

THE INFLUENCE OF THE PARTICLE'S SIZE ON THE SETTING REACTION OF SOL-GEL DERIVED CALCIUM SILICATE PARTICLES

A influência do tamanho de partícula na reação de presa de cimentos de silicate de cálcio produzidos por sol-gel

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ABSTRACT

Introduction: This study aims to analyze the influence of particles size of sol-gel derived calcium silicate particles in the setting reaction of bioactive endodontic cements.

Materials and Methods: Sol-gel derived calcium silicate particles were synthesized and sieved to separate the particles in different sizes: CS400, CS200, and CS100. A commercial MTA (Control) was used as control. The particle size and the specific surface area were assessed by laser diffraction and nitrogen adsorption. The cements were prepared with water as the liquid for the reaction. The setting time was conducted according to ISO 6876, and the setting kinetics was analyzed by Fourier transformed infrared spectroscopy (FTIR) at different time points between 120s to 72h. **Results:** The particle size varied from 9.45µm (CS400) to 31.01 (Control). The higher specific surface area valuer reached 15.14g/cm² in the CS400. The smallest particle sizes, the higher specific surface area, and the lowest setting time were found for CS400 (p < 0.05). Control presented the highest setting time (p < 0.05). The FTIR analyses showed the differences in materials structure over time, with faster hydration and crystallization for CS400. The setting kinetics was slower for Control even when compared to a sol-gel derived group with similar particle size. **Conclusion:** The route of synthesis and the particle size influences the setting reaction of calcium silicate-based cements. The reduction of particle size for sol-gel derived calcium silicates lead to the acceleration of the setting reaction of the produced bioactive endodontic cement.

Keywords: Regenerative endodontics. Silicate cement. Pulp capping and pulpectomy agents. Materials science. Fourier analysis. Spectroscopy, Fourier transform infrared.

RESUMO

Introdução: Este estudo tem como objetivo analisar a influência do tamanho de partículas de silicato de cálcio derivadas de sol-gel na reação de presa de cimentos endodônticos bioativos. **Materiais e Métodos:** Partículas de silicato de cálcio derivadas de sol-gel foram sintetizadas e peneiradas para separar as partículas em diferentes tamanhos: CS400, CS200 e CS100. Um MTA comercial (Controle) foi usado como controle. O tamanho das partículas e a área superficial específica foram avaliados por difração a laser e adsorção de nitrogênio. Os cimentos foram preparados com água como líquido para a reação. O tempo de presa foi conduzido de acordo com a ISO 6876, e a cinética de presa foi analisada por espectroscopia de infravermelho transformada de Fourier (FTIR) em diferentes pontos de tempo entre 120s a 72h. **Resultados:** O tamanho de partícula variou entre 9,45µm (CS400) e 31,01 (Controle). A maior área de superfície foi encontrada nas partículas do grupo CS400 (15.14g/cm²). Os menores tamanhos de partícula, a maior área de superfície específica e o menor tempo de presa foram encontrados para CS400 (p < 0,05). O Control apresentou o maior tempo de presa (p < 0,05). As análises de FTIR mostraram as diferenças na estrutura dos materiais ao longo do tempo, com hidratação e cristalização mais rápidas para CS400. A cinética de presa foi mais lenta para Control mesmo quando comparado a um grupo derivado de sol-gel com tamanho de partícula semelhante. **Conclusão:** A rota de síntese e o tamanho das partículas influenciam a reação de endurecimento dos cimentos à base de silicato de cálcio. A redução do tamanho de partícula para silicatos de cálcio derivados de sol-gel leva à aceleração da reação de pega do cimento endodôntico bioativo produzido.

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Palavras-chave: Endodontia regenerativa. Cimento de Silicato. Agentes de capeamento da polpa dentária e pulpectomia. Ciência dos materiais. Espectroscopia de infravermelho com transformada de Fourier.

