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Research Report

Characterization of a novel calcium phosphate cement–calcium sulfate hemihydrate–acemannan for vital pulp therapy

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ABSTRACT

Background: *Dental caries remains as one of the most prevalent oral diseases worldwide, especially in developing nations like Indonesia. Untreated caries may lead to pulp involvement, prompting vital pulp therapy (VPT) to protect pulp vitality. The success of VPT is dependent on the biological and physical properties of the capping materials. Traditional pulp capping materials, such calcium hydroxide $Ca(OH)_2$ and mineral trioxide aggregate (MTA), show bio-activity but are limited by issues of solubility, manipulation, and bio-compatibility. Consequently, calcium phosphate cement (CPC) and calcium sulfate hemihydrate (CSH) with acemannan (Ace) have been explored as potential alternatives.* ***Purpose:*** *This research aimed to evaluate the potential of a mixture containing CPC-CSH-Ace as a bio-active material for vital pulp therapy.* ***Methods:*** *CPC–CSH–Ace was formulated by combining 70 wt% CPC and 30 wt% CSH with acemannan concentrations of 3 wt%, 5 wt%, and 10 wt%. Calcium hydroxide (Dycal, Dentsply) and MTA (Bio MTA+, Cerkamed) were used as controls. Particle size was measured using a Particle Size Analyzer (Horiba SZ-100), surface morphology and calcium ratio were tested with SEM–EDS, and crystal structure was determined using XRD (Rigaku Miniflex). Data were examined utilizing one-way ANOVA and subsequent post-hoc testing ($p < 0.05$).* ***Results:*** *CPC–CSH–Ace exhibited smaller particle sizes, smoother surfaces, higher Ca/P ratios, and more defined hydroxyapatite peaks than $Ca(OH)_2$ and MTA.* ***Conclusion:*** *The combination of CPC–CSH-Ace showed significant chemical and physical characteristics and has promise as a new bio-active material for vital pulp therapy.*

Keywords: *Acemannan; calcium phosphate cement; calcium sulfate hemihdrate; particle size; pulp capping*

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INTRODUCTION

Dental caries remains one of the most prevalent and persistent oral health concerns, presenting a significant public health risk.[1] The condition is caused by an imbalance in the oral micro-bacteria, which happens when carbohydrates from sugars accumulate on the surface of teeth over time.[2] This situation makes bacteria make more acid, which causes the enamel and dentin to undergo periods of demineralization and remineralization.[1] The World Health Organization (WHO) Global Oral Health Status Report in 2022 reported that 3.5 billion people worldwide experience dental and oral health problems, with 3 out of 4 sufferers living in middle-income countries, while the World Health Organization (WHO) target is a def-t index ≤ 2 significantly in 2020.[3] Untreated caries can progress to the dental pulp, where inflammation serves as the primary defense mechanism against bacterial invasion, trauma, and other irritants.[2]

Vital pulp therapy (VPT) is for preserving the health and vitality of dental pulp with the application of bio-active materials that promote healing and dentin regeneration.[4] Direct pulp capping (DPC), a prevalent VPT treatment, involves the application of a bio-active material directly on exposed pulp tissue to promote reparative dentin development and maintain pulp vitality.[5] The efficacy of DPC is dependent on the bio-compatibility of the material, sealing capability, and bio-activity of the used pulp capping material.[6]

Calcium hydroxide $Ca(OH)_2$ has historically been called the standard for direct pulp capping (DPC) because of its antibacterial characteristics and capacity to stimulate tertiary dentin development.[7] However, it exhibits multiple disadvantages, such as inadequate dentin adaptation, elevated solubility, and possible cytotoxicity.[8] Mineral trioxide aggregate (MTA) became known as a superior alternative, providing enhanced sealing, increased bio-compatibility, and superior bio-activity. However, MTA's lengthened setting time, handling challenges, and susceptibility to discoloration restrict its clinical utility.[6]

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Calcium phosphate cement (CPC) and calcium sulfate hemihydrate (CSH) have recently become of interest as potential choices for pulp capping materials due to their superior bio-compatibility and capacity to generate hydroxyapatite, the principal mineral structure of dentin.[9,10] The combination of CPC and CSH improves mechanical strength, increases setting time, and increases calcium and phosphate ion release, therefore promoting rigid tissue development.[9]

