the acrylic mold with a cement spatula (HuFriedy, Chicago, IL, USA). While in the Ultracal XS® group, the material was loaded by NaviTip™ 29 ga, 25 mm (Ultradent, South Jordan, UT, USA) and the cement spatula. The mold had an internal diameter of 3 mm and a thickness of 1 mm. Then, a glass slide was put on top of the mold to control the material thickness.

After that, the mold was x-ray irradiated (Carestream, Atlanta, GA, USA) at 60 kV, 8 mA, 0.2 seconds with a fixed 30 cm distance between the x-ray tube and the target plate. The plate was processed by RVG 6200 imaging x-ray program (Carestream, Atlanta, GA, USA). At this step, samples showing radiographic non-homogeneity of the medication were excluded.

The ImageJ program was utilized for evaluating the grey value of the materials and aluminum step wedge. The Oval tool was applied to select the circular area at the core of the disc, while the Rectangular tool was used for the rectangular area at the center of the aluminum step. When using the Measure tool, the average grey value of the selected area displayed automatically. The obtained values were converted into mmAl and compared between groups.

Statistical analysis

The data was statistically analyzed using IBM® SPSS® software version 26.0.0.0 (IBM Corp., Armonk, NY, USA). The distribution of data was evaluated using the Shapiro-Wilk test, and the homogeneity of variance was tested using Levene's test. The results indicated that the variance was not homogeneous, therefore, the mean radiopacity among all six groups was compared using a Welch ANOVA test, and pairwise comparisons were made using the Games-Howell test with the significance level of 0.05.

Results

The means and standard deviations of all samples' radiopacity are presented in Figure 2, ranging from 0.675-2.642. NCH without a radiopacifier exhibited the lowest radiopacity at 0.675±0.012 mmAl. Increasing the concentration of $BaSO_4$ added to NCH resulted in a proportional increase in radiopacity. The highest radiopacity was observed in NCH with 40% by weight of $BaSO_4$, measured at 2.642±0.065 mmAl. Ultracal XS® showed lower radiopacity compared to NCH from 30% by weight of $BaSO_4$. Statistically significant differences in radiopacity were observed among all examined materials.

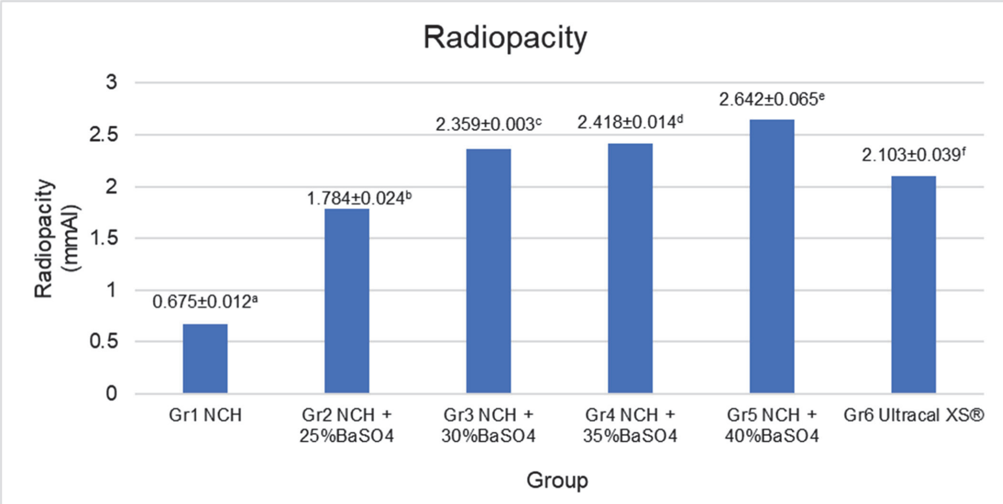

Figure 2 Means and standard deviations of the radiopacity (mmAl) of the experimental materials.
* Different lowercase letters indicate statistically significant differences ($p<0.05$) among experimental groups.

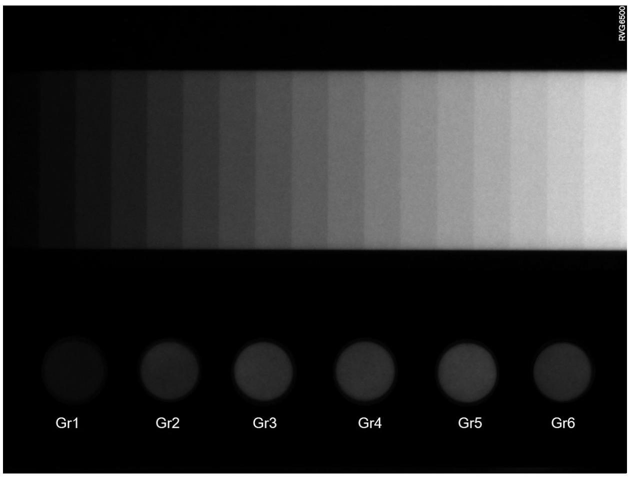

Figure 3 Radiographic image of specimens from 6 groups and aluminum step wedge.

