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Comparison of particle morphology between commercial- and research-grade calcium hydroxide in endodontics

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Abstract: $Ca(OH)_2$ aqueous slurry is widely used as an inter-appointment antimicrobial dressing in root canal treatment. The aim of this study was to quantify the particle size and shape of commercial-grade UltraCal XS (UC) and to compare it with that of research-grade $Ca(OH)_2$ (RG) using a flow particle image analyzer (FPIA). The morphology and penetration inside the dentin tubules of the UC and RG particles were examined using a scanning electron microscope (SEM). UC and RG (10 mg) were mixed with 15 mL of alcohol, and were sonicated. Five milliliters of the dispersion was subjected to FPIA, and particle length, width, perimeter and aspect ratio were analyzed. In addition, UC paste and RG aqueous slurry were agitated on dentin discs and were prepared for SEM examination. There were significant differences between UC and RG with regard to the frequency of different length groups (*P* < 0.0001). UC contained smaller particles than RG (*P* < 0.0001). Under SEM, the agitated UC and RG particles occluded the opening of dentin tubules and penetrated inside the dentin tubules. The size of UC particles is smaller than those of RG. Both UC and RG particles were able to penetrate into open dentin tubules. (J Oral Sci 56, 195-199, 2014)

Keywords: calcium hydroxide; dentin tubule; image analysis; morphology; UltraCal XS.

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Introduction

Calcium hydroxide aqueous slurry is widely used as an inter-appointment antimicrobial dressing in root canal treatment (1-5). Dentin tubules in root canal walls have been shown to harbor microorganisms (6,7). The penetration of microorganisms into dentin tubules in infected teeth is generally reported between 50 and 100 µm (8). Application of calcium hydroxide in instrumented and irrigated root canals effectively eliminates microorganisms (1).

The antimicrobial action of calcium hydroxide depends on the concentration of hydroxide ions in the solution (1,9). Less than 0.2% of calcium hydroxide slurry dissociates at body temperature into calcium ions (Ca^{2+}) and hydroxide ions (OH^-). Most of the calcium hydroxide aqueous slurry is composed of undissolved particles (10). If direct mechanical penetration of calcium hydroxide particles into open dentin tubules occurs, penetrating particles may act as a direct source of dissociated calcium hydroxide by continuing to dissolve into the aqueous forms of calcium hydroxide. This may result in a continuing high local pH for enhanced antimicrobial effectiveness (11).

Our previous study investigated the particle morphology of research-grade calcium hydroxide (RG, Sigma C-7887, St. Louis, MO, USA) (11). However, little is known about commercial $Ca(OH)_2$ products, which are used daily in endodontic treatment. In general, commercial $Ca(OH)_2$ products contain other particles in addition to $Ca(OH)_2$. UltraCal XS (UC; Ultradent, South Jordan, UT, USA) is used as an inter-appointment antimicrobial dressing and for apexification procedures. It is a paste-type material consisting of calcium hydroxide ($Ca(OH)_2$), barium sulfate ($BaSO_4$), water and other ingredients. There have been no reports on the particle morphology of commercial $Ca(OH)_2$ products such as UC.

The aim of this study was to quantify the particle size and shape of UC using a flow particle image analyzer (FPIA), as compared with those of RG. Penetration of UC and RG particles into dentin tubules was examined using a scanning electron microscope.

