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# Developing an Injectable Calcium Hydroxide/ Hydroxyapatite Cement as a New Potential Material For Tooth Tissue Regeneration

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## ABSTRACT

**Background:** In this study, the physical properties of a calcium hydroxide/hydroxyapatite (CH/HA) cement were investigated as a novel bioactive material for the repair of bone and dental defects.

**Methods:** The powder phase, consisting of varying proportions of CH and HA, was mixed with glycol disalicylate as the liquid phase. The setting reaction produced an amorphous matrix containing dispersed HA particles adjacent to unreacted CH particles, as confirmed by X-ray diffraction (XRD) and scanning electron microscopy (SEM) analyses. Elemental phosphorus mapping from X-ray analysis of SEM images further confirmed the presence and distribution of HA particles within the cement matrix.

**Results:** The cements exhibited setting times ranging from 2.9±0.5 to 7.8±0.8 min, depending on composition. Injectability increased from 68±3% to 86±6% as HA content increased, with no evidence of filter pressing or phase separation. After 24 h of setting, compressive strengths ranged from 55±2.6 to 62.3±2.8 MPa, which are higher than those typically reported for conventional calcium phosphate cements (CPCs). After 3 days of immersion in distilled water, the compressive strength decreased to 43.0–49.2 MPa due to partial matrix dissolution. The cements exhibited controlled calcium ion release over 28 days, with higher release observed in formulations containing greater amounts of CH. Furthermore, the pH of cement suspensions increased rapidly during the first 30 min and then stabilized in the alkaline range. Water solubility decreased from 6.85±0.12% to 2.95±0.12% with increasing hydroxyapatite content.

**Conclusion:** The developed CH/HA cement has promising mechanical and handling properties for potential biomedical applications in bone and dental tissue repair.

**Keywords:** Bone filler, Hydroxyapatite, Calcium hydroxide, Dental cement

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## Highlights

- Calcium phosphate bioceramics, such as HA, are widely used in medical and dental applications but they have limitations.
- CPCs have shown considerable potential for hard tissue repair and regeneration, but they have low mechanical strength, which restricts their use.
- In this study, a CH/HA cement was developed as a novel bioactive material for the repair of bone and dental defects.
- The results confirmed the mechanical and handling properties of the CH/HA cement for potential biomedical applications in bone and dental tissue repair

## Plain Language Summary

Calcium phosphate bioceramics are widely used in medical and dental applications; however, they have several limitations. To overcome their limitations, calcium phosphate cements (CPCs) have been developed for hard tissue repair and regeneration, but their low mechanical strength restricts their use. Considering the favorable biological properties of calcium hydroxide (CH) and the excellent bioactivity and osteoconductivity of hydroxyapatite (HA), in this study, an attempt was made to develop a novel CH/HA composite cement in which CH acts both as a reactive setting component and a source of biologically active calcium and hydroxyl ions, while HA serves as an osteoconductive and bioactive filler. The developed material utilizes a salicylate ester-based setting system, resulting in rapid setting, excellent injectability, and relatively high compressive strength. According to the results, the CH/HA composite cement has promising mechanical and handling properties for potential biomedical applications in bone and dental tissue repair.

## Introduction

Due to close chemical composition and structural resemblance to the mineral phase of hard tissues, calcium phosphate bioceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) are widely used in medical and dental applications [1, 2]. These biomaterials are commonly fabricated as granules or blocks; however, these forms have several limitations, including poor adaptability to irregular skeletal defects, inadequate structural integrity of granules, and difficulty shaping the implant to match the geometry of the damaged tissue [3]. To overcome these limitations, there has been growing interest in developing injectable, bioactive, and self-setting materials, particularly for minimally invasive procedures and defects in difficult-to-access sites. Calcium phosphate cements (CPCs), first introduced by Brown and Chow [4], are an important class of such materials. Since their introduction, extensive research has been conducted on various CPC formulations and systems [5–8]. CPCs have shown considerable potential for hard tissue repair and regeneration, including craniofacial reconstruction and frontal sinus augmentation [9–12], endodontic applications [13, 14], pulp capping [15], root canal treatments, and the repair of periodontal and dental defects [16, 17]. Despite these advantages, the major drawback of CPCs is their relatively low mechanical strength immediately after setting, which restricts their use primarily to non-load-bearing applications [18, 19].

Calcium hydroxide (CH)-based materials are another important group of bioactive dental materials widely used for pulp capping, cavity liners, bases, and other endodontic treatments [20, 21]. These materials are known for their ability to stimulate reparative dentin formation and provide antibacterial activity against cariogenic microorganisms [22]. Conventional CH cements generally consist of CH and other fillers, combined with salicylic acid esters, resulting in calcium phenolate matrices containing dispersed excess calcium hydroxide [23]. The clinical effectiveness of these materials is mainly attributed to the ionic dissociation of unreacted calcium hydroxide into calcium and hydroxyl ions [24].