Acemannan, a polysaccharide extracted from aloe vera included CPC–CSH formulations could improve biological performance.[11] Acemannan demonstrates bio-compatibility with diverse mesenchymal cells and facilitates osteogenic differentiation, proliferation, and tissue regeneration.[12] Research has shown that acemannan can expedite reparative dentin production and improve pulp healing. Combination of CPC–CSH-Ace can be claimed to produce a bio-active and bio-compatible material appropriate for vital pulp therapy.[9,13]

There is a limited amount of data on the mechanical properties of CPC–CSH–Ace in comparison to common pulp capping materials. This study wants to evaluate the potential of a mixture containing calcium phosphate cement (CPC) and calcium sulfate hemihydrate (CSH) with acemannan as a bio-active material for vital pulp therapy.

MATERIALS AND METHODS

This experimental study was designed with a post-test-only control group design to determine the particle size, surface morphology of the bio-active material, and the calcium phosphate crystallization ratio in the CPC-CSH combination with acemannan in vitro. These factors influence the potential of pulp capping materials in vital tooth pulp therapy. The research was conducted from June 2025 to July 2025 and took place at the Biomolecular Laboratory, BioCORE, and Labotopia Laboratory. The study analyzed 3 types of samples to determine the particle size, surface morphology of the bio-active material, and the calcium phosphate crystallization ratio. The independent variables in this study are particle size, the surface morphology of the bioactive material, the calcium crystallization ratio, and the material's crystal shape. The dependent variable of this study is CPC-CSH with acemannan (CPC-CSH-Ace).

The experimental material was prepared by combining calcium phosphate cement (CPC) and calcium sulfate hemihydrate (CSH) in a ratio of 70:30 by weight (CPC_7–CSH_3). The mixture was subsequently combined with acemannan powder that had been dissolved in distilled water at concentrations of 3 wt%, 5 wt%, and 10 wt% to produce three formulations: CPC–CSH–Ace 3%, CPC–CSH–Ace 5%, and CPC–CSH–Ace 10%. The mixtures were blended for 1–2 minutes until a homogeneous cement-like phase was achieved. Calcium hydroxide (Dycal, Dentsply, USA) and mineral trioxide aggregate (Bio MTA+, Cerkamed, Poland) were used as control materials. Dycal consisted of a base and catalyst mixed in a 1.17:1 ratio until a paste-like consistency was achieved, while MTA was prepared by mixing 0.14 g of powder with one drop of liquid.

Each material was placed into molds (diameter 10 mm, height 2 mm) on 96-well plates and allowed to set. The specimens were sterilized under ultraviolet light for 15 minutes and subsequently divided into experimental groups for physicochemical characterization. The mean particle size of each material was determined using a dynamic light scattering analyzer (Horiba SZ-100). Approximately 50 mg of each sample was suspended in distilled water, ultrasonicated to prevent aggregation, and analyzed under ambient temperature. The mean diameter and particle size distribution were recorded in micrometers (μm) and nanometers (nm).[13,14]

The surface morphology of the materials was examined using a scanning electron microscope (SEM; Thermo Scientific Quantra 650). The samples were sputter-coated with a thin layer of gold prior to analysis. The elemental composition of each specimen was determined using energy-dispersive spectroscopy (EDS) to quantify calcium (Ca), phosphorus (P), oxygen (O), and sulfur (S) content. The calcium-to-phosphate (Ca/P) ratio was calculated from atomic percentage (At%) data to assess the bio-activity potential of each material.[10,15] The crystalline phases of the materials were characterized using X-ray diffraction (XRD; Rigaku Miniflex). The diffraction patterns were recorded between 2θ (°) and I (cps). The diffraction peaks were compared with standard hydroxyapatite reference data to identify the crystalline phases present in each group.[16]

Data normality was evaluated using the Shapiro–Wilk test, while homogeneity was assessed using Levene's test. One-way ANOVA followed by post-hoc analysis was used to compare mean differences among the groups, with a significance level of $p < 0.05$.

RESULTS

The particle size analysis showed CPC–CSH–Ace formulations exhibited smaller and more uniform particle sizes compared to calcium hydroxide and MTA. In the experimental groups, the CPC–CSH–Ace 5% formulation produced the smallest average particle diameter, indicating improved dispersion and uniformity. The results demonstrated that the addition of acemannan influenced particle aggregation and homogeneity, enhancing the overall consistency of the cement matrix.