Materials and Methods

Flow particle image analyzer

UltraCal XS (UC; Lot B6KSJ, Ultradent) and research-grade calcium hydroxide (RG; Lot #31H3445, Sigma C-7887) were examined with a flow particle image analyzer (FPIA-3000; Sysmex, Kobe, Japan). The particle size and shape were analyzed using the particle size parameters of length (length of longer axis when the particle image is bound by two pairs of parallel lines), width (length of shorter axis when the particle image is bound by two pairs of parallel lines), perimeter (length of particle perimeter) and particle shape aspect ratio (ratio of width/length). Polystyrene latex particles (2 μm in diameter; Polymer Microspheres 5200A; Duke Scientific Corporation, Fremont, CA, USA) were used as test objects to adjust the focus before calcium hydroxide samples were tested. An alcohol suspension of UC and RG provided a fluid particle suspension that prevented the FPIA machine flow chamber from becoming clogged. Ten milligrams of UC and RG were mixed with 15 mL of alcohol, and were then sonicated for 1 min to create a homogeneous fluid. Five milliliters of the dispersion was subjected to FPIA. The final analyzed volume was set at 0.35 μL. Five sample groups were randomly prepared and analyzed. The mean and standard deviation in length (μm), width (μm), perimeter (μm) and aspect ratio were calculated for UC and RG particles. Length was classified into five categories: category 1 (0.5-1.0 μm), category 2 (1.0-1.5 μm), category 3 (1.5-2.0 μm), category 4 (2.0-2.5 μm) and category 5 (more than 2.5 μm). Student's *t*-test was conducted to investigate whether there were any significant differences in length (μm), width (μm), perimeter (μm) and aspect ratio between UC and RG. Chi-squared tests were used to test whether there were significant differences in the frequency of particles between UC and RG in each of the five length categories.

Scanning electron microscope preparation and examination

Three fully erupted, defect-free human anterior teeth, approved by the research ethics committee (No. 2011-06), were obtained from local dentists. After being cleaned and washed with water, the middle one-third of each root was sliced parallel to the long axis of root with a slow-speed diamond saw (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA) at 300 rpm, and was hand-polished using 400-grit silicon carbide abrasive paper to obtain dentin discs of 0.5 mm thick. Smear layers were removed by immersing the discs in 17% EDTA (1 min), followed by in 5% NaOCl (1 min). Sections were washed with distilled water, dried and divided into three groups of five discs each. One group was treated with UC paste, one group was treated with RG aqueous slurry, and one group was left untreated and served as a control. For UC or RG treatment, the UC paste or RG aqueous slurry was placed onto the dentin discs and agitated in a sonicator for 2 min. Dentin discs were wiped with a cotton swab to remove any excess material, rinsed with deionized water, air dried, then sputter coated and examined using scanning electron microscopy (SEM, JSM-6300; JEOL USA, Peabody, MA, USA) at 15 kV by sputter coating the surface with a thin gold coating under a vacuum (Desk II; Denton Vacuum LLC, Moorestown, NJ, USA). SEM was used to examine the morphology and penetration inside dentin tubules of the UC and RG particles.

Results

Flow particle image analyzer

A total of 53,661 and 46,818 particles from UC and RG were analyzed. The averages for particle length, width, perimeter, and aspect ratio of UC were 1.66 μm, 1.17 μm, 5.08 μm and 0.72, respectively (Table 1). There were significant differences between UC and RG in all four parameters, including particle length, width, perimeter and aspect ratio ($P < 0.0001$). UC contains smaller particles than RG (Table 1).

The frequency of particles among the five length categories in both UC and RG is shown in Table 2. There were significant differences between UC and RG in the frequency of particles in all five length categories ($P < 0.0001$). The proportion of smaller particles in UC was larger than that in RG (Table 2).

Table 1 Comparison of particle morphology between UltraCal XS (UC) and research-grade $Ca(OH)_2$ (RG)

	UltraCal XS (UC)	Research grade $Ca(OH)_2$ (RG)	*P*-value
Length (μm)	1.66 (1.32)	2.26 (1.99)	$P < 0.0001$
Width (μm)	1.17 (0.92)	1.62 (1.46)	$P < 0.0001$
Perimeter (μm)	5.08 (3.63)	6.70 (5.60)	$P < 0.0001$
Aspect ratio	0.72 (0.15)	0.74 (0.15)	$P < 0.0001$
Total particle #	53661	46818	

Mean (S.D.)