Considering the favorable biological properties of CH and the excellent bioactivity and osteoconductivity of HA, it may be possible to develop a novel injectable self-setting composite with enhanced clinical performance [25]. Therefore, in the present study, different mixtures of CH and HA powders were combined with a

salicylate ester liquid to produce injectable self-setting materials. The effects of HA filler content on the physical and mechanical properties of the prepared composites were systematically investigated. Although calcium phosphate cements and CH-based dental materials have been extensively investigated independently, studies combining these two bioactive systems into a single injectable and self-setting composite remain very limited. The present work introduces a novel calcium hydroxide/hydroxyapatite (CH/HA) composite cement in which CH acts both as a reactive setting component and a source of biologically active calcium and hydroxyl ions, while HA serves as an osteoconductive and bioactive filler. Unlike conventional calcium phosphate cements that rely on aqueous setting reactions and often suffer from limited injectability and low early mechanical strength, the developed material utilizes a salicylate ester-based setting system, resulting in rapid setting, excellent injectability, and relatively high compressive strength. Therefore, this study provides a new strategy for developing injectable bioactive cements for both bone and dental tissue regeneration.

## Materials and Methods

The liquid phase of the cement consisted of a mixture of methyl salicylate and glycol salicylate prepared through a transesterification process described previously [26]. Briefly, 4.20 equivalents of 1,3-butylene glycol were reacted with 4.41 equivalents of methyl salicylate under reflux at 110–210 ˚C for 24 h, during which approximately 160 mL of methanol was collected as a by-product. Sodium methylate was used as the catalyst for the synthesis of the salicylate ester.

The powder phase consisted of a homogenized mixture of calcium hydroxide (Merck, 102047) and 50–80 wt% precipitated HA (Merck, 102143). CH, with an average particle size of 2 µm, served as the reactive component, while HA, with an average particle size of 10 µm, was used as a bioactive, osteoconductive, and bone-compatible filler. The cement paste was prepared by mixing the powder phase (P) with the liquid phase (L) at a powder-to-liquid (P/L) ratio of 1:1.

The X-ray diffraction (XRD) patterns of the powdered and dried samples were obtained using a diffractometer system (Philips PW 3710) equipped with Ni-filtered Cu-Kα radiation generated at 30 kV and 10 mA. Data were collected at a scanning rate of 1° (2θ) $min^{-1}$. The external and fractured surfaces of the set cements were characterized morphologically using a scanning electron microscope (SEM; JXA-840, JEOL Co., Japan). In addition, elemental mapping of phosphorus, corresponding to the SEM images of the external surfaces, was performed to evaluate the presence and distribution of HA particles within the cement matrix. The final setting times of the cements were determined in accordance with the ASTM C266-89 standard. According to this standard, the cement is considered set when a needle with a tip diameter of 1.06 mm, loaded with a 453.6 g weight, fails to produce a perceptible circular indentation on the cement surface. Five specimens were evaluated for each composition.

Injectability was evaluated by extruding a predetermined amount of cement paste through a disposable syringe using a modified method previously described [27]. The syringes had a capacity of 10 mL and a nozzle diameter of 2.0 mm. For each test, 8 g of paste was loaded into the syringe, and injectability was defined as the percentage by weight of the paste that could be manually extruded through the syringe. The tests were performed in a water bath, and all measurements were repeated three times to ensure reproducibility.

The mechanical properties of the set CH/HA composite cements were evaluated in terms of compressive strength. Measurements were performed at two time points: 24 h after incubation and 3 days after soaking in distilled water. For specimen preparation, the cement paste was placed into Teflon molds measuring 6 mm in diameter and 12 mm in height. The molds were then incubated at 37 ˚C under 100% relative humidity. After 24 h, the specimens were removed from the molds. A portion of the samples was tested immediately, while the remaining specimens were immersed in distilled water and tested after 3 days. Compressive strength measurements were carried out using an Instron universal testing machine at a crosshead speed of 1 mm $min^{-1}$. Six specimens were tested for each composition.

The water solubility test was conducted to evaluate the cement's resorption behavior. In this experiment, disc-shaped specimens were immersed in 50 mL of distilled water. The concentration of released calcium ions ($Ca^{2+}$) in the solution was measured after 1, 3, 7, 14, and 28 days using inductively coupled plasma (ICP) analysis. To maintain sink conditions, the immersion solution was refreshed every 24 h throughout the evaluation period. In addition, the mass loss of the cement specimens was determined at the end of the 28-day period as an additional indicator of water solubility.

