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Particle size and shape of calcium hydroxide

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Abstract

The aim of this study was to examine the particle length, width, perimeter, and aspect ratio of calcium hydroxide powder using a flow particle image analyzer (FPIA). Five sample groups each with 10mg calcium hydroxide were mixed with 15mL of alcohol and sonicated. Digital images of the particle samples were taken using the FPIA and analyzed with a one-way ANOVA. The overall averages $\pm$ S.D. among the five groups for particle length (μ m), width (μ m), perimeter (μ m), and aspect ratio were 2.255 ± 1.994 , 1.620 ± 1.464 , 6.699 ± 5.598 , and 0.737 ± 0.149 , respectively. No statistical significance was observed among the groups for all parameters. When the total of 46,818 particles from all five groups were classified into the five length categories of 0.5 μ m increments, there were significant differences in width, perimeter, and aspect ratio (all p-values < 0.0001). In conclusion, calcium hydroxide particles have a size and shape that may allow direct penetration into open dentin tubules.

Keywords

Calcium hydroxide; Image Analysis; Particle size/shape; Dentin tubule; Aspect ratio

Introduction

Calcium hydroxide aqueous slurry is widely used as an interim (interappointment) antimicrobial dressing in root canal treatment (1–5). Less than 0.2 % of the calcium hydroxide slurry dissociates at body temperature into calcium ions (Ca^{2+}) and hydroxide ions (OH^-), leaving most of the particles undissolved (6).

Studies indicate that the dentin tubules of the root canal walls harbor microorganisms (7,8). The penetration of microorganisms into infected dentin tubules is reported to extend generally from 50 to 100 μ m (9). However, it has been shown that the application of calcium hydroxide into instrumented and irrigated root canals eliminates microorganisms effectively (1).

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The antimicrobial action of calcium hydroxide depends on the concentration of hydroxide ions in the solution (1,10). This selective permeability of the hydroxide ions in the dentin tubules is known because of the buffering capacity of hydroxyapatite (11). Depending on the concentration of hydroxide ions, the antimicrobial effect of calcium hydroxide in the dentin tubules may or may not be effective.

A newly proposed theory about using calcium hydroxide in endodontic treatment suggests that if the calcium hydroxide particles are inserted into the open dentin tubules, the particles may act as a direct source of dissociated calcium hydroxide by continuing to dissolve into the aqueous form of calcium hydroxide. This theory may result in the maintenance of a high local pH, which will enhance the antimicrobial effectiveness. While most calcium hydroxide aqueous slurry is comprised of undissolved particles, information about the morphology of these particles is scarce (12–15). The reports do not sufficiently answer the clinical endodontic questions with respect to particle penetration into the dentin tubules. Further, evaluating particle morphology based on representative SEM images, which has been the conventional technique used in the past, is challenging. Therefore, in the present study, a novel technique of image analysis to characterize the particles will be described.

Image analysis technology is used in the ceramics, pharmaceutical, and toner cartridge industries for optimizing product and process performance (16–18). This technique provides statistically valid information and images showing the size and shape of each particle in large numbers of samples. A flow particle image analyzer (FPIA-3000; Sysmex, Kobe, Japan) analyzes the size and shape of particles (1.5 μm to 160 μm) in emulsions and suspensions and produces quantitative shape information expressed as the morphological parameters of particles (19). The low-power field mode allows the analysis of coarse particles ranging in size from 6 to 160 μm . The high-power field (HPF) mode allows analysis of fine particles between 1.5 and 40 μm with a minimum detectable level of 0.5 μm for selected analysis parameters. Because several studies reported that calcium hydroxide particles vary from 0.5 μm to 20 μm (12–15), the HPF mode was used for this study.

The aim of this study was to quantify the particle length, width, perimeter, and aspect ratio of calcium hydroxide powder using the HPF mode of the flow particle image analyzer.

Materials and Methods

Calcium hydroxide powder (Product Number C7887, Lot Number 31H3445; Sigma-Aldrich, St. Louis, MO) was examined with a flow particle image analyzer (FPIA-3000). Polystyrene latex particles (2 μm in diameter; Polymer Microspheres 5200A; Duke Scientific Corporation, Fremont, CA) were used as test objects to adjust the focus before the calcium hydroxide samples were tested. An alcohol suspension of calcium hydroxide provided a fluid particle suspension that prevented clogging the FPIA machine flow chamber. Ten milligrams of calcium hydroxide were mixed with 15 mL of alcohol and then sonicated for 1 minute to create a homogeneous fluid. Five milliliters of the dispersion was added to the FPIA. The final analyzed volume was set at 0.35 μL .

