Keywords

Apatite-forming ability, Bioactivity, Surface mineralization, Ca/P ratio, Surface chemical reactivity, Dental cement.

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Bone substitute nanomaterials for enhancing the apatite-forming capacity of dental repair material

ABSTRACT

***Objective*:** To analyse the apatite-forming ability of mineral trioxide aggregate (MTA Angelus) modified with nano-carbonated hydroxyapatite (nCHAp) as a bioactive endodontic biomaterial.

***Methods*:** Disc specimens (10.0 ± 0.1 mm diameter; 2.0 ± 0.1 mm thickness) were made-up from unmodified MTA (control) and MTA hold 2, 3, and 4 wt% nCHAp (n=12/group). After setting for 24 h at 37 °C and relative humidity, six specimens per group were immersed in 20 mL sterile phosphate-buffered saline (PBS, pH 7.4) at 37 °C for 21 days with PBS renewal every 3 days; the remaining six were stored dry. Surface mineralization was measured by field-emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDX; five spots/specimen) with Ca/P ratio calculation, and X-ray diffraction (XRD) for phase finding. Statistical analysis was performed using ANOVA/Tukey tests ($\alpha = 0.05$). ***Results*:** PBS exposure evoked calcium-phosphate deposition on all immersed specimens. Unmodified MTA showed porous spherical Ca–P aggregates agreeable with amorphous calcium phosphate; phosphorus emerged (13.60 wt%) and Ca/P was 2.49, indicating calcium-rich, immature surface deposits. nCHAp incorporation enhanced mineralization in a concentration-dependent manner: 2 wt% produced an early nucleation layer change into lamellar/plate-like crystallites, whereas 3–4 wt% yielded continuous nano-crystalline apatite layers with needle/plate morphologies and rosette-like aggregates. Post-immersion Ca/P ratios decreased to 1.80, 1.76, and 1.70 for 2%, 3%, and 4% nCHAp, respectively, approaching biologically relevant apatite. XRD confirmed hydroxyapatite/carbonated hydroxyapatite reflections with decreased silicate/portlandite intensities after immersion.

***Conclusions*:** Adding 2–4 wt% nCHAp speeding biomimetic apatite formation on MTA, encouraging its potential for regenerative endodontic repair and reinforced dentin–material interfacial mineralization.

Clinical Relevance: Boosting the bioactivity to make the seal tighter and help the hard tissue heal more effectively. these could make MTA last longer and work more reliably in root canal treatments.

INTRODUCTION

In recent years, there's been a lot of exciting progress in dental materials. The rise of new restoratives and products that help to regenerate bone. These advancements have really changed the way dentists approach treatments and have improved results for patients[1]. Lately, there's been a lot more focus on bioactive materials because of their beneficial interactions with biological tissues, because these materials can actually trigger helpful biological responses when they come into contact with cells and tissues. They aid natural repair and regeneration processes, which ultimately lead to better oral health[2] . Calcium hydroxide, the first endodontic material known for its ability to encourage the formation of a dentinal bridge over exposed pulp tissue[3]. Over time, newer materials like mineral trioxide aggregate (MTA) and similar calcium silicate cements were developed. These are basically portland cement