For pH measurements, 2 mg of ground and sieved set cement (passed through a 325-mesh sieve) from each composition was separately suspended in 50 mL of distilled water. The pH variation of the cement slurry was monitored over time using a pH/Ion Analyzer (MA 235, Mettler-Toledo, Switzerland).

Human dental pulp stem cells (hDPSCs) were cultured in Dulbecco's modified eagle medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin under standard culture conditions (37 ˚C, 5% $CO_2$). Cement extracts were prepared according to ISO 10993-12. Briefly, the set cement specimens (50/50, 40/60, 30/70 and 20/80 CH/HA ratios) were immersed in complete culture medium at a surface area-to-volume ratio of 3 $cm^2$/mL for 24 h at 37 ˚C. The extracts were collected and filtered through a 0.22 µm membrane filter. hDPSCs were seeded into 96-well plates at a density of $1\times10^4$ cells/well and incubated for 24 h. The culture medium was then replaced with cement extracts. After 1, 3, and 7 days of incubation, 20 µL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) solution (5 mg/mL in phosphate-buffered saline) was added to each well and incubated for 4 h. Subsequently, the medium was removed, and the resulting formazan crystals were dissolved in 150 µL dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader. A control group consisting of hDPSCs cultured in complete growth medium without cement extract was included in all experiments. Cell viability was expressed in percentage. Cell viability in the control group was defined as 100%, and the viability in the experimental groups was expressed relative to the control group. All experiments were performed in triplicate.

To evaluate odontogenic/osteogenic differentiation, hDPSCs were seeded in 24-well plates at a density of $5\times10^4$ cells/well and cultured in the presence of cement extracts prepared as described above. After 1, 3, and 7 days of culture, the cells were washed with phosphate-buffered saline and lysed using cell lysis buffer. Alkaline phosphatase (ALP) activity was quantified using a commercial ALP assay kit based on the conversion of p-nitrophenyl phosphate to p-nitrophenol. The absorbance was measured at 405 nm using a microplate reader. The obtained ALP values were normalized to the total protein content determined using a BCA protein assay kit and expressed as U/mg protein. A control group containing hDPSCs cultured in complete medium without exposure to cement extracts was also evaluated. ALP activity of all experimental groups was compared with that of the control cells cultured under identical conditions. All measurements were performed in triplicate.

## Results

Figure 1 presents the XRD patterns of the set cements containing different amounts of HA filler. In the set cement containing 50 (weight percent) wt% CH in the powder phase, characteristic CH peaks were observed alongside those of HA. As the proportion of CH in the powder phase decreased, the intensity of the CH peaks gradually diminished. No detectable CH peaks were observed in the XRD pattern of the cement containing 20 wt% CH and 80 wt% HA.

These results suggest that most of the CH participated in the setting reaction, thereby forming a calcium phenolate matrix, while HA primarily acted as an inert filler. Consequently, the final structure consisted of an amorphous calcium phenolate matrix with dispersed HA particles.

Figures 2a and 2b present SEM micrographs of the fractured cross-section of set cements prepared with a powder phase consisting of 50 wt% CH and 50 wt% HA. Large pores observed on the fractured surfaces are attributed to air bubbles entrapped within the cement paste during mixing. Fine CH and HA particles were found to be dispersed and partially agglomerated within the amorphous matrix. Figure 2c shows the corresponding X-ray elemental map of phosphorus overlaid on the SEM image of the external surface. The bright regions show the distribution of phosphorus, indicating the presence of HA particles. This confirms the incorporation and homogeneous distribution of the bioactive filler within the cement surface, which may contribute to the material's bioactivity.

The results of setting time, injectability, compressive strength, and water solubility are summarized in Table 1. The cements exhibited relatively rapid setting behavior, and a reduction in setting time was observed as the CH content in the powder phase decreased. The self-setting property of the cement is attributed to the reaction between CH and the salicylate ester, which forms an amorphous calcium phenolate matrix. In preliminary experiments, cement formulations containing only HA did not exhibit any setting behavior, indicating that HA does not participate in the reaction with the salicylate ester. Accordingly, partial substitution of CH with HA reduced the amount of reactive CH available for chelate formation, which would be expected to delay setting. However, an overall decrease in setting time was observed as HA content in the cement powder increased, suggesting that HA may indirectly influence setting kinetics, possibly by modifying packing, microstructure, or ion diffusion within the system.