Particle size analysis of the five tested materials showed significant differences among groups ($p < 0.05$) as shown on Table 1. The mean particle size of $Ca(OH)_2$ (696.7–1007.7 nm) was smaller than MTA (1134.3–1976.4 nm), CPC–CSH–Ace 3% (2130.7–2771.5 nm), CPC–CSH–Ace 5% (1007.7–1613.8 nm), and CPC–CSH–Ace 10% (1329.2–1625.6 nm). Data were normally distributed (Shapiro–Wilk, $p < 0.05$) and homogeneous (Levene's test, $p > 0.05$). One-way ANOVA revealed significant differences in particle size among the materials ($p < 0.05$), with CPC–CSH–Ace 3% showing the largest mean particle size

(2493.4 ± 328.7 nm) as shown on Table 2. Post-hoc Tukey analysis indicated significant differences between $Ca(OH)_2$ and CPC–CSH–Ace 3%, and between CPC–CSH–Ace 3% and CPC–CSH–Ace 5% ($p < 0.05$) as shown on Table 3.

Scanning Electron Microscopy (SEM) revealed notable morphological variations among the tested materials. Crystalline deposits were observed on all specimen surfaces after hydration. The micrographs showed relatively smooth crystal structures resembling plate-like formations in the central regions, indicating crystal growth. Surrounding areas displayed more granular and coarse textures, signifying regions of partially reacted or incompletely hydrated particles. In contrast, other areas consisted of fine, spherical particles of varying sizes.

Ca(OH)2 shows relatively homogeneous irregular small grain-shaped particle aggregates with hexagonal and trigonal shapes. EDS analysis shows calcium particles (33.4 Wt%), phosphorus (7.1 Wt%) and oxide (59.5 Wt%). Mineral Trioxide Aggregate (MTA) shows relatively homogeneous spherical particle aggregates with monoclinic shapes. EDS analysis shows calcium particles (39.8 Wt%), phosphorus (1.9 Wt%) and oxide (58.3 Wt%).

CPC-CSH-Ace 3% showed granular particle aggregates with a mixture of relatively larger particles and relatively smaller particles with hexagonal and monoclinic shapes. EDS analysis showed calcium particles (32.4 Wt%), phosphorus (13.2 Wt%) and oxide (54.4 Wt%). CPC-CSH-Ace 5% showed relatively homogeneous spherical particle aggregates with smooth surfaces with orthorhombic and hexagonal shapes. EDS analysis showed calcium particles (34.8 Wt%), phosphorus (10.8 Wt%) and oxide (54.4 Wt%). CPC-CSH-Ace 10% showed relatively homogeneous spherical particle aggregates that were relatively large with monoclinic and hexagonal shapes. EDS analysis showed calcium particles (36.6 Wt%), phosphorus (13.1 Wt%) and oxide (50.3 Wt%).

The XRD analysis provided a detailed description of the crystalline phases present in all tested materials as shown on Figure 1. Calcium hydroxide ($Ca(OH)_2$) exhibited diffraction peaks at 18.10°, 18.64°, 28.76°, 31.80°, 34.12°,

Table 1. Particle size analysis (PSA) test results

Material	PSA Test		
	1	2	3
Ca(OH)2	1007.7 nm	696.7. nm	832.4 nm
MTA	1134.3 nm	1976.4 nm	1532.5 nm
CPC-CSH-Ace 3%	2130.7 nm	2771.5 nm	2578.6 nm
CPC-CSH-Ace 5%	1613.8 nm	1007.7 nm	1138.6 nm
CPC-CSH-Ace 10%	1329.2 nm	1468.4 nm	1625.6 nm

Table 2. One-way ANOVA analysis results for particle size

Particle *size analysis*					P Value
(CPC7+CSH3) + Ace			$Ca(OH)_2$	MTA	
3%	5%	10%			
2493.4±328.7	1253.4±318.9	1474.4±149.2	845.6±155.9	1314.4±313.5	<.001*

