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Carbonation inhibitor by polyethylene glycol encapsulation of calcium hydroxide fine particles to improve antimicrobial and root canal penetration properties

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ABSTRACT

The carbonation of calcium hydroxide ($Ca(OH)_2$) is affected by humidity and a saturated atmosphere. $Ca(OH)_2$ from nature is easily carbonation and self-aggregates into calcium carbonate ($CaCO_3$), resulting in larger particle size impairing the antimicrobial properties due to lack of penetration into the dentinal tubules and lower ion dissociation. To reduce the particle size, the wet beads milling process with distilled water as the medium is commonly used, but often results in great carbonation of the final product. Polyethylene Glycol (PEG) may inhibit the carbonation process as well as re-agglomeration. However, it requires intensive drying of the fine $Ca(OH)_2$ particles. As an alternative, we used ethanol as a medium in the milling process, which is easily dried and compatible with PEG as a surfactant. This study aimed to evaluate PEG 400 as a dispersing agent in ethanol medium in the beads milling process to prevent carbonation of the

Abbreviation: CaC, Calcium Hydroxide Commercial; $Ca(CO)_3$, Calcium Carbonate; CaO, Calcium Oxide; $Ca(OH)_2$, Calcium Hydroxide; CaP, Calcium Hydroxide Palimanan; CaPC, Calcium Hydroxide Palimanan Carbonated; CaP-DW, Calcium Hydroxide Palimanan beadsmill with Distilled Water; CaP-Eth, Calcium Hydroxide Palimanan beadsmill with Ethanol; CaP-PEG, Calcium Hydroxide Palimanan beadsmill with Polyethylene Glycol; DW, Distilled Water; FTIR, Fourier Transform Infrared; OH, Hydroxyl; PEG, Polyethylene Glycol; PSA, Particle Size Analysis; SEM, Scanning Electron Microscopy; XRD, X-Ray Diffraction.

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fine $Ca(OH)_2$ particles. The following groups were analysed CaP-PEG ($Ca(OH)_2$-PEG) with ethanol as a medium, CaP-Eth ($Ca(OH)_2$ with ethanol as a medium), CaP-DW ($Ca(OH)_2$ with distilled water as a medium), CaPC ($Ca(OH)_2$-carbonated) as the negative control and CaC ($Ca(OH)_2$ analytical grade) as the positive control The final particle results were characterized to evaluate the crystal structure, functional groups, and particle size. The corresponding pH and antimicrobial activity against *Enterococcus faecalis* were assessed at 1, 3, 7, and 14 days. The penetration ability was evaluated by Scanning Electron Microscope. The data obtained were analysed by ANOVA with a significance level of 5%. PEG was able to inhibit carbonation and stabilize pH for up to 14 days, providing increased antimicrobial activity against *E. faecalis.* PEG also facilitates the ability of fine $Ca(OH)_2$ particles to penetrate deeper into the dentine tubules by reducing particle size.

1. Introduction

$Ca(OH)_2$ is a white, odourless powder with a molecular weight of 74.08, limited water solubility (approximately 1.2 g/L at 25 °C) and high pH (12.5–12.8). Although the overall mechanisms of action $Ca(OH)_2$ are not fully understood, many articles have been published describing its biological activity [1–4].

The primary biological actions are accomplished through the ionic dissociation of Ca^{2+} and hydroxyl ions (OH^-) and their effect on inducing hard-tissue deposition and antimicrobial properties. $Ca(OH)_2$ inhibits the growth of bacteria by denaturing proteins and damaging the DNA and cytoplasmic membranes of bacteria. In close interaction with the bacteria, $Ca(OH)_2$ changes the physicochemical condition of intercellular substances and causes protein denaturation via glycoprotein rupture [2]. These events are related to the alkaline environment to its surroundings through the release of OH^-ions, which has a lethal effect on bacteria [3,5]. The ability of $Ca(OH)_2$ to dissociate into an ionic form and maintain close contact with the infected site where the bacteria are retained is crucial to have an optimum antimicrobial effect [4,6,7].

The root canal system is a complex structure with small and narrow areas and dentinal tubules forming the dentinal walls [8,9]. Therefore, the particle size of the intracanal medicament should be small enough to penetrate and come into direct contact with residing bacteria in those areas. Nanoparticles $Ca(OH)_2$ have superior antimicrobial activity against *Enterococcus faecalis* compared to conventional $Ca(OH)_2$ because they can effectively penetrate deeper into narrow gaps in the dentinal tubules [10]. Medicaments that can penetrate deeper into the dentinal tubules not only function as inhibitory agents and prevent microbial repopulation but also inactivate bacteria in the tubules [11]. Nanoparticle size is expected to increase the free flow capability until the apical section of root canal teeth with irregular direction and density [12]. Thus, a high local pH may be produced, increasing the antimicrobial activity in small areas [13]. In addition, reducing particle size will improve the active surface area and consequently enhance chemical and biological reactivity [10,14].

Natural limestone ($CaCO_3$) is a potential raw material that can be used as a precursor for synthesizing $Ca(OH)_2$. In this process, the $Ca(OH)_2$ obtained by hydrating CaO in distilled water yielded a more reactive hydroxide than hydration in the vapour phase. $Ca(OH)_2$ transforms to $CaCO_3$ when in contact with air, and this carbonation process increases as environmental humidity rises [15,16]. Our previous study successfully synthesized $Ca(OH)_2$ from natural limestone in Palimanan area in West Java, Indonesia [17]. This Palimanan $Ca(OH)_2$ (CaP), was further optimized by reducing its particle size through a wet beads milling process. Despite the satisfactory particle size obtained, the CaP was easily carbonized to $CaCO_3$ when in contact with air. The $CaCO_3$ aggregation will obliterate the penetration of $Ca(OH)_2$ into the dentinal tubules and restrict their antimicrobial action, particularly *E. faecalis,* known as a robust bacteria able to penetrate deep into the dentinal tubules [18]. Consequently, the prevention of carbonation and re-agglomeration must be considered when reducing particle size.

Polyethylene glycol (PEG) 400 is a water-soluble polymer, non-irritating molecule, with minimal toxicity that does not transform the structure and is widely used in biological applications [19]. PEG may prevent the carbonation process and re-agglomeration of the CaP during the milling process. However, we expected the final product of the milling process to be in a dry powder form, whereas using PEG alone or combined with distilled water will result in a paste form requiring an intensive drying process. We proposed beads milling with ethanol medium, which is more easily evaporated together with PEG to encapsulate the particle and prevent carbonation. Therefore, the use of ethanol as a medium should be evaluated to confirm the optimal size reduction and suitability with PEG as a surfactant.