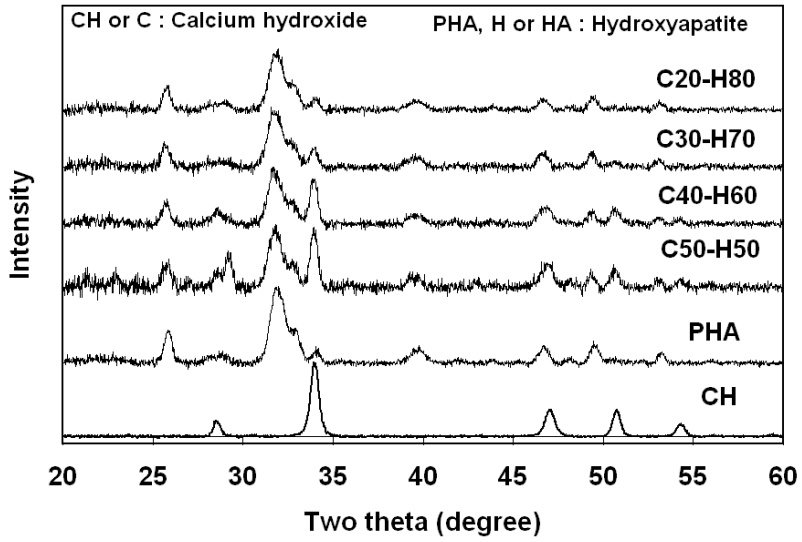


**Figure 1.** Powder XRD patterns of set CH/HA cements with different ratios of CH to HA in the cement powder phase

Note: The diffraction patterns of pure CH and precipitated HA were included for comparison.

The injectability of CH/HA cements increased by increasing the HA content in the cement composition. In conventional CPCs, which typically consist of calcium phosphate powders and water as the liquid phase, extrusion through a wide-bore needle may induce a filter-pressing effect, leading to phase separation between the liquid and solid components [6]. Therefore, adequate paste cohesion is essential to prevent this phenomenon.

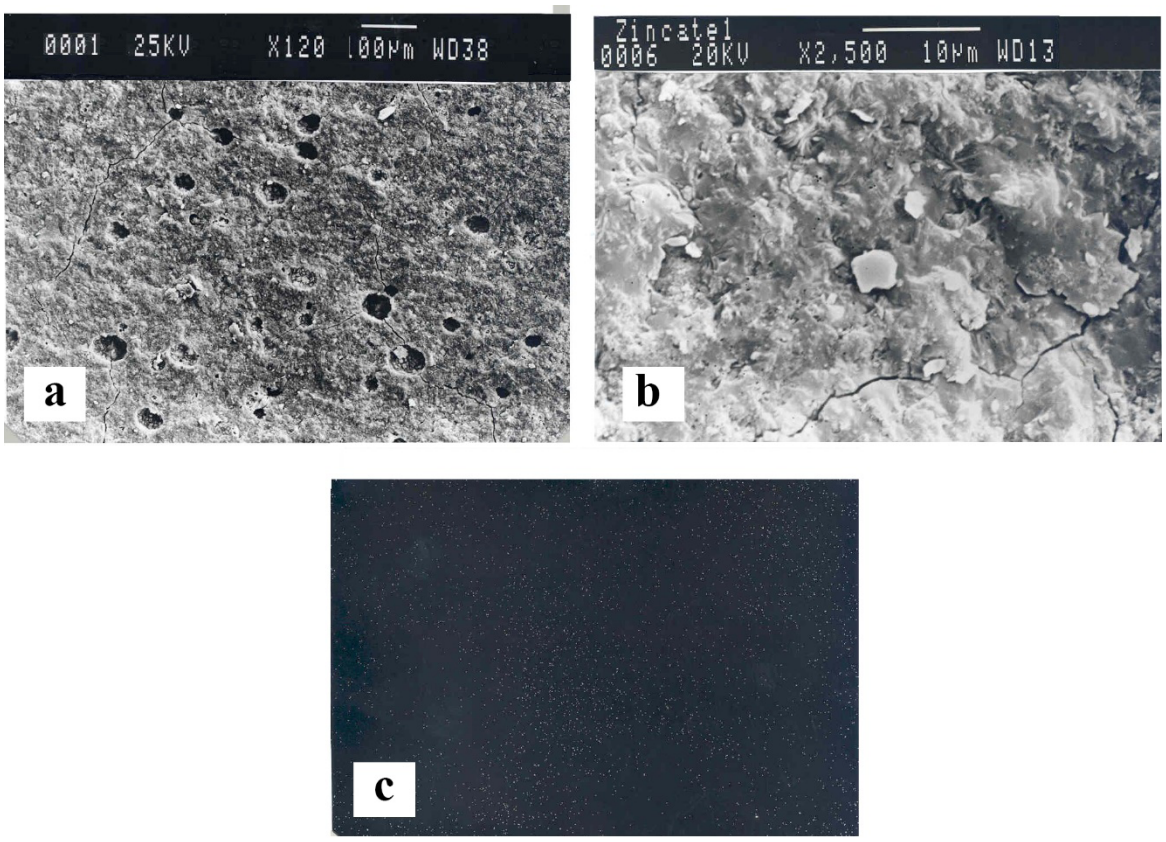


**Figure 2.** The SEM images of the fractured surface of the set CH/HA cement (a, b), and the X-ray elemental map of phosphorus corresponding to the SEM image of the external surface of the cement (c)