INTRODUCTION

Bioactive endodontic cements (BECs) are primarily composed of calcium silicate particles that are responsible for the bioactivity, the sealing ability, and the setting of these materials^{1,2}. BECs were first developed for root-end filling procedures, but due to their bioactivity and adequate physicochemical properties, they have been used in different regenerative endodontic procedures such as direct pulp capping³, root perforation sealing⁴, apexification⁵, and pulpotomy⁶. Due to their versatility, BECs have been increasingly used and studied in endodontics. Tailoring cement properties by modifying its setting reaction may be an alternative to increase the clinical applicability of these materials.

Several modifications are made in the material's structure to overcome the long setting time of BECs⁷⁻¹². Most of these modifications are related to the addition of new components as catalysts of the setting reaction. Changes in powder: liquid ratio, the addition of additives into the calcium silicate particles, and the addition of catalysts into the water are commonly used strategies. Controlling the morphological features of calcium silicate particles is another strategy that may be used to modify the setting reaction, promoting its acceleration^{13,14}.

Calcium silicate particles are usually produced via the melt-quenched method with melting precursors in high temperatures, leading to dense particles' production¹⁵. Sol-gel has recently emerged as an alternative route to produce calcium silicate particles for endodontic purposes^{12,16}. The formation of silicate bonding at room temperature and the solvent removal lead to the production of highly porous particles with increased surface area^{12,17}, enhancing the reactivity of cements with water. It is known that controlling the particle size impacts the surface area available for the reaction in melt-quenched particles¹³, reducing the setting time. This effect may be especially considered for sol-gel derived particles with a high specific area. The optimization of particle size in these cements could contribute to facilitate their clinical application and thus, this study aims to analyze the influence of the size of sol-gel derived calcium silicate particles in the setting reaction of BECs.

MATERIALS AND METHODS

Particle synthesis

The calcium silicate particles used in this study were synthesized by the sol-gel route, as previously described¹². The hydrolysis of tetraethylorthosilicate (TEOS – Aldrich Chemical; St Louis, MO, USA) in an acidic solution of nitric acid (HNO_3 – Merck KGaA, Darmstadt, Germany) was used to produce the sol under stirring for 1h. Calcium nitrate (CaNO_3 – Êxodo Científica, Sumaré, SP, Brazil) was used as a mineral modifier under stirring for 30min. The obtained sol was submitted to thermal treatment at 60°C for 24h, 120°C for 24h, and 700°C for 24h. The calcium silicate particles (CS) were manually grounded to obtain different particle sizes for the analysis.

The CS particles were divided according to their size by grounding and sieving. Sieves with Mesh 400, Mesh 200, and Mesh 100 were used, and the bypass was collected to produce three experimental groups: CS400 – Mesh 400; CS200 – Mesh 200; and CS100 – Mesh 100.

A commercial bioactive endodontic cement was used as control (Control - MTA Angelus - Angelus, Londrina, PR, Brazil).

Particle characterization

The size and surface area of particles were analyzed by laser diffraction and nitrogen adsorption, respectively. A particle size analyzer (CILAS 1180, France) was used, and the particles were dispersed in isopropyl alcohol (Aldrich Chemical; St Louis, MO, USA) for measurements. A laser was focused on the particle dispersion, and the laser diffraction was measured to characterize the particle size distribution for each group. Nitrogen adsorption measurements were carried out using an Autosorb Quantachrome Nova 1200 (Quantachrome Instruments Corporate Headquarters, USA) instrument. The powder-specific surface area was calculated by the Brunauer–Emmett–Teller (BET) method based on the Nitrogen adsorption isotherm data.

Cement preparation

The cements were produced by mixing CS particles in each particle size with distilled water in an mg:µl proportion of 1.25, as established previously¹². The cements were hand-mixed by 30s until all the powder was incorporated into the water leading to a homogenous mixture. The Control group was manipulated according to the manufacturer's instructions. The proportion, in this case, was 1:3, and the mix of cements was made manually as well.

Setting time

The setting time analysis was conducted according to ISO 6876:2012¹⁸. The powder and liquid were dispensed and mixed. After 120s from the beginning of cement preparation, samples were produced (n=3) into a mold measuring 4mm diameter x 1mm height. With the aid of a Gilmore needle (100g), indentations were produced sequentially in the surface of the specimen until no visible indentation was seen. The time between the mixture and the absence of indentation was recorded as the setting time of the cements. The mean value between the three analysis was considered as the setting time of cements in minutes.