mixed with bismuth oxide to make them visible on X-rays. These hydraulic bioceramics have become popular for use in procedures like vital pulp therapy,
repairing perforations, and other dental repairs[4] . Their bioactivity comes from releasing calcium ions, which react with phosphate in the body fluids, This creates hydroxyapatite crystals on the surface of the material to support tissue repair[5] . Bioactivity refers to ability of dental materials to interact with tissues and help tissues to heal by encouraging the formation of apatite at the material–tissue interface. This feature is useful in endodontic applications[6] .
Mineral Trioxide Aggregate (MTA) is a calcium silicate-based cement used in root treatments, known for its good biological properties. But, its bioactivity has some issues. For instance, forming an apatite layer on MTA slow and uneven process, which might impact the material to seal properly and support the remineralization of dentin[7]. Its ability to interact biologically comes from releasing calcium and hydroxyl ions. These ions then react with phosphate in the nearby fluids, forming a calcium-phosphate layer on the surface. This layer helps the material bond better with dental tissues[8] . However, since MTA doesn't have phosphate inside itself, it depends on phosphate from outside sources. That means it takes longer for it to start forming apatite compared to materials that include phosphate-releasing components right in their structure[9, 10] . Researchers are constantly working to make MTA better by boosting its ability to interact with tissues. One exciting idea is to bone-like materials, like nano carbonate-substituted hydroxyapatite (nCHA), it is similar to the minerals in bones and teeth. Research shows that nCHA helps formation of more organized and quicker-growing apatite crystals, ultimately improving bioactivity of materials and clinical performance[11] . Thus, incorporating nCHA into MTA could provide extra nucleation sites, accelerates calcium-phosphate deposition, and enhances mineral induction.

MATERIAL AND METHODS

Bioactivity assessment (apatite-forming ability) The bioactivity of the cement formulations evaluated following the ISO 23317:2014 [12], standard called "Implants for surgery – In vitro evaluation for the apatite-forming capacity of implant materials." Basically, if a material can develop a noticeable layer of apatite on its surface within 21 days when soaked in a simulated body fluid, it's considered bioactive. Phosphate-buffered saline (PBS) used as the soaking solution because it closely mimics the ionic makeup of the body's fluids.

Specimen preparation and immersion protocol

Disc-shaped specimens about (2.0 ± 0.1) mm in thick and (10.0 ± 0.1) mm in diameter.These were prepared for both control and experimental groups. Cements were mixed per the manufacturers' instructions and placed into plastic molds on polyethylene-lined glass slabs to prevent adhesion. A second polyethylene-covered glass slab was applied to ensure surface uniformity by removing excess material. Thus, the resulting specimens with smooth flat surfaces. The specimens were then incubated at 37 °C with 95% relative humidity for 24 hours to simulate intraoral conditions and allow complete setting. Following setting, specimens were demolded, and surface irregularities were refined using fine abrasive paper.
Twelve specimens (n = 12 per group) for each of the Angelus MTA without nanoparticles (control group positive) and the Angelus MTA with 2, 3, and 4% by weight of nCHA (experimental groups) were prepared. Six specimens per group were immersed in 20 mL of sterile phosphate-buffered saline (PBS) in screw-capped containers and incubated at 37 °C for 28 days to mimic long-term physiological exposure. The remaining six specimens were stored dry as negative controls.

Groups:
1.Control positive group (n=12): Angelus MTA without nanoparticles (before and after immersion). Six specimens per group were immersed and the remaining six specimens were stored dry.
2.Experimental groups: Angelus MTA with 2, 3, and 4% wt nCHA (before and after immersion), for each concentration (n=12). Six specimens per group were immersed. The remaining six specimens were stored dry as negative controls.
Phosphate-buffered saline (PBS) was prepared by dissolving 8.58 g of PBS powder (pH 7.4) in 1000 ml of distilled water under continuous stirring until completely dissolved.
The pH was adjusted to 7.4 and the solution was sterilized by autoclaving at 121 °C and 15 psi for 15 minutes, according to manufacturer's guidelines. To simulate renewal physiological fluids in vivo, PBS was replenished every 3 days during the immersion period[13] . PBS act as a physiological storage solution without calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions. Its ionic makeup included: Sodium chloride (NaCl, 7.650 g/L), Disodium hydrogen phosphate (Na_2HPO_4 ,0.724), and Dipotassium hydrogen phosphate ($K2HPO_4$, 0.210), with the pH maintained at 7.4 at 25°C.