Table 2 Comparison of the frequency of particles between UltraCal XS (UC) and research-grade $Ca(OH)_2$ (RG) in five length categories

Length categories	UltraCal XS (UC)	Research grade $Ca(OH)_2$ (RG)	*P*-value
0.5-1.0 μm	28.96%	19.15%	$P < 0.0001$
1.0-1.5 μm	30.75%	27.13%	$P < 0.0001$
1.5-2.0 μm	20.96%	17.09%	$P < 0.0001$
2.0-2.5 μm	8.63%	10.73%	$P < 0.0001$
>2.5 μm	10.70%	25.90%	$P < 0.0001$

Scanning electron microscopy

UC particles were smaller and more evenly distributed than RG particles (Figs. 1a, 1b). Open dentin tubules without peri-tubular dentin erosion were observed in the control dentin disc (Figs. 1c, 1d). Agitated UC and RG on dentin disc occluded open dentin tubules (Figs. 1e, 1f, 1g, 1h). While some open dentin tubules remained on the RG treated dentin disc (Figs. 1e, 1f), UC covered almost all open dentin tubules (Figs. 1g, 1h). Under high magnification, both UC and RG particles were seen inside the dentin tubules (Figs. 1f, 1h).

Discussion

The results of this study show that the particle size and shape of UC are smaller than those of RG. The difference stems from the smaller $Ca(OH)_2$ particles within UC and the inclusion of barium sulfate ($BaSO_4$). UC consists of 35 wt% calcium hydroxide ($Ca(OH)_2$), 2 wt% barium sulfate ($BaSO_4$), and other ingredients. Barium sulfate, which is one of the most common contrast agents in medicine (12), is added to calcium hydroxide to increase radiopacity. The particle size (diameter) of commercially available barium sulfate varies from nanoscale (80-500 nm) to micron (2 μm) scale, depending on the manufacturing sources (13). Previous studies have shown that nanoparticles of $BaSO_4$ can improve X-ray contrast properties of increased surface areas (14) and that they were more radiopaque than microparticles (13). The addition of barium sulfate thus increases the percentage of smaller particles in UC. The smaller $Ca(OH)_2$ particle size in UC is also attributed to the particles in UC being smaller than RG particles. Different sizes of $Ca(OH)_2$ powders have been observed by various researchers in recent clinical/material studies (15-18).

Dentin tubule density and size in root dentin have been studied by various investigators (19-25). Dentin tubules are generally considered to have a diameter of 2 to 5 μm. Our previous study found that the size of RG particles is correlated with the size of the dentin tubules, and it is proposed that the $Ca(OH)_2$ particles may penetrate and occlude dentin tubules when used in endodontic treatment (11). FPIA results from the current study indicate that the geometry of the smaller particles in UC also make it possible for calcium hydroxide particles to enter the open dentin tubules. SEM results in the present study confirm that both UC and RG particles penetrate into the dentin tubules.

Regarding the organization of particles on the dentin surface, a rough surface was observed, indicating poor wetting and adhesion to the dentin surface. The UC image looked smoother than that of RG, indicating that some characterization was performed by the manufacturer. As the particles become smoother, wetting to the dentin surface was improved. With respect to the behavior of particles flowing into open dentin tubules, not all dentin tubules were filled with RG particles. As the RG aqueous slurry is composed of calcium hydroxide and water, the results of this study suggest that the properties of the RG aqueous slurry need additional modification to facilitate the $Ca(OH)_2$ particles flowing into dentin tubules. The