Setting kinetics

The setting reaction was studied considering the changes in chemical bonding in the cements after the hydration process as previously reported¹¹. Fourier Transformed Infrared Spectroscopy (FTIR) was used for the identification of chemical groups during the reaction. A spectrometer (Vertex 70 - Bruker Optics, Ettlingen, Germany), equipped with an attenuated total reflectance device (Platinum ATR-QL; Bruker Optics) was used, and the prepared cements were placed on the top of the ATR device after 120s of mixing. Measurements were sequentially performed between 400 and 4000cm⁻¹ and started at 120s after cements mixing until 72h after setting. During the time between the analysis, the samples were stored at 37°C in a 100% humidity environment to avoid sample dehydration. The differences in each sample's chemical profile were used to understand changes in material structure during a long-term setting.

Statistical analysis

Descriptive analyses were performed for the particle size, the specific surface area, and the FTIR analysis. For the setting time, the normality of data was assessed by the Shapiro-Wilk test, and the groups were compared by One Way ANOVA and Tukey test with 5% significance.

RESULTS

The particle size of sol-gel derived particles and the control group is shown in Table 1. The values ranged between 31.01 μm for Control group and 9.45 μm for CS400 group ($p > 0.05$). The experimental cements presented a higher specific surface area, ranging between 15.14 g/cm^2 and 10.48 g/cm^2 , while the specific surface area for the control group was 4.27 g/cm^2 . Statistically significant higher setting times were observed for Control, where a commercial bioactive endodontic cement was tested. CS200 and CS100 groups presented reduced setting times compared to Control and increased setting time compared to CS400 without a statistically significant difference. CS400 group showed a setting time of 16min and 54s, which are statistically significantly lower than all other groups in the present study ($p > 0.05$).

Table 1: The particle size results are shown by the median value and 10%-90% quartiles. The specific surface area in g/cm^2 is shown for all powders and mean setting time evaluated in accordance with the ISO 6876:2012.

	Particle size (μm)	Specific surface area (g/cm^2)	Setting time (min:s)
Control	31.01 (4.64 – 66.98)	4.27	31:23 ($\pm 3:59$) ^C
CS400	9.45 (1.73 – 22.20)	15.14	16:54 ($\pm 1:01$) ^A
CS200	15.18 (2.49 – 49.72)	10.48	22:22 ($\pm 0:29$) ^B
CS100	25.08 (3.45 – 69.54)	10.98	25:59 ($\pm 1:59$) ^B

Different capital letters indicate a statistically significant difference between groups.

The FTIR analysis of setting reaction is observed in Figure 1. After 120s of mixing, similar peaks were found for the sol-gel derived cement groups (CS400, CS200, CS100) on FTIR analysis. The peaks around 489 cm^{-1} , 640 cm^{-1} , and 997 cm^{-1} are assigned to the Si-O bonding in different vibrations and are more evident for the sol-gel derived particles when compared to Control. Carbonate peaks ($\text{CO}_3^{-2}\text{V}_3$ and $\text{CO}_3^{-2}\text{V}_2$) were observed in all groups. The presence of water was observed on the 1632 cm^{-1} , and on the region between 2840 cm^{-1} and 3700 cm^{-1} . These peaks are associated with the presence of OH^- and are reduced overtime during the setting reaction. A slower reduction on OH^- intensity was found for Control, CS100, and CS200 groups when compared to CS400. As the reaction takes place, the $\text{CO}_3^{-2}\text{V}_3$ reduced its intensity and a shift for a higher wavelength around 930 cm^{-1} assigned to the formation of Si-OV₃ asymmetrical stretching. The A $\text{CO}_3^{-2}\text{V}_2$ peak in the region of 1380 cm^{-1} was observed after 5min of reaction in CS400 group. On CS200, CS100 and Control, this change was found only after 10min, 35min, and 24h, respectively. After 72h, all groups presented Si-O peaks in the region 450–600 cm^{-1} and 900–1015 cm^{-1} and a C-O peak at the region between 1380 cm^{-1} and 1450 cm^{-1} indicating the complete setting reaction.