**Table 1.** Variations in the basic properties of CH/HA cements with different CH to HA ratios in the powder phase

| Properties | Ratio of CH to HA in Powder Phase | | | |
|---|---|---|---|---|
| | 50/50 | 40/60 | 30/70 | 20/80 |
| Setting time (min) | 2.9±0.5 | 3.7±0.6 | 5.3±1.1 | 7.8±0.8 |
| Injectability (%) | 68±3 | 77±4 | 85±4 | 86±6 |
| Compressive strength at 24 h after incubation (MPa) | 62.3±2.8 | 57.9±2.4 | 59.2±2.7 | 55±2.6 |
| Compressive strength at 3 days after soaking (MPa) | 49.2±2.3 | 45.5±2.1 | 42.2±3.1 | 43±3.3 |
| Water solubility (mass loss %) | 6.85±0.12 | 4.8±0.15 | 3.4±0.13 | 2.95±0.12 |

In the present study, no demixing or filter pressing was observed during the extrusion of the cement pastes from the syringe. The formulations maintained good cohesion and exhibited improved injectability compared with conventional CPCs. Although injectability in the present work was evaluated based on the percentage of paste extruded from the syringe, the obtained results clearly demonstrated the excellent extrusion capability of the developed formulations. Future studies can incorporate quantitative rheological characterization and force–displacement measurements to provide a more comprehensive evaluation of injection behavior and extrusion resistance.

These cements exhibited a relatively higher compressive strength than previously reported CPCs [28–31] after 24 h of setting. However, a reduction in compressive strength was observed after 3 days of immersion in distilled water. This decrease can be attributed to partial dissolution of the cement matrix, leading to the formation of micropores within the microstructure. No significant differences in compressive strength were observed among samples with different compositions. The water solubility of the developed cements, expressed as weight loss after 28 days of storage in distilled water, decreased with increasing HA content in the cement formulation.

Figure 3 illustrates the changes in calcium ion ($Ca^{2+}$) concentration released from the cement samples into the aqueous medium over a 28-day soaking period. In general, calcium ion concentration increased with increasing soaking time across all compositions. This indicates a progressive ion exchange and partial dissolution of the cement matrix over time.

Among the different formulations, the amount of released $Ca^{2+}$ was strongly dependent on the CH content in the powder phase. Samples containing a higher pro-

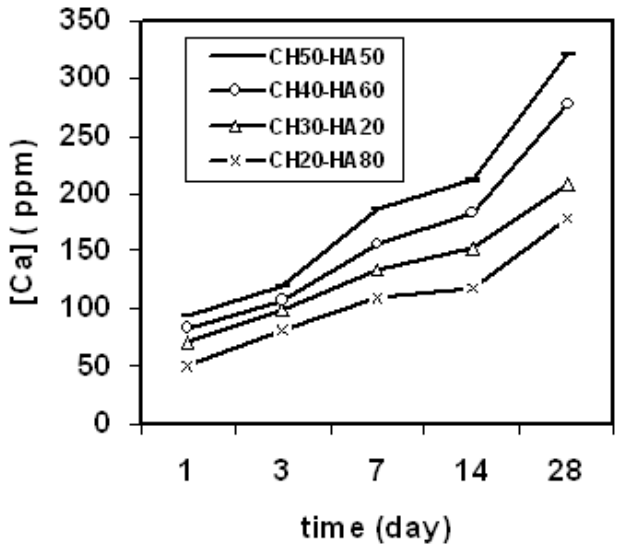


**Figure 3.** Variation in calcium ion ($Ca^{2+}$) concentration released from CH/HA cements with different powder phase compositions over time