This research aimed to evaluate PEG 400 as dispersing agents to prevent the carbonation effect during the beads milling process to obtain fine $Ca(OH)_2$ particles. The investigation of encapsulated $Ca(OH)_2$ particle include crystal structure, functional groups, particle size, corresponding pH value, and antimicrobial activity in comparison with using only distilled water and only ethanol medium. The results of the milling process with the different mediums will also be evaluated by Scanning Electron Microscope (SEM) to see the penetration of the material in a root canal model.

2. Materials and method

$CaCO_3$ was collected from Palimanan, Jawa Barat, Indonesia (Latitude, Longitude: 6°42′40″, 108°26′17"E). CaCO3 was rinsed with distilled water and dried in the oven (MTI X1200, China) for an hour at 120 °C. $CaCO_3$ was calcined for 4 h at 900 °C, to produce CaO powder. Thereafter, the CaO powder was hydrated with Milli Q (Specification: PRD.2.ZQ5.10000054299, Sigma-Aldrich, Germany) on

a hot plate rotary stirrer (Thermo Scientific Model No. SP88857105, USA) for 1 h at a speed of 300 rpm, 50 °C. The $Ca(OH)_2$ paste was dried at 120 °C, then crushed using a mortar and pestle and sieved using 400 mesh to obtain a powder of $Ca(OH)_2$ Palimanan (CaP) [17]. The fine particle of $Ca(OH)_2$ powder was obtained by beads milling the CaP powder for 4 h with different active agents and medium in each group (Fig. 1). Group1 $Ca(OH)_2$ Palimanan-PEG 400 with ethanol 95% as medium (CaP-PEG); group 2 $Ca(OH)_2$ Palimanan-Ethanol 95% (CaP-Eth); group 3 $Ca(OH)_2$ Palimanan-Distilled water (CaP-DW) as the negative control, and $Ca(OH)_2$ (Merck, EMSURE® ACS, Reag. PhEur, Germany) as the reference of analytical grade $Ca(OH)_2$.

The result product of fine powder samples was subjected to characterization by X-Ray Diffraction (XRD) (Bruker D8, Advance, Germany) with Cu Kα radiation generated at 45 kV and 30 mA, 5 and 65° to determine the mineralogy of different beads milling surfactants. The quantitative analysis was conducted using Match Version 3.x software [20], FTIR (Nicolet Is5 Thermo, Thermo Scientific, USA), Particle Size Analyzer (PSA) (SZ-100, Horiba, Japan), and Scanning Electron Microscope (SEM) (JEOL JSM IT300, Japan). The initial pH of the $Ca(OH)_2$ pastes was measured by mixing 0.055 g of the test material in 10 mL of distilled water (Milli Q, Sigma Aldrich, Germany). A calibrated digital pH meter (Lutron, pH-201, Taiwan) was used to determine the pH value at 0, 1, 3, 7, and 14 days for each group. The antimicrobial activity was assessed by the agar diffusion test. A suspension of *E. faecalis* (ATCC 29212) with 0.5 Mc Farland was standardized and inoculated on an agar plate culture media (Tripticase Soy Agar media enrichment) ($n = 5$). Five wells (4 mm diameter) were promptly filled with freshly mixed $Ca(OH)_2$ paste. $Ca(OH)_2$ paste was prepared by mixing the powders with distilled water ratio of 0.8 [1]. One of the four wells was filled with analytical grade $Ca(OH)_2$ as the positive control (Merck, EMSURE® ACS,Reag. Ph Eur, Germany) while another was filled with CaP-DW, CaP-Eth, and CaP-PEG. The plates were incubated at 37 °C for 1, 3, 7, and 14 days under anaerobic conditions and the inhibitory haloes and diffusion was measured using a digital calliper on each evaluation day. The measurement of the inhibition zone diameter around each well was done three times, and the mean value of the inhibition zones were recorded.

Evaluation of penetration of the CaP-PEG and the positive control $Ca(OH)_2$ paste was carried out on a root canal model obtained from the apex of extracted upper anterior premolar teeth recently extracted due to orthodontic or periodontal reasons. A total of 1.0 mg of powder was mixed with 0.8 ml of distilled water (Milli Q, Sigma Aldrich, Germany) to form a homogeneous paste, then injected into the root canals of the teeth. Two parallel grooves were made on opposite external surfaces of each root with mark point using diamond discs, without penetrating the canal space. The roots were then split using end-cutting pliers. The resulting samples were placed in a vacuum chamber in a SEM (JEOL JSM IT300, Japan) and coated with Au. The microscope was operated at 20 kV and 2500× magnification to observe the maximum penetration depth of the CaP-PEG and CaC into the dentinal tubules in the coronal, middle, and apical thirds of each sample. To ensure uniformity of measurement, a single operator evaluated all the specimens. The maximum penetration depth is evaluated by measuring the maximum distance between the dentin canal wall and $Ca(OH)_2$ present in the dentinal tubules. The data were averaged to obtain a single value per section to eliminate any discrepancies. The obtained data were subject to statistical analysis using ANOVA and Post hoc *t*-test at a 5% significance level.