Post-Incubation Treatment and Analytical Procedures

After the 21-day of immersion, the specimens were retrieved out of the PBS and placed in a desiccator with phosphorus pentoxide (P_2O_5) at 37 °C for 24 hours to ensure they were completely dry. The specimen surfaces were subsequently examined for apatite formation using field-emission scanning electron microscopy (FESEM) for surface morphology of the specimens and the Ca/P ratio was assessed using energy-dispersive X-ray spectroscopy (EDX) analysis, while X-ray diffraction (XRD) used to detect structural phase changes.

Evaluation of apatite-forming ability

The apatite-forming ability was evaluated by analyzing surface morphology and microstructural features before and after PBS immersion using field-emission scanning electron microscopy (FESEM) (TESCAN MIRA3

France), combined with Energy-Dispersive X-ray Spectroscopy (EDX) for elemental analysis. Specimens were air-dried at room temperature, sputter-coated with a 20 nm gold–palladium layer to enhance conductivity, and mounted on aluminum stubs with double-sided carbon tape. EFSEM imaging was performed at 15 kV accelerating voltage and ~10 mA beam current, with micrographs captured at magnifications ranging from 10.0× to 25.0×, and up to 100× to visualize apatite-like surface deposits.

EDX analysis quantified the weight percentages of chemical elements and to rank the surface calcium (Ca) / phosphorus (P) ratio as an indicator of apatite-phase formation. Both PBS-immersed and non-immersed specimens were analyzed to detect chemical composition alterations and to detect the bioactivity-induced morphological and chemical changes. The samples were analyzed for each group, and five analysis spots were examined for each sample surface. The results were the averages of the data calculated[14] . Additionally, phase identification and crystallinity were assessed via X-ray Diffraction (XRD) using Cu Kα radiation (λ = 1.5406 Å), operating at 30 kV and 30 mA, with a 2θ scanning range of 10°–80°, a step size of 0.02°, and 0.5 s per step. The hydroxyapatite (HAp) phase was identified using the International Center for Diffraction Data (ICDD) database: $Ca_{10}(PO_4)_6(OH)_2$ [ICDD 00-009-432][15] .

RESULTS

Field emission scanning electron microscope analysis

The FESEM images at various magnifications showed clear differences in the surface textures between the control and experimental samples, both before and after soaking in PBS, Figue 1. The set MTA looked after 21 days in PBS, the cement surface was coated with a continuous layer of calcium-phosphate deposits, composed mainly of large, highly porous spherical aggregates with a nanosponge like architecture. This morphology is typical of amorphous calcium phosphate (ACP). Plate-like crystallites consistent with octacalcium phosphate (OCP), a transitional phase that appears during the early stages of calcium-phosphate maturation. MTA with 2% nCHA showed a noticeable mineral layer after soaking in PBS. The surface displayed rounded calcium-phosphate deposits that act as the initial mineral growth. A porous network of thin lamellar structures, suggesting that the initial amorphous calcium-phosphate was beginning to change into OCP. The stacked crystalline structures on the surface are a typical of carbonated hydroxyapatite. These features shows that adding nCHA enhances and speed up the formation of apatite on MTA when exposed to phosphate-rich environments. Modified MTA with 3% nCHA revealed a noticeable progression of calcium-phosphate deposits across the surface. The surface became completely covered by a tightly packed layer of nano-crystalline with needle-like and short plate morphologies, which is typical as the apatite matures. While modified MTA with 4% nCHA revealed a continuous, apatite layer composed of needle- and plate-like crystallites arranged in rosette-like aggregates. It's clear that the mineral particles are forming and growing faster when nano-carbonated hydroxyapatite is added. This display that the modified MTA is more biologically active and advisable at encouraging mineral development.