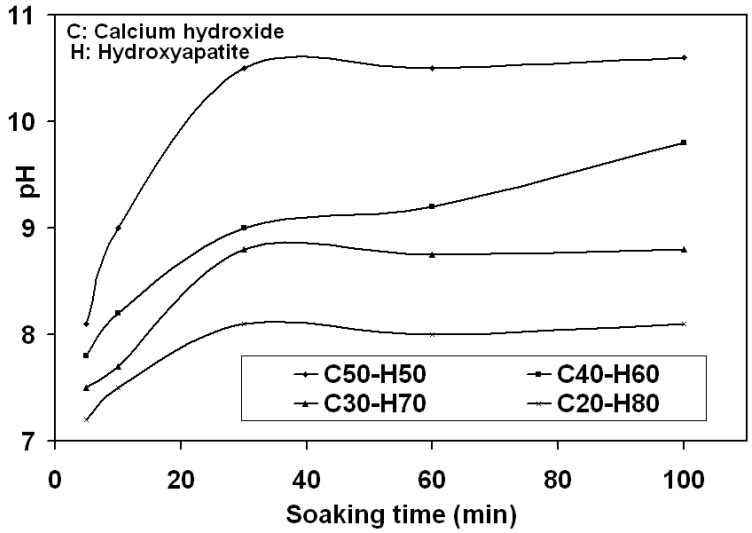


**Figure 4.** Plot of pH variation across different soaking times for CH/HA cements containing different ratios of CH to HA in the powder phase

portion of CH exhibited a significantly greater calcium ion release throughout the entire immersion period. Conversely, formulations with higher HA content showed lower $Ca^{2+}$ release levels.

This behavior can be attributed to the higher solubility and reactivity of CH compared to HA. The CH readily dissociates into $Ca^{2+}$ and $OH^-$ ions upon contact with aqueous media, contributing to sustained ion release. In contrast, HA is relatively stable and less soluble under physiological conditions, acting mainly as a bioactive but less reactive filler phase.

The sustained release of calcium ions is particularly important for bioactivity, as $Ca^{2+}$ plays a key role in promoting mineralization, cell signaling, and tissue regeneration. Therefore, the higher calcium release observed in CH-rich formulations may enhance biological performance, although an optimal balance between ion release and structural stability is required for clinical applications.

Figure 4 presents the variation in pH over time for slurries containing different CH/HA cement compositions. In all formulations, an initial increase in pH was observed during the first 30 min, followed by a stabilization phase in which the pH remained nearly constant.

The water solubility, pH evolution, and biological performance of the cements can be influenced by the amount of unreacted CH present in the formulation. The dissociation of unreacted CH into calcium and hydroxyl ions is primarily responsible for the antibacterial activity and tissue interactions of these materials, thereby contributing to their clinical effectiveness [24]. In addition, the antibacterial effect may be associated with the induction and upregulation of odontoblast-like cell differentiation, promoting new matrix formation, as well as the release of growth factors from the dentin matrix [32]. Furthermore, the low crystallinity of HA particles, together with the esteric and hydrolytic nature of the bonds within the amorphous calcium phenolate matrix, may enhance the cement's resorption behavior. In vivo, the resorption rate is expected to be further accelerated by the activity of osteoclasts, macrophages, and other biological factors [33].

The cytocompatibility of the CH/HA cements was evaluated using hDPSCs cultured in cement extracts for 1, 3, and 7 days. The results demonstrated that all cement formulations maintained cell viability above 80% throughout the experimental period, indicating acceptable cytocompatibility according to ISO 10993-5 criteria.

As shown in Figure 5a, cell viability increased with increasing culture time across all groups. On day 1, the CH50/HA50 formulation exhibited the lowest viability (82±4%), whereas the CH20/HA80 group showed the highest value (95±3%). The slightly reduced viability observed in formulations with higher CH content may be attributed to the initial alkaline environment induced by the release of hydroxyl ions. After 3 days of incubation, a significant increase in cell viability was observed across all groups, ranging from 88±5% to 108±4%. The improvement in cell proliferation may be associated with the continuous release of calcium ions from the ce-