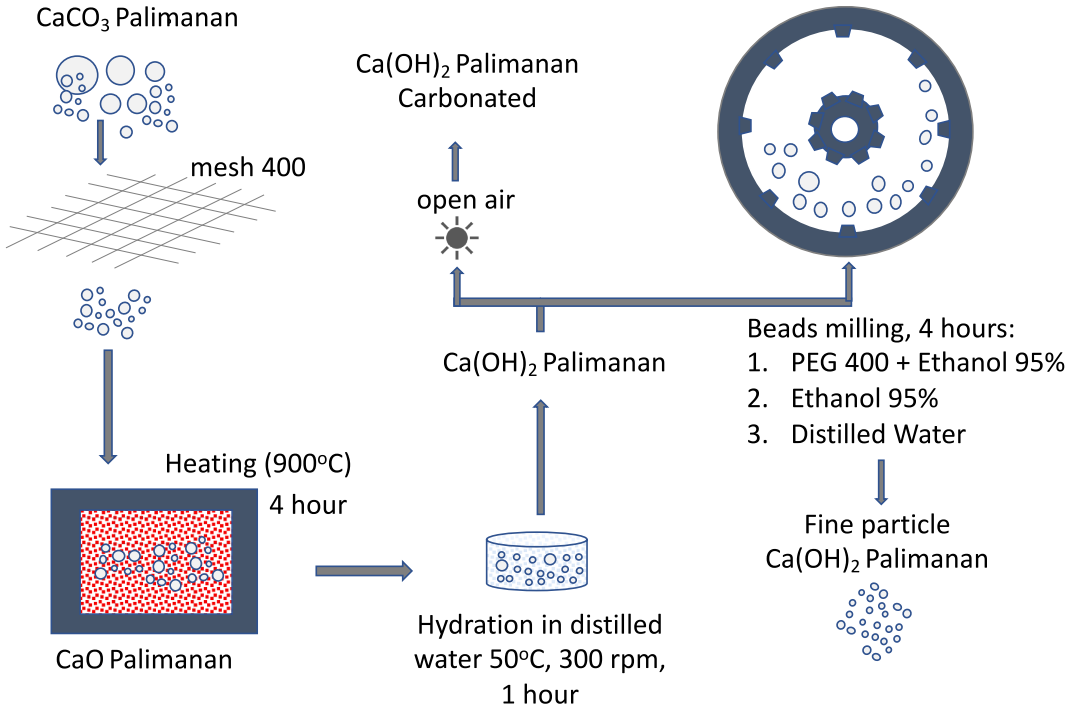

Fig. 1. Experimental procedure for synthesizing fine particle $Ca(OH)_2$ from natural limestone (CaP).

3. Result

The crystal structure of all samples was evaluated by XRD as shown in Fig. 2 [20]. The XRD pattern formed on CaP-Eth, CaP-DW, and CaPC was rhomboid calcite as the major phase with portlandite $Ca(OH)_2$ as the minor phase. In contrast to the CaP-PEG pattern, which only forms the portlandite $Ca(OH)_2$. The CaP pattern showed that CaP-PEG and CaP were similar to the CaC as control which is a typical peak of $Ca(OH)_2$ with high crystallinity.

Based on the data in Table 1. CaC has a crystal size of 29.7 nm on average. The crystal size is greater than CaP-PEG, which is 23.8 nm. Furthermore, CaPC, CaP-Eth, CaP-DW contained a higher percentage of rhombohedral calcite, 87.5, 90.0, and 88.7% respectively. Meanwhile, the majority of CaP-PEG and CaP are hexagonal $Ca(OH)_2$ phases, estimated to a percentage of 78.9 and 97.6% of the total. Based on the quantitative XRD characterization, it can be seen that the largest percentage of hexagonal $Ca(OH)_2$ among beads milling groups is found in the CaP-PEG.

Furthermore, all IR-spectra of the $Ca(OH)_2$ showed calcium characteristics related to both hydroxide and carbonate groups, as shown in Fig. 3. The relatively strong absorption band at ~3642 cm^{-1} corresponded to the O–H stretching mode. While the peak at ~2869 cm^{-1} corresponded to C–H stretching of PEG functional groups. The O–H stretching absorption band was not so sharp on the CaP-Eth, CaP-DW and CaPC. The sharp peak at ~879 cm^{-1} corresponded to the m^2 symmetric deformation of the CO_3 group. The absorption peaks of the out-of-plane bending of CO_3^{2-} in $CaCO_3$ were also detected at ~1457 cm^{-1} corresponding to the asymmetric stretching of the CO_3 group. The minor peaks at ~2502–2535 cm^{-1} were due to the adsorption of the atmospheric CO_2 and C═O stretching. The results indicate that PEG successfully inhibits the carbonation process of fine particle CaP.

The particle size of all samples are shown in Fig. 4. Beads milling treatment using active agent resulted in smaller particle size where CaP-Eth produces the smallest particle size (150–400 nm), while CaP-PEG produces particle sizes of 200–400 nm, and CaP-DW produces particle sizes of 800–1000 nm. CaC has the largest particle size among all groups, with an average particle size of 3 μm. Fig. 5a–f shows the SEM characterization results, which provide a comprehensive morphological analysis of the particles across all groups. According to the observations, the majority of particles in the CaPC, CaP-Eth, and CaP-DW groups have a spherical morphology. In contrast, hexagonal shapes predominate in the CaP-PEG, CaP, and CaC groups indicated the formation of hexagonal crystal phase $Ca(OH)_2$ as observed in XRD spectra. Furthermore, the CaP-Eth and CaP-PEG groups appear to have smaller particles than