Table 3. Tukey's post-hoc test for particle size

Particle *size analysis*			(CPC7+CSH3) + Ace			$Ca(OH)_2$	MTA
			3%	5%	10%		
(CPC7+CSH3) + Ace	3%	Mean difference	-	1240.2	1019.2	1648.0	1079.2
		P Value	-	.005	.017	.001	.012
	5%	Mean difference		-	-221.0	407.7	-161.0
		P Value		-	.904	.534	.967
	10%	Mean difference			-	628.8	60.0
		P Value			-	.178	.999
$Ca(OH)_2$		Mean difference				-	-568.8
		P Value				-	0.248
MTA		Mean difference					-
		P Value					-

Table 4. XRD results of bioactive material content

Material	$Ca(OH)_2$ Wt%	MTA Wt%	CPC-CSH-Ace 3% Wt%	CPC-CSH-Ace 5% Wt%	CPC-CSH-Ace 10% Wt%
Calcium	43.4	16.6	35.1	30.3	35.6
Phosphorus	14.0	12.5	12.2	15	16
Oxide	41.8	36.7	46.1	47	40.5
Other elements	0.8	34.2	6.6	7.7	7.5

34.44°, 36.30°, and 47.10°, corresponding to calcium tris-phosphate hydroxide and calcium hydroxide phases. Mineral trioxide aggregate (MTA) showed diffraction peaks at 26.82°, 27.20°, 28.12°, 29.44°, 31.56°, 32.52°, 34.30°, and 41.28°, indicating the presence of fluorarrojadite (KFe) and tricalcium silicate crystal reflections.

The CPC–CSH–Ace 3% demonstrated peaks at 11.72°, 20.76°, 26.00°, 29.16°, 31.80°, 32.28°, 32.86°, and 34.18°, corresponding to hydroxyapatite and calcium sulfate dihydrate phases. For CPC–CSH–Ace 5%, diffraction peaks appeared at 11.66°, 20.78°, 25.88°, 29.20°, 31.10°, 31.68°, 32.16°, and 32.86°, which were associated with ortho-phosphate, carbonate hydroxyapatite, calcium sulfate dihydrate (gypsum), and calcium tris-phosphate hydroxide. The CPC–CSH–Ace 10% group showed distinct peaks at 11.66°, 20.66°, 23.88°, 29.20°, 31.80°, 32.28°, 32.98°, and