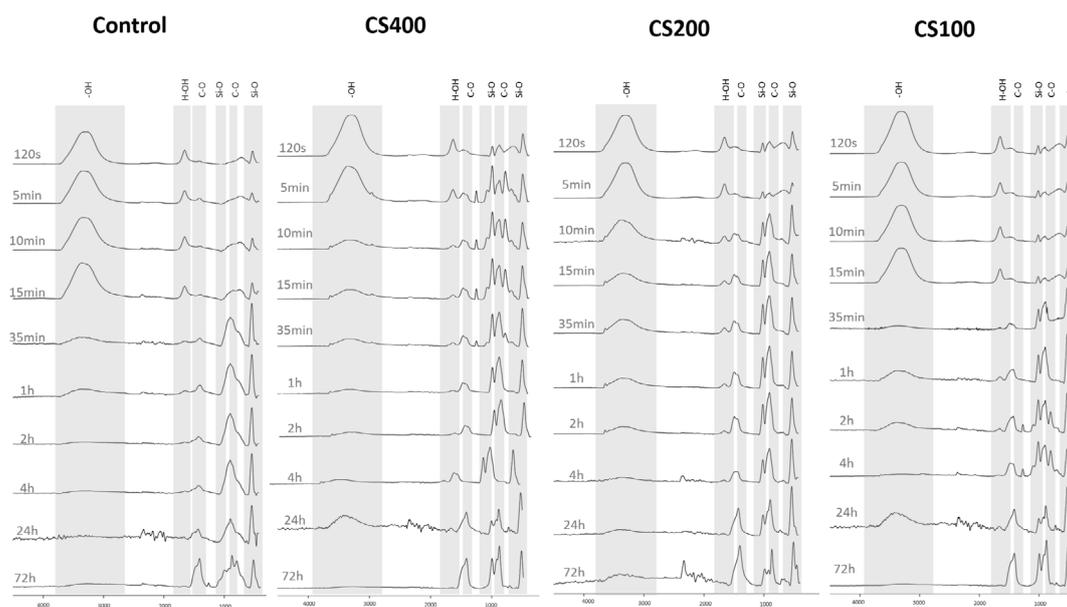


Figure 1: FTIR spectra of cements from 120s after mixing with water to 72h of setting. Spectra were obtained between 400-4000 cm^{-1} for Control (A) and sol-gel derived calcium silicate cements with different particle sizes obtained through sieving in CS400 (B), CS200 (C), and CS100 (D).

DISCUSSION

Setting time is still a challenge for the clinical application of BECs^{1,7,19}. Among the strategies to facilitate the application of these cements in daily endodontic procedures, the control of material setting is used in several studies^{7,9,12,20}. Tailoring surface particle may be an alternative to adjust the setting of the cement. In this sense, the setting reaction of newly developed calcium silicate properties was tested, and sol-gel derived particles with a high specific surface area were used in different particle sizes and compared to a commercial BEC. The smaller particle size led to a higher specific surface area and resulted in lower setting times with more rapid modifications in setting reaction.

Smaller particle sizes were known to promote an increase in the specific surface area of particles²¹. The influence of the production route in the morphology of particles is studied^{22,23}, and remarkable differences are found between melting technique and sol-gel route derived particles. While the first one is based on the bonding of oxide precursors in high temperatures (1500-2000°C), the second is based on the formation of silicate network through hydrolysis of alkaloids at low temperatures (20-100°C). The melting technique leads to the formation of dense structures producing particles with a reduced specific surface area due to the low mesoporosity found for these particles¹⁷. Under other conditions, the high specific surface area of sol-gel derived particles is related to the gel aging in the particle synthesis²². As observed in the present study, higher values for the specific surface area were found for CS400, CS200, and CS100, when compared to the melt-quenched commercial cement used as control. This is explained by the formation of a silicate network during the sol-gel synthesis due to the entrapment of the reaction products (ethanol, nitrates) into the formed gel. During the heat treatment (120-700°C), they are removed from the structure, leaving a vacancy in the material structure, which becomes a pore after the cooling²⁴. These pores are responsible for an irregular surface characteristic in these particles leading to the surface area with higher values (Table 1).