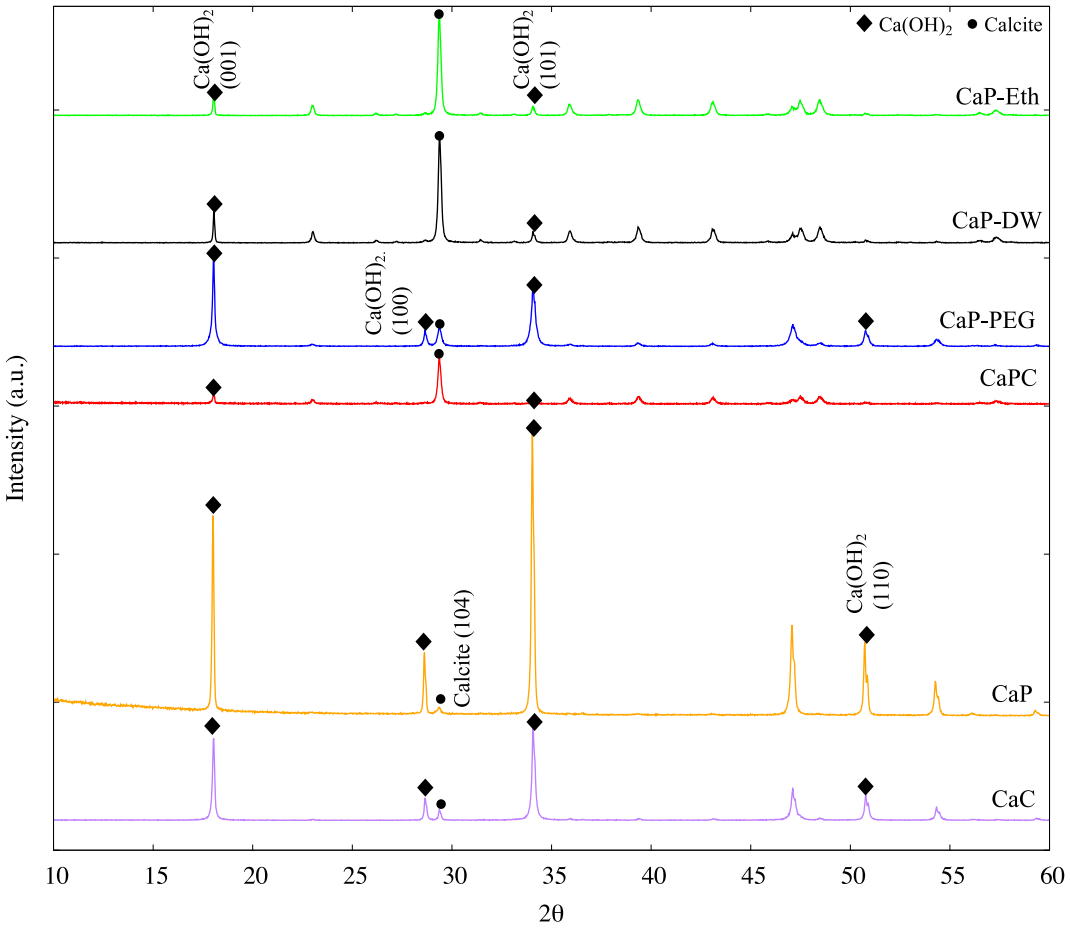

Fig. 2. XRD patterns of all sample.

Table 1
The Quantitative Analysis of XRD spectra of samples by Match at various beads milling treatment.

Group sample	Average Crystalline Size (nm)	Data Entry	Formula	Nama	Quantitative (%)
CaPC	33.4	96-101-5391	$CaCO_3$	Calcite rhombohedral	87.5
		96-900-0114	$Ca(OH)_2$	$Ca(OH)_2$ hexagonal	12.5
CaP-PEG	23.8	96-101-0963	$CaCO_3$	Calcite rhombohedral	20.2
		96-702-0139	$Ca(OH)_2$	$Ca(OH)_2$ hexagonal	79.8
CaP-Eth	35.8	96-101-0963	$CaCO_3$	Calcite rhombohedral	90.0
		96-702-0139	$Ca(OH)_2$	$Ca(OH)_2$ hexagonal	10.0
CaP-DW	36.0	96-901-6707	$CaCO_3$	Calcite rhombohedral	88.7
		96-702-0139	$Ca(OH)_2$	$Ca(OH)_2$ hexagonal	11.3
CaP	40.3	96-101-5391	$CaCO_3$	Calcite rhombohedral	2.4
		96-900-0114	$Ca(OH)_2$	$Ca(OH)_2$ hexagonal	97.6
CaC	29.7	96-101-0963	$CaCO_3$	Calcite rhombohedral	8.8
		96-702-0139	$Ca(OH)_2$	$Ca(OH)_2$ hexagonal	91.2

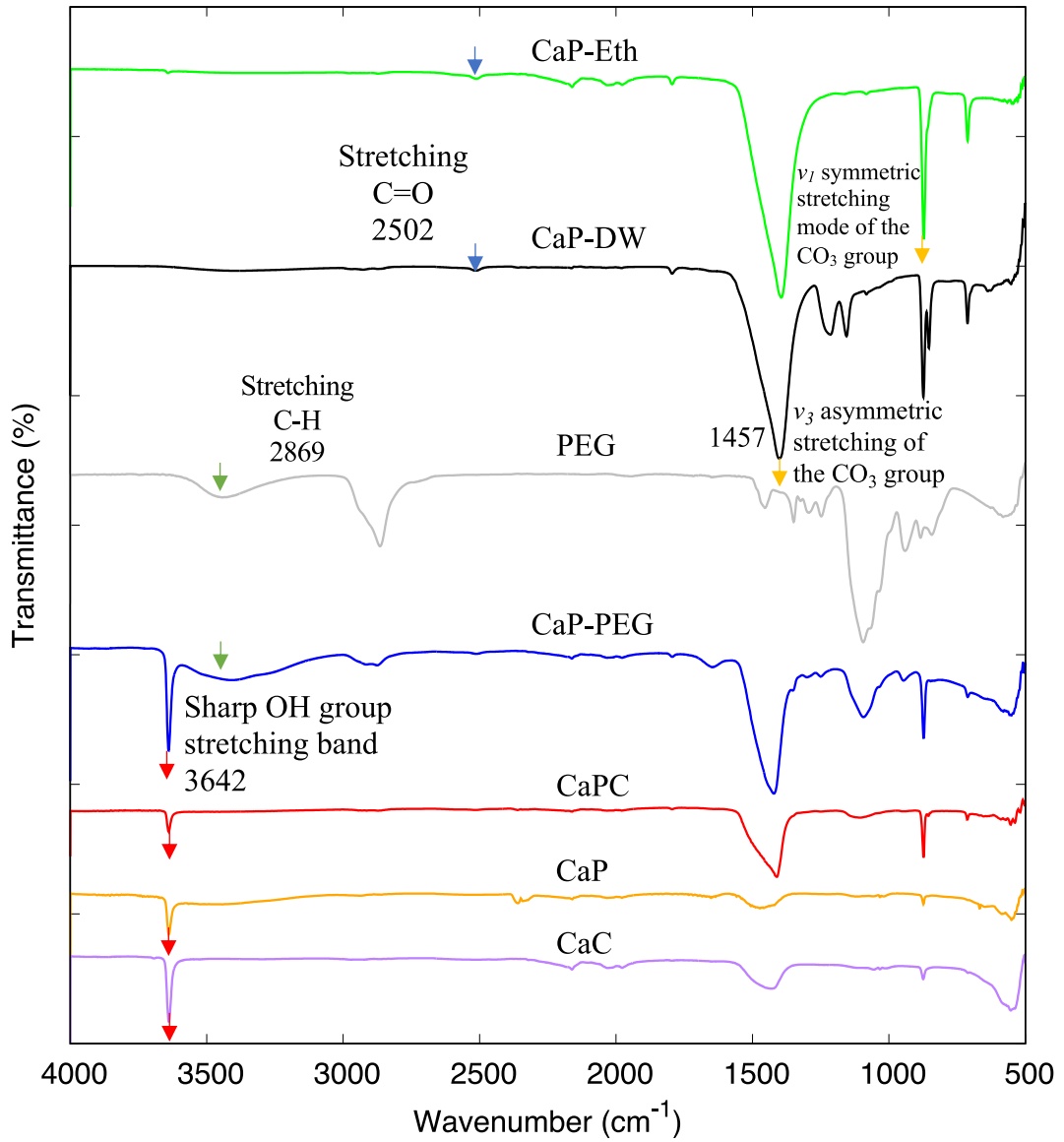

Fig. 3. FTIR spectra of CaP-Eth, CaP-DW, PEG, CaP-PEG, CaPC, CaC.