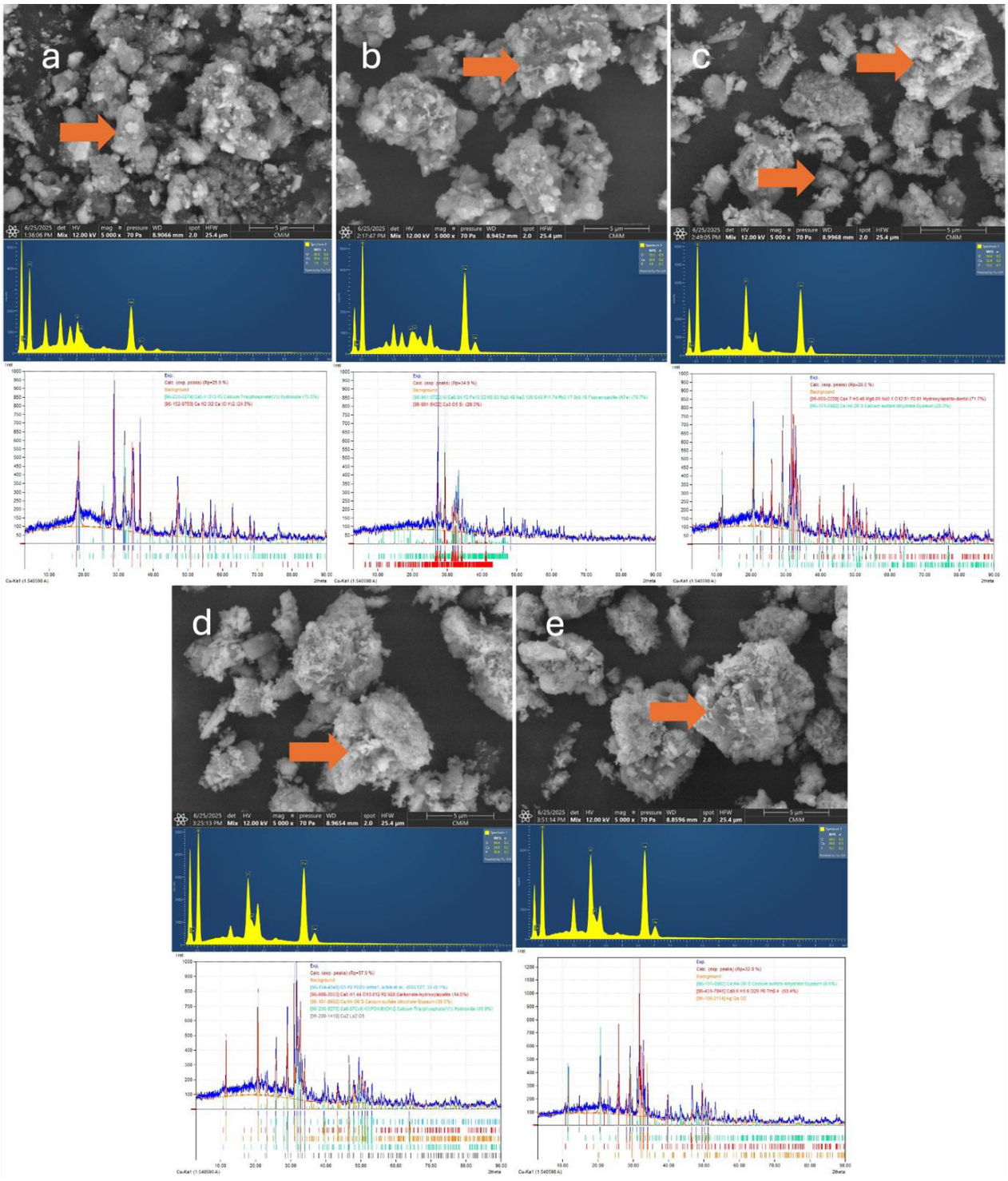

Figure 1. The sample results (a). SEM, EDS and XRD $Ca(OH)_2$, (b). SEM, EDS and XRD MTA, (c). SEM, EDS and XRD CPC-CSH-Ace 3%, (d) SEM, EDS and XRD CPC-CSH-Ace 5%, (e). SEM, EDS and XRD CPC-CSH-Ace 10%.

49.58°, confirming the formation of hydroxyapatite and calcium sulfate dihydrate.

XRD analysis confirmed that the CPC–CSH–Ace exhibited distinct diffraction peaks corresponding to hydroxyapatite and calcium phosphate phases, indicating successful formation of bio-active crystalline structures. The intensity of hydroxyapatite peaks increased with higher acemannan concentrations, suggesting enhanced crystallinity. In contrast, calcium hydroxide and MTA showed lower-intensity peaks and less-defined patterns, reflecting limited crystalline organization.

Quantitative element analysis revealed the following calcium concentrations: $Ca(OH)_2$ (43.4%) > CPC–CSH–Ace 10% (35.6%) > CPC–CSH–Ace 3% (35.1%) > CPC–CSH–Ace 5% (30.3%) > MTA (16.6%). The CPC–CSH–Ace 10% also exhibited higher phosphorus content compared to the other groups. The presence of hydroxyapatite and calcium sulfate phases indicates that acemannan incorporation enhances mineralization potential and crystalline stability, supporting its role as a bioactive pulp capping material.

DISCUSSION

The present study showed that the potential of acemannan with CPC–CSH significantly improved the physicochemical characteristics relevant to pulp capping applications. The reduction in particle size observed across all experimental groups suggests enhanced dispersion and material uniformity, which are critical for achieving intimate adaptation to dentin and effective sealing against bacterial ingress.[17] Smaller particles can penetrate dentinal tubules more effectively, potentially promoting remineralization and reparative dentin formation.[18] These findings are consistent with previous studies reporting that finer particle distributions enhance cell adhesion, proliferation, and differentiation within dentin-pulp complex environments.[19]

Vital pulp therapy (VPT) aims to maintain the vitality of pulp tissue after exposure to caries or trauma.[5] Direct pulp capping (DPC) requires a bio-active material that is bio-compatible, anti-bacterial, anti-inflammatory, and capable of stimulating dentin mineralization and pulp regeneration.[7] Common materials such as calcium hydroxide ($Ca(OH)_2$) and mineral trioxide aggregate (MTA) are effective but have limitations, including solubility and handling difficulties.[4,20] Therefore, bio-active materials such as calcium phosphate cement (CPC), calcium sulfate hemihydrate (CSH), and acemannan have been developed to overcome these weaknesses.[9,12] In this study, CPC–CSH combined with acemannan (CPC–CSH–Ace) was compared with $Ca(OH)_2$ and MTA.