An increased surface area in calcium silicate particles allows the formation of a higher number of hydration sites on the surface of particles. The initial surface dissolution leads to the production of a calcium-silicate-hydrated (CSH) and calcium hydroxide (CaOH_2)^{14,25,26} as a first step that is essential for the primary setting of cements and further hardening. This reaction results in the formation of silica gel and calcium hydroxide over the surface of the particles. The further steps in the cement hardening involve the precipitation of minerals on the gel layer. As observed in Figure 1, the reduction in the O-H-O and the $(\text{CaOH})_2$ peak was faster for CS400 cements, indicating that the formation of the gel phase and increased reaction rate were more rapid smaller particles used in this study. For the bigger particle sizes, the changes in the FTIR analysis were observed in later times of examination. When considering the CS100 and the Control it is found that despite the closer values for particle size, the setting time is statistically higher for the Control. This result corroborates to the understanding of the role of the surface properties on the initial hydration and the setting of these cements.

After the first step of hydration, several phase transformations take place into the cement structure. The shift in Si-O peak from $\sim 930\text{cm}^{-1}$ to higher wavelengths, the reduction in the OH⁻ peak (2840cm^{-1} to 3700cm^{-1}), and the increase in CO_3^{2-} (1380cm^{-1} to 1450cm^{-1}) indicate the formation of C-H-S and the subsequent crystallization reaction^{11,14}. All these modifications are observed in the tested cements and happened in early time points as the particle size reduces in the sol-gel derived materials. In Figure 1 analysis, it can be observed that the modifications in these peaks occurred slower for the Control group, especially when the formation of CaCO_3 is considered. The C-O bonding in the 1450cm^{-1} is related to the increased crystallinity of cements and may lead to enhanced mechanical properties for BECs²⁷. This peak appeared only after 24h after mixing on Control (Figure 1), whereas in the sol-gel derived materials, the appearance of the C-O crystalline structure was faster. For example, in the CS400 group, the formation of this structure started at 5min. These findings contribute to the understanding of the influence of morphology in the reaction of BECs, showing how the entire setting reaction may be influenced by the reactivity and the micro and nano-scaled structure of particles. This could also contribute to future steps on the development of calcium silicate nanoparticles via the sol-gel route with optimized properties for endodontic cements.

The production route and the particle size are the main reason to explain the differences in morphology of studied particles; however, the commercially available MTA used as the control group presents a different composition. While the CS400, CS200, and CS100 were composed of calcium silicate particles alone, the Control is composed of calcium silicate and tricalcium aluminate¹⁹. Tricalcium aluminate is used on the mixture to accelerate the MTA reaction^{20,28}. The hydration of tricalcium aluminate is faster and leads to the formation of a diffusion barrier that impairs the further hydration of calcium silicate particles¹⁵. The faster hydration of calcium aluminate is desired in this case for a rapid initial hardening of the materials promoting the initial, which allows this material to be used on clinical application but may be involved with the slow overall reaction of setting. For the sol-gel derived particles, the setting occurred without the addition of any other component into the powder, showing that the CS particle characteristics may control the calcium silicate hydration and the formation of crystalline phases during the reaction and that this strategy may avoid the need of additives and catalysts for the reaction. Although this was not investigated in this study, maintaining the calcium silicate particles as the major component of these cements may contribute to the maintenance of physicochemical and biological properties.

The reduction of setting time is a strategy to avoid one of the limitations of the clinical application of BECs²⁹. The development of novel compositions may result in materials with better properties. Understanding these mechanisms behind this is essential to the future application of these cements in Dentistry. The setting reaction of calcium silicate-based

cements may be further studied, and exploiting this reaction may help explain the physico-mechanical and biological properties and the cement's behavior *in vivo*. In the present study, the effect of particle morphology on the setting reaction was shown, and the smaller sol-gel derived particles presented lower setting times. Changing the production route and controlling particle size makes it possible to achieve a faster and effective setting for bioactive endodontic cements.

CONCLUSIONS

The route of synthesis and the particle size influences the setting reaction of calcium silicate-based cements. The reduction of particle size for sol-gel derived calcium silicates lead to the acceleration of the setting reaction of the produced bioactive endodontic cement.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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