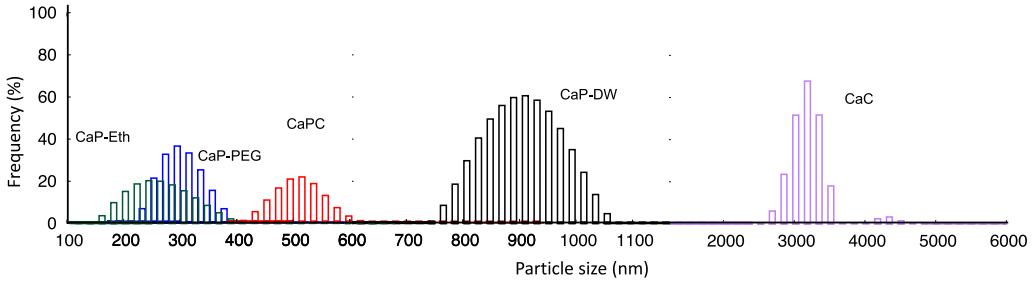

Fig. 4. PSA of CaPC, CaP-PEG, CaP-Eth, CaP-DW and CaC.

the other groups.The data obtained on pH evaluation on days 1, 3, 7, and 14 are shown in Fig. 6. All samples were stable under alkaline conditions until day 14. In the initial period of measurement on day 1, there was no significant difference for CaPC, CaP-PEG, dan CaC. However, on day 1, CaP-DW and CaP-Eth differed from other groups ($p < 0.05$). Only CaP-PEG showed a significant difference on day 3 ($p < 0.05$). On days 7 and 14, all groups showed similar results ($p > 0.05$). CaP-PEG produced the highest pH values, ranging from (8.63 ± 0.03) to (11.85 ± 0.55) on day 1 to day 14.

Antimicrobial evaluation by agar diffusion method showed that all groups had an antimicrobial effect against *E. faecalis* as shown in Fig. 7a1-e14. The means of inhibition zone in each group are demonstrated in Fig. 8. All groups showed a stable antimicrobial action that persisted for up to 14 days. On day 7, the antimicrobial activity of CaC declined, but on day 14, it increased. The highest efficacy belonged to CaP-PEG, while the lowest was for CaP-Eth. The efficacy of medicaments from the highest to the lowest in descending order was CaP-PEG > CaC > CaP-DW > CaPC > CaP-Eth.

The CaP-PEG particles appeared smaller and more uniform than the CaC (Fig. 9a–f). SEM evaluation showed that the dentinal tubules in the CaP-PEG group were filled and closed. CaP-PEG and CaC were seen to penetrate inside the dentinal tubules (Fig. 9b,e), as highlighted in the white arrow. Some dentinal tubules in CaC appear to be open without being filled, highlighted in the red arrow (Fig. 9c,f).

Table 2 shows the mean ± SD penetration depth (nm) for CaP-PEG and CaC in three different sections. The results of the ANOVA analysis showed significant differences in the depth of penetration in the three sections, in the CaC groups ($p = 0.004$). Post hoc Tukey test showed lower penetration depth of CaC in the apical than in the middle and the coronal section ($p < 0.05$). However, the difference between the middle and apical sections was not significant ($p = 0.711$). Nevertheless, CaP-PEG group penetration depth increased from apical to coronal ($p = 0.436$). All sections in the CaP-PEG group had greater penetration depth values than the same sections of the CaC group ($p < 0.05$).

4. Discussion

The natural resource form of $Ca(OH)_2$ can be easily converted to calcium carbonate ($CaCO_3$) through carbonization, resulting in a larger particle size that prevents them from reaching the dentinal tubules, limiting their ability to act as an ions-releasing antimicrobial agent. To address this problem, we propose to use and obtain fine $Ca(OH)_2$ particles using PEG as a dispersant to prevent carbonization, which can provide pH stabilization, antimicrobial enhancement and deeper penetration into the causes dentinal tubules.

The XRD pattern of CaPC was similar to CaP-DW and CaP-Eth with a high percentage of hexagonal $Ca(OH)_2$ phase. Meanwhile, CaP-PEG follows the same pattern as CaP and CaC with a mixture of calcite rhombohedral and hexagonal $Ca(OH)_2$ phases. The carbonation characteristics of $Ca(OH)_2$ relate to the carbonation mechanism that occurs in several stages in which CO_2 from the air speed up $CaCO_3$ transformation [21–24-]. The wet beads milling procedure to modify the particle surface is a simple technique to improve the properties and quality of the material. Particle surfaces that interact with one another under mechanical stresses can exhibit mechanochemical reactions that result in physical and mechanical changes. This can result in surface activity and energy release of surface particles and increase interphase reactivity [25]. PEG can be an alternative to prevent the aggregation of nanoparticles and as stabilizing capping agent [26]. PEG can maintain the stability of $Ca(OH)_2$ against environmental influences [22,27]. The hydrophobic and hydrophilic chain characteristics in PEG make it suitable as a stabilizer. PEG inhibits carbonation by encapsulating fine particles of $Ca(OH)_2$, keeping the $Ca(OH)_2$ powder dry and preventing the carbonation process from continuing [19]. Based on the XRD, it can be seen that CaP-PEG has a comparable peak intensity with CaP and CaC. Meanwhile, CaPC has a similar peak intensity, but the formation is not as high as CaC. Based on these results, it can be concluded that CaP-PEG has superior stability by preventing carbonation compared to the other groups.