Elemental analysis using energy-dispersive X-ray spectroscopy before and after PBS immersion

Energy-dispersive X-ray spectroscopy (EDX) used to look at what elements are on the surface of materials—both the original MTA and the MTA mixed with nCHA— before and then again after soaking in PBS for 21 days. Before soaking, the cement had a lot of calcium—about 35.91% by weight—and also contained significant amounts of silicon (18.28%), oxygen (23.99%), carbon at 8.97%, along with smaller amounts of bismuth (9.96%) and aluminum (2.89%). Phosphorus was not noticed at this stage. However, after 21 days immersion in PBS, clear changes in elemental composition were detected. Phosphorus (P) appeared at 13.60% by weight, and calcium (Ca) slightly decreased to 33.87 %, resulting in a calculated Ca/P ratio of 2.49, suggesting calcium phosphate phase was forming, likely hydroxyapatite. Silicon (Si) and (Al) levels dropped markedly to 6.91 and 0.89% respectively. Bismuth content falling to 8.89% while carbon increased slightly to 9.89%, as shown in Table 1. and Figure 2

In contrast, the elemental constitution of modified MTA, as measured by EDX both before and after 21 days in PBS, is shown in Tables 2, 3 and 4 and Figure 2 Phosphorus levels increased noticeably after immersion, with higher nCHA content, while calcium showed a slight reduction, resultant in a Ca/P ratio close to that of mature hydroxyapatite. This point to the formation of a stable and well-balanced calcium phosphate layer and reflects improved bioactivity compared to the unmodified material. At the same time, silicon, bismuth, and aluminum levels decreased, whereas oxygen and carbon showed a slight increase.

Statistical analysis was performed using ANOVA/Tukey tests (α = 0.05), as shown in Table 5, the mean Ca/P ratios after 21 days revealed a significant difference among the control and the experimental groups (modified MTA). On the other hand, the groups that were modified with 2%, 3%, and 4% nCHAp showed much lower ratios—1.80 ± 0.064, 1.76 ± 0.167, and 1.70 ± 0.089, respectively. The control group possess a higher Ca/P ratio (2.49 ± 0.138, $P < 0.05$), whereas the groups modified with 2%, 3%, and 4% nCHAp showed significantly lower values of 1.80 ± 0.064, 1.760 ± 0.167, and 1.70 ± 0.089, respectively. Although the differences among the modified groups were not statistically significant ($P > 0.05$), all had significantly lower Ca/P ratios than the control.

These findings suggest that the addition of nCHAp modulates the apatite chemistry, upgrade the fabrication of a calcium-deficient or carbonated apatite phase, which more closely mimics natural biological

apatite. Calcium and phosphorus were the dominant elements at all concentrations, confirming calcium phosphate formation. As nCHAp increased, phosphorus content rose from 20.48 % (2%) to 20.97 % (3%) and 21.54 % (4%). also calcium slightly increased from 35.87% at (2%), 35.81 % at 3%, to 35.72 % at 4%, leading to a progressive reduction in the Ca/P ratio from 1.80 (2%) to 1.70 (4%), toward the hydroxyapatite stoichiometric value, indicating improved bioactivity. Silicon and aluminum contents decreased with higher nCHAp, from 3.13% at 2% to 1.06 % at 3%, further to 0.40 % at 4% and from 0.52 % to 0.21 % respectively. Whereas carbon and oxygen showed slight increases from 11.63 % to 12.61 % for carbon and from 25.00% to 26.03% for oxygen, consistent with carbonated apatite deposition. Bismuth levels remained stable, reflecting retention of the radiopacifier.