Five sample groups were randomly prepared by using material from one calcium hydroxide package (samples A, B, C, D, and E). The reproducibility of the calcium hydroxide preparations mimicked the clinical setting in which calcium hydroxide from the same package is prepared for each patient. The samples were tested once at HPF mode. The particle size and shape were analyzed using the particle size parameters of length, width, and perimeter; the shape parameter was considered the aspect ratio. The analysis of the parameters, units, calculation methods and comments are summarized in Table 1. Digital images of the particle samples were automatically collected by the FPIA machine, along with the above-described parameters.

Two statistical analyses were performed. In part 1, the mean, standard deviation, and the number of particles were calculated for each sample group. A one-way analysis of variance (ANOVA) was conducted to identify any significant differences in length (μm), width (μm), perimeter (μm), and aspect ratio among the five sample groups. A p value less than 0.05 was considered statistically significant.

In part 2 of the statistical analysis, the 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). The chi-square test was used to test for significant differences between the number of particles and the five length categories. An ANOVA was used to investigate whether there were any significant differences in width, perimeter, and aspect ratio among the five categories.

Results

The results of particle length, width, perimeter, and aspect ratio in five groups are summarized in Table 2. A total of 46,818 particles from all five sample groups were analyzed. No statistical significance was observed among the groups for all the parameters.

Table 3 shows the results of the analyses of particle number, width, perimeter and aspect ratio. The chi-square test showed that there was a significant association between the number of particles and the five categories of length ($p < 0.0001$). The highest number was found for category 2 (1.0–1.5 μm). The cumulative percentage of particles between 0.5 and 2.0 μm was 63%, while 74% of the particles were between 0.5 and 2.5 μm . The ANOVA showed that there were significant differences in width, perimeter and aspect ratio among the five categories (all p-values < 0.0001). The aspect ratio in category 1 (0.5–1.0 μm) was the highest among all the categories, and category 5 (over 2.5 μm) was the lowest of all. As the particle length decreased, the particles changed to a rounder shape. As the particle lengthened, the particle shape became more rectangular.

Discussion

The permeability of dentin is governed largely by dentin tubule anatomy, density, diameter, and length as well as features of the solute such as size and charge (20). Dentin tubule density and size in root dentin have been studied by various investigators (21–27). Mjör et al. reported that at the apical portion of permanent human teeth, the dentin tubules were irregular in direction and density (22). In general, the dentin tubules are considered to have a diameter of 2 to 5 μm .

Dentin is a substrate, whereas calcium hydroxide is a material. The size of the dentin tubules correlates with the size of the calcium hydroxide particles. This study found that the cumulative percentage of particles between 0.5 and 2.0 μm was 63%. Therefore, in theory, the geometry of these small particles makes it possible for calcium hydroxide to enter the open dentin tubules. The penetrating particles 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. An even higher pH would result in a stronger and more effective antimicrobial action.

The solubility of calcium hydroxide in water is very low. Aqueous slurry contains many undissolved particles together with the calcium and hydroxide ions. Since calcium hydroxide is not soluble in ethanol, using ethanol for the suspension of calcium hydroxide particles is also beneficial to prevent clogging of the flow chamber of the FPIA machine.

A newly proposed theory in endodontic treatment suggests that if calcium hydroxide particles penetrate into open dentin tubules, the particles may act as a direct source of dissociated calcium

hydroxide as they continue to dissolve into the aqueous form of calcium hydroxide. Based on the particle size data collected in this study, it is speculated that undissolved fine particles may play an important role in the antimicrobial action inside dentin tubules. These particles may steadily dissolve and ionize the dissolute in and around the dentin tubules and function as a continuing source of hydroxide ions to maintain a high pH locally for a prolonged time period in the dentin.

An advantage of FPIA analysis is the capacity to determine not only the particle size but also the particle shape (28,29). The data in the present study show that the particle shape is not round but irregular. 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 particle. It may also determine, wholly or partially, the flow of the particle into a dentin tubule. In addition, the direction and orientation of the particle may control the depth of penetration. For example, the length may prevent deep penetration of a thin elongated particle. The enhanced FPIA capabilities allowed advanced statistical analysis, which was classified according to the five different particle lengths (Table 3). The results show that the aspect ratio was the highest in category 1 and the lowest in category 5. As the particle length decreased, the particle shape became rounder. As the particle lengthened, the particle shape changed to a more rectangular shape. Thus, in theory, short particles are more desirable for deep penetration into the dentin.

The choice of one-visit versus two-visit root canal therapy for necrotic teeth with apical periodontitis is a current topic of debate (30). While two-visit root canal therapy uses calcium hydroxide as an interim (interappointment) antimicrobial dressing, one-visit therapy does not. To resolve this dilemma, one line of thought is that the potential new development of effective calcium hydroxide slurry through enhancing its biologic quality may improve the treatment modality. Aspect ratio may be one important factor determining the suitability of the calcium hydroxide preparation. The influence of particle size and shape might increase the surface area, hence increasing the amount of potential reactivity for clinical and biological quality control.