As seen from the Fourier Transform Infrared (FTIR) characterization, the O–H functional groups were found in the CaP, CaP-PEG, and CaC groups. These OH^- ions elevate the pH level and provide antimicrobial properties against bacteria, particularly *E. faecalis*. The result of CaP is in line with the pH measurements in the CaP-PEG and CaC groups, which released higher OH^- ions than CaP-Eth, CaP-DW and CaPC groups up to 14 days of measurement. Additionally, CaP-PEG has good stability and maintained the highest pH up to day 7. Even on day 3, CaP-PEG released more OH^- ions than other groups. This is consistent with previous studies reporting that nanoparticles can release ions faster than bigger particle sizes, which could be due to the higher concentration of particles per volume [28–30]. Small particle size can increase the solubility and mobility of Ca^{2+} and OH^- ions, indicated by an increase of bacterial

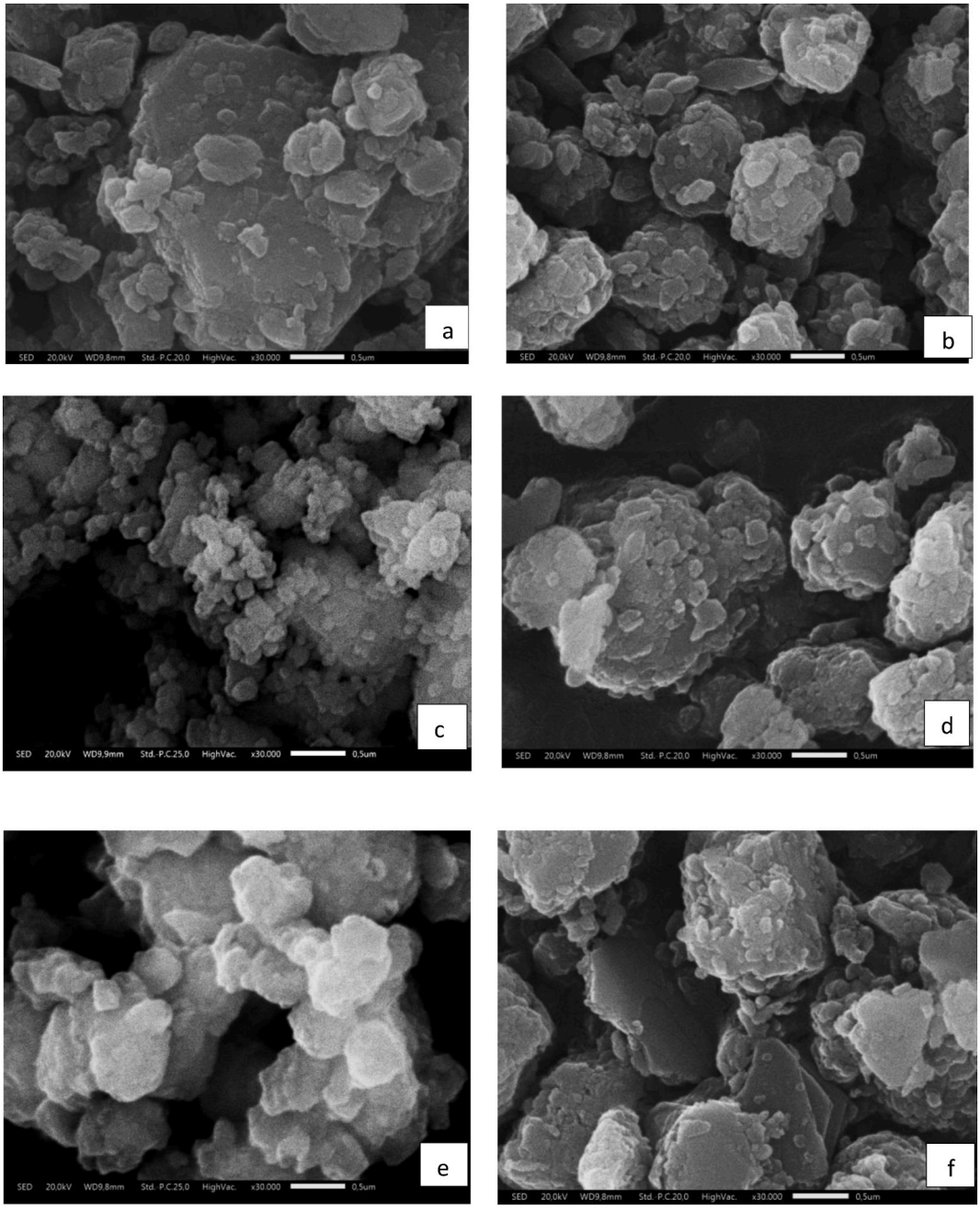

Fig. 5. SEM characterization of CaPC (a), CaP-PEG (b), CaP-Eth (c), CaP-DW (d), CaP (e), and CaC (f) particle.

inhibition zone in CaP-PEG [31,32]. In contrast, CaP-PEG revealed a higher inhibition zone than CaP-Eth though they have similar average particle sizes. The presence of PEG increased the antimicrobial activity of the CaP-PEG group [33,34]. Although CO_3^{-} has a slower reactivity than OH^{-}, CO_3 free radicals can contribute to aging, persistent inflammation, and several infectious diseases related to the DNA and protein damage caused by CO_3 groups [35,36]. As a result, the measurement of the bacterial inhibition zone continues to produce antimicrobial activity in response to the presence of CO_3 in CaP-DW and CaP-Eth. Although CaP-DW has good antimicrobial properties against *E. faecalis* in this in vitro study, the CaP particle size is still large. Thus, it will be challenging for the particle to penetrate complex anatomy and narrow root canals. Similarly, CaP-Eth despite having an antimicrobial effect and particle size that is nearly comparable to CaP-PEG, is unable to inhibit CaP carbonation.