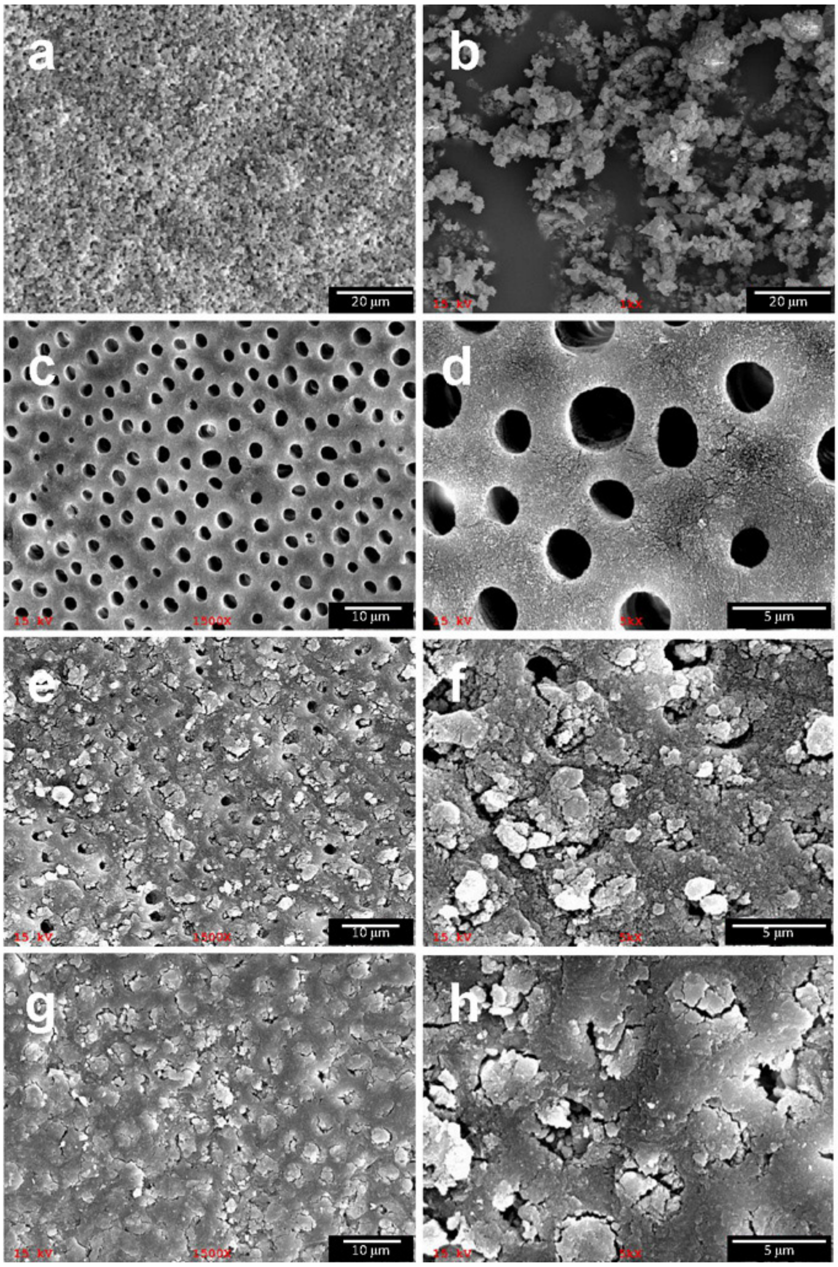

Fig. 1 Particles (a: UltraCal XS (UC), b: Research grade $Ca(OH)_2$ (RG)) (×1,000). Open dentin tubules (c: ×1,500, d: ×5,000). Agitated RG particles on dentin surface (e: ×1,500, f: ×5,000). Agitated UC particles on dentin surface (g: ×1,500, h: ×5,000).

$Ca(OH)_2$ particles inside the dentin tubules may act as a direct source of dissociated calcium hydroxide, resulting in a high local pH with a slight chance of being reduced by dentin buffering. The $Ca(OH)_2$ particles may also function as a continuing source of hydroxide ions to maintain a high pH locally for a prolonged period in the dentin. For facilitating $Ca(OH)_2$ particles penetrating into dentin tubules, the smear layer needs to be removed before placing $Ca(OH)_2$ into root canals.

The data show that the particle shape in UC is not round but irregular. Only two types of $Ca(OH)_2$ (UC and RG) were investigated in this study, which makes the wider application of the findings difficult. The aspect ratio may relate to the rate of dissolution and ionization in and around the dentin tubules due to the increased surface area of the particles (26). Exposure of the root dentin to the bioactive effects of $Ca(OH)_2$ may affect its physical characteristics and could have important clinical implications for the root canal treatment. The potential influence of particle size and shape on increasing the surface area, and the amount of potential reactivity, need to be investigated in the future.

This study found that UC particles are smaller than RG particles. Both UC and RG particles could penetrate into open dentin tubules.

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