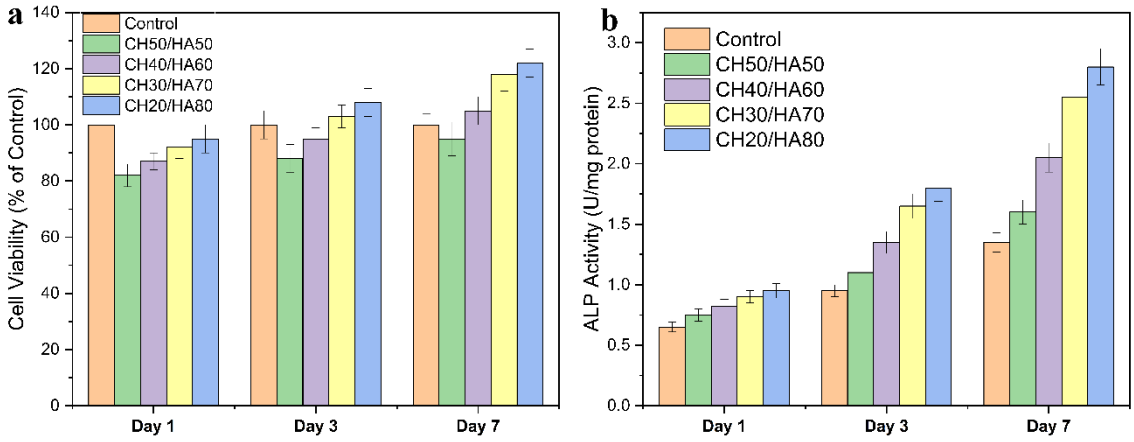


**Figure 5.** In vitro biocompatibility and osteogenic activity of hDPSCs exposed to CH/HA cement extracts

a) Cell viability of hDPSCs, b) ALP activity of hDPSCs cultured with different extracts of CH/HA cements after 1, 3, and 7 days

ment matrix, which are known to regulate cell signaling pathways and stimulate cellular activity. By day 7, all formulations supported excellent cell growth. The CH30/HA70 and CH20/HA80 groups exhibited the highest viability values (118±6% and 122±5%, respectively), compared to the control group. These findings suggest that increasing the HA content enhances the biological response of hDPSCs, likely due to the favorable surface chemistry and bioactive nature of HA particles. Furthermore, the sustained release of calcium ions may promote cellular proliferation and enhance the material's regenerative potential. The control group maintained a normal viability of approximately 100% throughout the study period.

Compared to the control group, the CH50/HA50 and CH40/HA60 groups exhibited slightly lower viability on day 1, which may be related to the initial alkaline environment generated by CH dissolution. However, cell viability progressively increased with culture time and reached values comparable to or exceeding those of the control group by day 7. Notably, the CH30/HA70 and CH20/HA80 formulations demonstrated viability values of 118% and 122% of the control, respectively, indicating that these compositions not only supported cell survival but also promoted cell proliferation. Overall, the results indicated that the developed CH/HA cements are non-cytotoxic and provide a suitable microenvironment for hDPSC growth. The formulations containing 70–80 wt% HA exhibited the most favorable biological performance.

ALP activity was measured to evaluate the odontogenic/osteogenic differentiation potential of hDPSCs cultured in the presence of CH/HA cement extracts. As shown in Figure 5b, ALP activity increased progressively with increasing culture time across all formulations. At day 1, relatively low ALP values were observed, ranging from 0.75±0.05 U/mg protein for CH50/HA50 to 0.95±0.06 U/mg protein for CH20/HA80. The differences among groups became more pronounced after 3 days of culture, with ALP activity increasing to 1.1±0.08, 1.35±0.09, 1.65±0.1, and 1.8±0.11 U/mg protein for CH50/HA50, CH40/HA60, CH30/HA70, and CH20/HA80, respectively. After 7 days, a substantial enhancement of ALP activity was observed in all groups. The highest activity was measured for the CH20/HA80 formulation (2.8±0.15 U/mg protein), followed by CH30/HA70 (2.55±0.13 U/mg protein). In contrast, the CH50/HA50 formulation exhibited the lowest value (1.6±0.10 U/mg protein).

The observed increase in ALP activity can be attributed to the synergistic effects of CH and HA. Calcium ion release from the cement matrix plays an important role in regulating odontogenic differentiation and mineralization processes. In addition, hydroxyapatite provides a biomimetic environment that resembles the mineral phase of hard tissues and supports cell attachment and differentiation. Interestingly, formulations containing higher HA levels exhibited significantly higher ALP activity despite releasing lower overall calcium concentrations. This observation suggests that the presence of HA contributes not only to cytocompatibility but also to the promotion of early differentiation events. The results are consistent with previous studies reporting that HA-containing biomaterials stimulate osteogenic and odontogenic marker expression in stem cells.

Regarding the ALP activity, the control group exhibited basal enzyme activity that increased gradually from 0.65±0.04 U/mg protein on day 1 to 1.35±0.08 U/mg protein on day 7. All CH/HA cement formulations showed higher ALP activity than the control at all time points. The difference became more pronounced as culture duration increased, particularly for the CH30/HA70 and CH20/HA80 groups. After 7 days, ALP activity in the CH20/HA80 formulation was approximately twofold higher than that of the control group, indicating enhanced odontogenic differentiation of hDPSCs induced by the bioactive cement extracts.

Overall, the MTT and ALP results indicate that the developed CH/HA cements support both cell proliferation and early differentiation of hDPSCs. Among the studied formulations, CH30/HA70 and CH20/HA80 exhibited the most promising biological performance, suggesting their potential suitability for vital pulp therapy and dentin–pulp complex regeneration.