SEM imaging showed the penetration of the homogenous paste into the dentinal tubules in the coronal, middle, and apical thirds. This can be attributed to the different particle sizes of CaP-PEG (318.0 nm), and CaC (2616.7 nm) and also to the anatomical complexity associated with the apical third of the root canal, less tubular density than that could be found in coronal dentin and

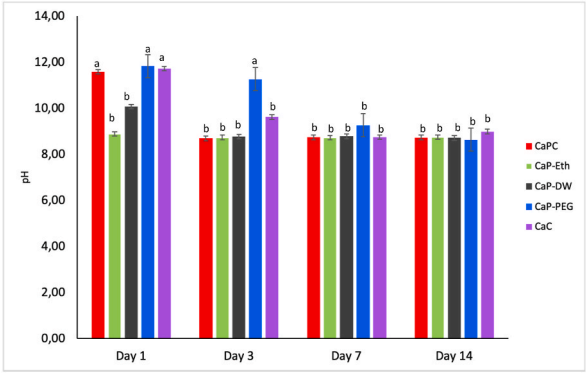

Fig. 6. pH all samples on days 1, 3, 7, 14. *Different letters indicate significant difference between the groups ($p < 0.05$).

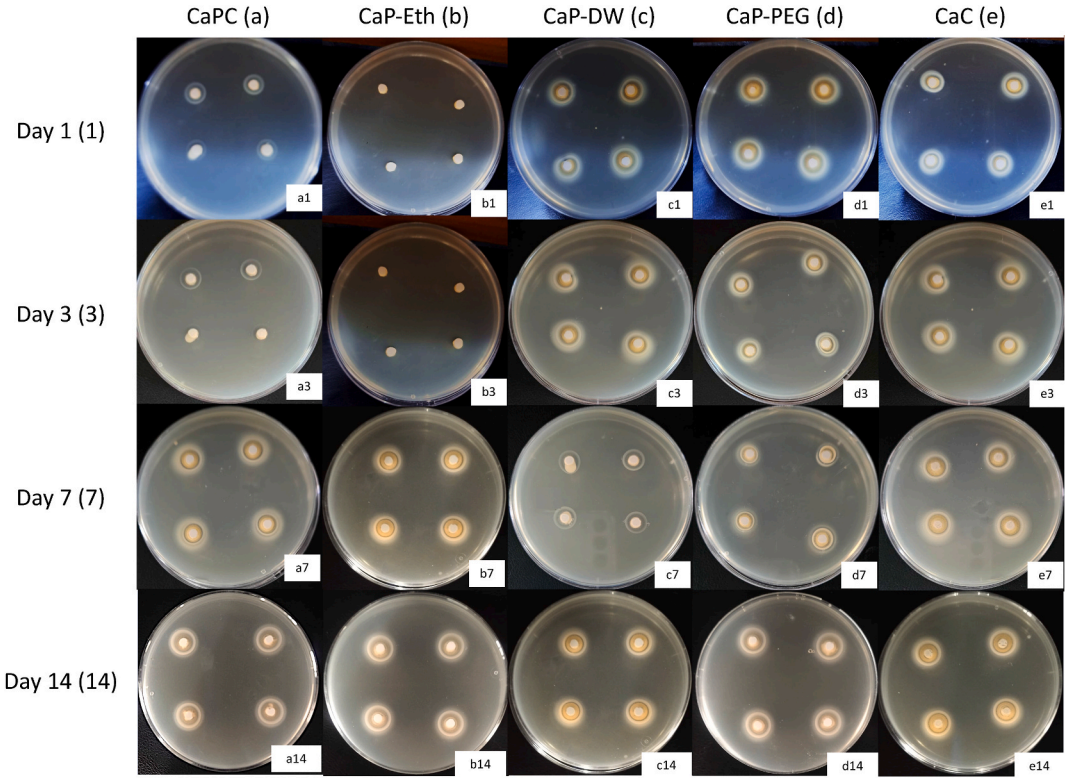

Fig. 7. Agar plate with zone inhibition around CaPC (a), CaP-Eth (b), CaP-DW (c), CaP-PEG (d), and CaC (e) on day 1 (1), day 3 (3), day 7 (7), and day 14 (14).

operator bias during the procedure interpretation [12]. Dentin tubules are considered to have a diameter of 2 to 5 μm [13]. The geometry of small particles should theoretically allow $Ca(OH)_2$ to penetrate the open dentin tubules because the size of the particles are smaller than the size of the dentin tubules [37].

Direct contact of $Ca(OH)_2$ to the dentinal tubular surface can have important clinical implications for root canal treatment, where bacteria as the source of infection are commonly found in the dentinal tubules and protected from disinfection procedures. A high local pH may arise from the penetrating particles acting as a direct source of OH- ions from the dissociated CaP-PEG, with a small probability that the pH will be decreased by dentin buffering. The potential effect of particle size and shape will increase the surface area and the amount of potential reactivity [38]. The higher concentration of fine particles per volume favours the penetration of $Ca(OH)_2$ into the dentinal tubules and provides a faster ions diffusion rate, resulting in an immediate increase of the pH level around the environment in a shorter time.

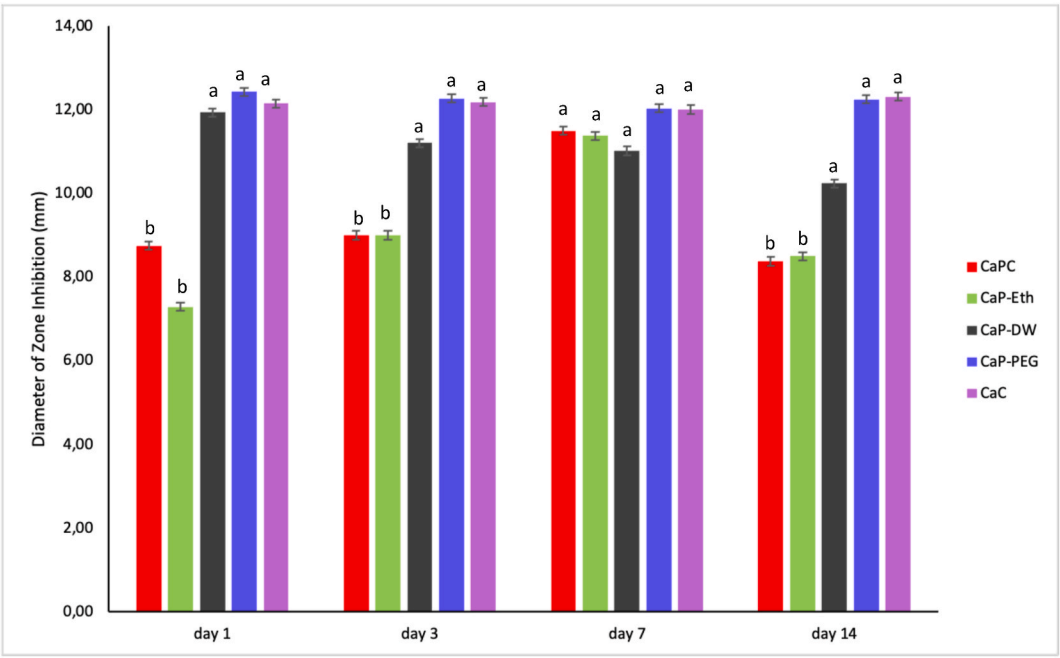

Fig. 8. Diffusion Zone of *E.faecalis* bacteria. *Different letters indicate significant difference between the groups ($p < 0.05$).