The CPC–CSH–Ace are promising physicochemical characteristics that support their potential as vital pulp therapy materials. The particle size of CPC–CSH–Ace 3% (2.1–2.7 µm) was closest to the dentinal tubule diameter (3–4 µm), which may improve mechanical bonding and facilitate ion exchange during remineralization.[20,21] Although $Ca(OH)_2$ had the smallest particles (0.6 µm), its high solubility and tunnel defect formation reduce its ability to seal dentinal tubules effectively. [19–21]

SEM images showed that $Ca(OH)_2$ had small, irregular particles forming dense clusters, while MTA showed a smooth plate-like structure surrounded by a granular matrix typical of hydrated calcium silicate. In contrast, CPC–CSH–Ace (3%, 5%, 10%) exhibited larger and denser particles with hydroxyapatite and calcium sulfate dihydrate crystals, suggesting stronger inter-particle bonding and improved mechanical properties due to acemannan incorporation. EDS analysis revealed that CPC–CSH–Ace 10% had a calcium ratio (36.6 wt%) close to that of MTA (39.8 wt%). The presence of calcium, phosphate, and oxygen ions indicates good bio-activity, as these ions are essential for reparative dentin formation and pulp healing.[10,22] This micro-structural organization is beneficial for maintaining material stability and providing a conducive substrate for cellular attachment. The higher calcium and phosphate content, as reflected in the EDS results, further supports the bio-activity of the composite, as these ions are essential for initiating dentin bridge formation and tissue regeneration.[21]

XRD analysis showed that CPC–CSH–Ace contained hydroxyapatite and calcium sulfate dihydrate crystals, with main peaks around 11.7°, 20.7°, 26.0°, 29.1°, 31.8°, 32.2°, and 34.1°. Increasing acemannan concentration enhanced hydroxyapatite peak intensity, indicating better crystallinity and bio-active potential. The enhanced crystallinity observed in the XRD analysis of CPC–CSH–Ace formulations suggests the formation of stable hydroxyapatite phases, which are crucial for long-term bio-compatibility and integration with surrounding dentin tissue.[11,12] The presence of acemannan likely contributed to the regulation of crystal growth, resulting in improved mineral phase organization. Acemannan's known biological properties including stimulation of fibroblast proliferation, collagen synthesis, and osteogenic differentiation further support its potential role in promoting reparative dentinogenesis when combined with bio-active cement matrices.[9,13]

The CPC–CSH–Ace showed superior particle uniformity, smoother morphology, and higher calcium-to-phosphate ratios compared to common pulp capping materials like calcium hydroxide and MTA. While $Ca(OH)_2$ remains widely used for its anti-bacterial effects, its high solubility even though cytotoxic potential limit long-term performance. Long setting times and difficult manipulation hinder MTA, despite its bio-compatibility.[6] The current results indicate that CPC–CSH–Ace addresses several of these constraints by integrating favorable handling properties with increased bio-activity.

CPC–CSH–Ace showed particle sizes close to dentinal tubules, high calcium and phosphate content, and hydroxyapatite formation was a key feature for successful pulp healing. The addition of acemannan improved structure, ion release, and potential for dentin remineralization. Among all tested materials, CPC–CSH–Ace 10% showed the most favorable balance of structural and bio-active properties, suggesting it could serve as a promising alternative to MTA and $Ca(OH)_2$ for direct pulp capping.

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The limitation of this study is to provide the results of the characteristics of the material regarding dentin attachment with CPC-CSH-Ace material, further chemical reactions of the CPC-CSH-Ace combination and the bond between CPC-CSH-Ace material and dental restoration materials. Therefore, further research needs to be carried out using additional parameters such as Fourier Transform Infrared (FITR), research considering setting time, in vitro research to see anti-bacterial, pH test, cell viability, and cytotoxicity, as well as in vivo research to confirm the physiological conditions of the pulp and the formation of reparative dentin histologically.

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