## Discussion

The present study demonstrates that the incorporation of HA into CH-based self-setting cements significantly influences the structural organization, physicochemical behavior, and functional performance of the system. The findings indicate that CH is the primary reactive phase governing matrix formation, while HA mainly acts as a bioactive reinforcing filler that modifies the overall cement behavior. From a structural perspective, the system can be described as an amorphous calcium phenolate matrix in which HA particles are embedded and distributed throughout the bulk. The absence of crystalline CH in lower-CH formulations suggests extensive participation of CH in the setting reaction, leading to a predominantly amorphous network [34]. Within this structure, HA does not chemically contribute to the setting reaction but provides a stable inorganic phase that can enhance structural integrity and bioactivity.

Microstructural observations supported the formation of a heterogeneous composite in which inorganic filler particles are embedded within a continuous organic–inorganic matrix. The uniform distribution of HA, confirmed by elemental mapping, is particularly important for ensuring consistent interfacial interaction with the surrounding biological environment. Such homogeneity is expected to favor uniform bioactive responses, including mineral nucleation and tissue integration. The observed improvement in injectability with increasing HA content can be attributed to changes in powder packing behavior and reduced interparticle friction within the paste. The presence of well-dispersed solid particles likely improves paste cohesion during extrusion, thereby preventing phase separation commonly encountered in particle-based cement systems. This behavior is especially advantageous for minimally invasive clinical applications, where stable extrusion and shape retention are critical.

Mechanical performance appears to be governed by a balance between matrix formation and gradual structural relaxation in aqueous conditions. Although partial degradation of the matrix occurs over time, the overall mechanical integrity remains relatively stable across compositions, suggesting that HA contributes to structural reinforcement without compromising the cohesion of the amorphous matrix. The observed reduction in strength upon immersion is consistent with water-induced microstructural rearrangements and the development of porosity within the matrix, a common feature in partially degradable cement systems. The dissolution behavior indicates that increasing HA content enhances resistance to mass loss, reflecting the intrinsic stability of HA in aqueous environments [35]. This suggests that HA plays a key role in regulating the degradation rate of the cement, allowing for tunable resorption profiles depending on application requirements. Such control over degradation is particularly important in bone-related applications, where scaffold stability must be balanced with gradual replacement by newly formed tissue.

Ion release behavior highlights the dominant role of CH in providing bioactive calcium ions. The sustained release of $Ca^{2+}$ is governed by the solubility and dissociation of CH within the matrix. Calcium ion release from calcium-based injectable cements has been recognized as a key factor promoting apatite formation, regulating the local microenvironment, and supporting bone regeneration processes. [36]. This ionic release is known to play a critical role in stimulating cellular activity, promoting mineralization, and modulating the local biological environment. In contrast, HA contributes minimally to ionic release but provides a stable reservoir that may support long-term bioactivity through surface-mediated interactions.

The alkaline environment generated by the system is directly linked to the presence of reactive CH and is an important factor in antimicrobial activity and tissue response modulation. Such pH elevation is beneficial in eliminating bacterial contamination and may also contribute to signaling pathways involved in hard tissue regeneration. Over time, stabilization of pH suggests a dynamic equilibrium between ion release and matrix buffering effects.

Overall, the developed CH/HA cement is a tunable biomaterial in which the ratio of reactive to inert phases allows precise control over key properties, including cohesion, degradation behavior, ion release, and biological potential. The interplay between CH-driven reactivity and HA-induced structural stabilization provides a versatile platform for developing injectable bioactive cements suitable for hard tissue engineering applications

## Conclusion

A novel, workable, and injectable self-setting CH/HA cement was successfully developed in this study by combining CH and HA powders with a salicylate ester liquid phase. The experimental results confirmed that HA particles were uniformly dispersed within an amorphous matrix, indicating that the material can be considered bioactive due to the presence of HA as a functional phase. The cement also exhibited resorbable characteristics, which can be attributed to the partial solubility of CH, the low crystallinity of HA particles, and the hydrolytic nature of bonds within the amorphous calcium phenolate network. Overall, these results suggest that the developed CH/HA cement may act as an injectable, self-setting biomaterial for bone and dental repair applications. Nevertheless, further in vitro and in vivo studies are recommended to fully validate its clinical potential.

## Ethical Considerations

### Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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### Authors' contributions

Conceptualization, methodology, investigation and writing: Shokoufeh Borhan; Project administration and visualization: Alireza Mahboubian; Data curation and formal analysis: Seyed Alireza Parhiz; Supervision and resources: Mohammad Hossein Shahrezaee.

### Conflict of interest

The authors declared no conflict of interest.

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