5. Conclusion

It was determined that encapsulation of Ca $(OH)_2$ with PEG 400 during milling procedure successfully prevent the carbonation process, supporting stable pH elevation up to 14 days, increasing the antimicrobial activity against *E. faecalis* and increasing penetration into the root canal.

Authors contributions

Atia Nurul Sidiqa conceived, designed or planned the study, analysed the data, and drafted the manuscript.
Myrna Nurlatifah Zakaria analysed the data and helped interpret the results, and drafted the manuscript.
Arief Cahyanto acquired and analysed the data, drafted the manuscript.
I Made Joni conceived, designed or planned the study, analysed the data, drafted the manuscript.
Ani Melani acquired and analysed the data, drafted the manuscript.

Data availability statement

Data will be made available on request.

Ethics approval and consent to participate

The project was approved by Institutional Review by The Research Ethics Committee Universitas Padjadjaran, Bandung. Extracted teeth for the lab experiments approved by the ethics committee with registered No: 956/UN6.KEP/EC/2021.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

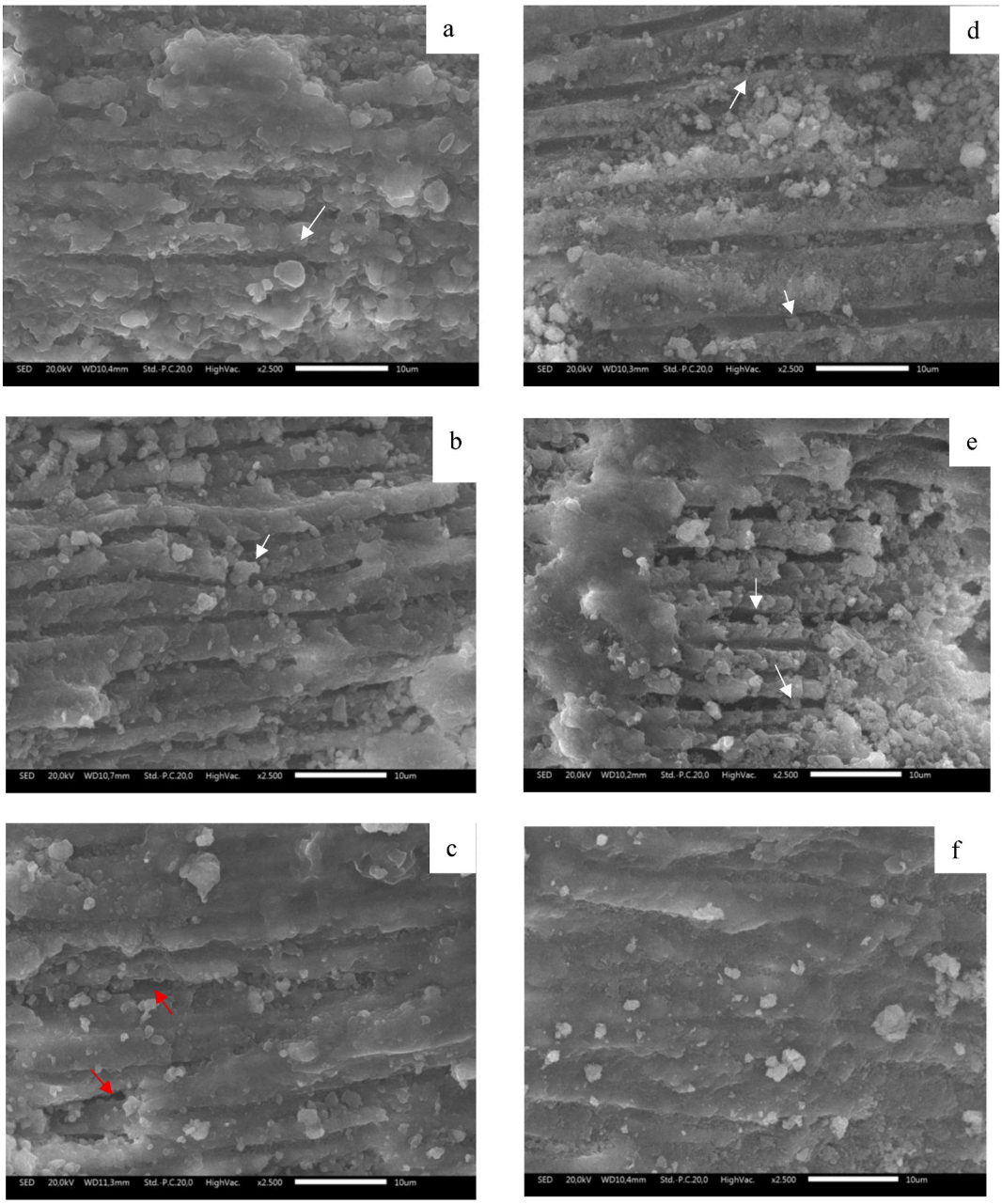

Fig. 9. SEM characterization showing the penetration of the paste into the dentinal tubules in the CaC groups (a,b,c) and in the CaP–PEG groups (d, e,f) coronal (a,d), middle (b,e) and apical thirds (c,f).

Table 2
The mean depth of penetration of the CaP-PEG and CaC.

Group	Particle size (nm)	Level of root	(Mean ± SD) nm
CaP-PEG	363.1 ± 156.9	Coronal	(406.1 ± 37.6)[a]
		Middle	(362.4 ± 68.7)[a]
		Apical	(388.1 ± 44.5)[a]
CaC	2616.7 ± 892.7	Coronal	(296.9 ± 55.8)[b]
		Middle	(209.0 ± 21.6)[c]
		Apical	(190.2 ± 43.2)[c]

*different letters indicate significant difference between the groups ($p < 0.05$).

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