Discussion

The primary reason for adding radiopacifiers to the calcium hydroxide paste is to enable the detection of lateral and accessory canals, resorptive defects, fractures, and other structures [8, 17]. Barium sulfate is commonly used as a radiopacifier in calcium hydroxide pastes. Insoluble $BaSO_4$ is inexpensive and nonpoisonous [8, 12, 18]. However, the impact of overflow of calcium hydroxide paste containing $BaSO_4$ or another radiopacifier into the periradicular tissues on the healing of periapical lesions is not completely comprehended [18]. When calcium hydroxide paste containing $BaSO_4$ is extruded over the apex, the $BaSO_4$ can obstruct the apex and is not easily resorbed over time [18, 19]. Consequently, placing calcium hydroxide paste might delay healing or interfere with radiographic interpretation of bone healing due to the inability to absorb insoluble $BaSO_4$ [8, 18]. Therefore, the amount of $BaSO_4$ added to calcium hydroxide should be the minimal amount that allows differentiation between the calcium hydroxide paste in the root canal and root dentin to assess the quality of medication placement, the characteristics of root canal anatomy, the quantity of paste to effectively eradicate bacteria and achieving various beneficial properties of calcium hydroxide when used as intracanal medication.

From the experiment, it was found that all six groups (Ultracal XS® and NCH with 0-40% by weight of of $BaSO_4$) yielded statistically significant differences in radiopacity, based on the ISO standard 6876:2012. Acrylic molds were utilized in this study due to their ease of availability and preparation, as well as to prevent artifacts at the edges of the images. Additionally, a clear acrylic box was used as a guide to align the film parallel to the horizontal plane, and a clear acrylic sheet was placed on the top after loading the material to control the volume and thickness of the material in each group. Furthermore, the use of a digital phosphor imaging plate reduced processing variations compared to conventional film [20]. The digital radiographic data could be easily transferred and analyzed with the ImageJ program, which automatically averaged gray values in the selected area and compared them with a reference step wedge, ensuring more accurate data readings compared to visual inspection [21].

In this study, the radiopacity of the NCH groups containing 30%, 35%, and 40% of $BaSO_4$, as well as Ultracal XS®, were above 2 mmAl, which is the ANSI/ADA specification No. 57 recommendation to be clearly visible in a radiograph for comparison with bone and dentin. The radiopacity of Ultracal XS® was also found to be similar to that of the previous study [22]. Several factors could affect the radiopacity of dental materials in different studies, such as exposure settings, X-ray beam angulation, X-ray film speed, film-source distance, and the methodology used for assessment [23]. However, the measured radiopacity of the NCH with 25% by weight of barium sulfate group was still less than 2 and less than Ultracal XS®. Although the company claimed that Ultracal XS® contains barium sulfate as a radiopacifier at approximately 10-25%, the differences in radiopacity may be due to other undisclosed components in the product, which are considered trade secrets. Moreover, this may be due to variations in the concentration of the solution. In this experiment, the powder was mixed with distilled water to a concentration of 1g/ml, as in a previous study [16], to achieve a consistency that was close to clinical usage.

The radiopacity of NCH was noticeably improved by adding $BaSO_4$, reaching higher than the acceptable level when mixed with at least approximately 30% by weight of $BaSO_4$. Nevertheless, based on the results, the difference in radiopacity between the 25% and 30% groups was 0.5-0.6 mmAl. On the other hand, the difference between the 30% and 35% groups, as well as between the 35% and 40% groups, was only 0.1-0.2 mmAl. This did not show a direct relation to the amount of $BaSO_4$ added. As a result, determining the proportion of the increase in $BaSO_4$ added to the increase in radiopacity when mixed with NCH may require further study in the future. However, in order to improve the radiopacity, the amount of NCH, the main active ingredient for antibacterial effects and other desirable properties as an intracanal medicament, was reduced. Increasing the amount of $BaSO_4$ may affect other properties such as flowability, injectability, pH in root dentin, and antimicrobial properties. Therefore, further studies on these additional properties are necessary to determine the optimal amount of $BaSO_4$ for clinical use while maintaining the desirable properties of an intracanal medicament.

Conclusion

The radiopacity of NCH increases with the addition of barium sulfate. Approximately 30% by weight of $BaSO_4$ should be added to achieve a radiopacity greater than 2 mmAl, based on an adaptation of the recommendation from the American National Standards Institute and the American Dental Association.

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