A research-grade type of commercial calcium hydroxide powder was used in this study. It should be noted that the geometry of the calcium hydroxide may be different, depending on the manufacturer, processing, country of origin, price, and mixing solution. For example, the calcium hydroxide powders used in recent clinical/materials studies were different in each case (30–33). Calcium hydroxide powder mixed with 2% chlorhexidine gel as an intracanal medication has also been studied (30,31); however, the dissociation into calcium ions and hydroxide ions might have been influenced by the chlorhexidine gel. Therefore, the results of various studies on calcium hydroxide powder may not be entirely comparable since the calcium hydroxide power products available on the world market cannot be considered identical.

In future research, it will be interesting to examine the relationship between the particle size and shape distribution, as well as the reaction speed and rate, as these factors relate to antimicrobial characteristics. The results of the present research are valuable for clinical endodontics, but the question remains as to the disposition of the calcium hydroxide particles. It is possible that they are ingested by bacteria, but some residual unreacted particles may remain. Taking into consideration the anatomical stability of dentin tubules, the penetration mechanism of residual calcium hydroxide particles is a clinical interest. Although the present study focused on calcium hydroxide as an intracanal medication, calcium hydroxide, together with a mineral trioxide aggregate, has been clinically studied for use in pulpotomies in primary molars (32), as a pulp capping agent in permanent premolars (33), and a regenerative treatment of an immature traumatized tooth with apical periodontitis (34). Therefore, further research on the calcium hydroxide particle size and shape will be applicable to other emerging areas of endodontic research, such as the dentin-material interface, biomineralization, stem cell/scaffold research, and nanotechnology.

Conclusion

1. The size and shape of calcium hydroxide particles may allow direct penetration into open dentin tubules.
2. No statistically significant differences were observed among the five groups (A–E) in the analysis of all parameters, including particle length, width, perimeter, and aspect ratio.
3. The number of particles in each of the five categories indicated that category 2 (1.0–1.5 μm) had the highest number of particles. The cumulative percentage of the fine calcium hydroxide particles between 0.5 and 2.0 μm was 63%; this length is less than the reported diameter of the dentin tubules in root dentin.
4. The aspect ratio was the highest in category 1 (0.5–1.0 μm), whereas it was the lowest in category 5 (over 2.5 μm). As the particle size increased, the particle shape became more rectangular.

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Table 1
The explanation of particle length, width, perimeter, and aspect ratio

Parameters	Unit	Explanation
Particle size		
Length	μm	The length of the longer axis when the particle image is bounded by two pairs of parallel lines.
Width	μm	The length of the shorter axis when the particle image is bounded by two pairs of parallel lines.
Perimeter	μm	Length of the particle perimeter.
Particle shape		
AspectRatio (W/L)	None	The ratio between length and width.

Table 2
The results of particle length, width, perimeter, and aspect ratio in five sample groups

Parameters	Group				ANOVA	
	A	B	C	D	E	p-value
Particle size						
Length (μm)	2.238 ± 1.936	2.235 ± 1.958	2.284 ± 2.054	2.253 ± 2.027	2.262 ± 1.984	0.4339
Width (μm)	1.609 ± 1.411	1.611 ± 1.423	1.636 ± 1.478	1.616 ± 1.542	1.623 ± 1.441	0.7162
Perimeter (μm)	6.646 ± 5.397	6.651 ± 5.571	6.768 ± 5.682	6.698 ± 5.756	6.720 ± 5.560	0.5444
Particle shape						
Aspect ratio (W/L)	0.737 ± 0.147	0.740 ± 0.148	0.737 ± 0.151	0.734 ± 0.149	0.736 ± 0.149	0.1989
The number of particles	8627	9153	9776	9274	9988	46818

Values are mean with standard deviation.

Table 3

The frequency of particles among five length categories together with ANOVA test results for width, perimeter and aspect ratio

Category	Length		Frequency (%)	Width	Perimeter	Aspect ratio
	µm	# of particles				
1	0.5–1.0	8967	19.15	0.714 ± 0.171	2.728 ± 0.411	0.808 ± 0.166
2	1.0–1.5	12699	27.13	0.943 ± 0.195	3.854 ± 0.603	0.743 ± 0.134
3	1.5–2.0	8002	17.09	1.273 ± 0.267	5.435 ± 0.762	0.724 ± 0.144
4	2.0–2.5	5025	10.73	1.604 ± 0.317	6.986 ± 1.099	0.716 ± 0.137
5	over 2.5	12125	25.90	3.233 ± 2.078	13.329 ± 7.376	0.694 ± 0.138
	(Total)	46818	100.00			
p-value			p<0.0001	p<0.0001	p<0.0001	p<0.0001

Values are mean with standard deviation.