Crystalline phase evolution before and after PBS immersion

The XRD analysis prior to PBS immersion in both unmodified and modified MTA Angelus groups Figure 3 A1, B1, C1 and D1 shows a largely amorphous profile with several well-defined crystalline peaks. Reflections near 18.16°, 28.72°, 34.19°, 36.80°, 54.47° and 64.43° correspond to $Ca(OH)_2$ (ICDD 01-076-0570), indicating that portlandite forms a major hydrated phase. The group of peaks at 25.02°, 25.80°, 26.11°, 28.66°, 28.86°, 29.51°, 30.10°, 31.53°, 32.69°, 33.91° and 35.02° matches Ca_3SiO_5 (ICDD 00-014-0693) and 26.36°, 28.08°, 31.77°, 32.75°, 34.71°, 45.69°, 49.29°, 50.58°, 51.65°, 53.24° and 54.26° for Ca_2SiO_4 (ICDD 00-024-0037), pointing to unreacted calcium silicates still present in the material. Additional features at roughly 28.34°, 32.84°, 40.51° ,47.12°, 50.17°, 53.09°, 55.90° and 58.63° align with Bi_2O_3 (ICDD 01-076-2478), confirming a bismuth-oxide phase. Weak peaks near 30.99°, 32.44° and 44.974° fit $CaCO_3$ (ICDD 00-051-1524) and indicate mild carbonation. No calcium phosphate peaks were observed in the unmodified group, consistent with the absence of phosphorus in EDX analysis. In contrast, the modified group exhibited distinct peaks around 18.29°, 18.904, 25.57 26.26°, 27.68°, 28.91°, 31.56°, 39.14°, 47.04°,, 59.17°—also coincide with calcium phosphate hydrate, $Ca_2(P_4O_{12})\cdot 4H_2O$ (ICDD 00-041-0483). After immersion of MTA in PBS for 21 days (Figure. 3 (A2, B2, C2 and D2)), a relative decrease in the intensity of the original cementitious peaks was observed, accompanied by an increase in the peak intensity within the 25–35° 2θ range. This indicates ongoing hydration and the formation of calcium-phosphate/apatite-like precipitates on the material's surface, consistent with the well-documented bioactivity of MTA. The broad background between ~10–35° 2θ is still present calcium silicate hydrate (CSH), but there is a slight increase in the intensity of the peaks within this region, appearing near its midpoint (approximately 25–35° 2θ). The peaks previously attributed to C_3S/C_2S and Bi_2O_3 remain detectable, although some of them have become relatively less intense compared with the newly enhanced peaks in the 25–35° range. Thus, XRD pattern exhibited substantial changes indicative of mineral phase transformation of unmodified MTA angelus. Most notably, new diffraction peaks emerged at 25.87, 31.74, 32.86, 34.04, 39.17, 39.75, 46.34 which are characteristic of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), as indexed to ICDD Card No. 01-072-1243. Also, some of unreacted Ca3(PO4)2 characteristic peaks appear at (θ)= 28.26°, 28.57°, 31.75°, 32.08°, 32.45° and 39.46° that match with standard (ICDD Card No. 01-070-0364). The development of these apatite-specific peaks strongly supports the precipitation of a calcium phosphate layer, consistent with the EDX observation of a phosphorus content of 13.60 wt% and a Ca/P atomic ratio of 2.49. The relatively high Ca/P ratio suggests the presence of calcium-rich phosphate phases, potentially amorphous calcium phosphate (ACP), but the phase may not yet resemble stoichiometric or mature hydroxyapatite (ideal Ca/P = 1.67). Concurrently, modified MTA angelus showed a relative enhancement in the diffraction peaks associated with calcium phosphate (Ca3(PO4)**2**) was observed. Specifically, reflections at 2θ 32.08°, 32.21°, 32.45°, 32.75°, 32.92°, 34.214°, 39.46°, 39.66° and 40.58° with ICDD Card No. 01-070-0364 increased in intensity especially with 3% nCHA, indicative of ongoing surface mineralization. A new and intensified peaks emerged around 2θ= 25.8°, 28.7°, 31.8°, 32.9°, 33.9°, 39.9°, and 46.7° with ICDD Card 12-0529**,** which are representative of carbonated hydroxyapatite (CHA)**.** These findings suggest active biomimetic mineral deposition on the cement surface in response to phosphate-rich PBS. Concomitant with the appearance of apatite after 21 days of immersion in PBS, XRD analysis revealed a significant decrease in the intensity of characteristic peaks of the modified and unmodified MTA angelus compared with no immersion, particularly tricalcium silicate (C_3S) and dicalcium silicate (C_2S). Peaks observed at 25.02°, 29.51°, 31.53°, 32.69° and 33.91° matches Ca_3SiO_5 (ICDD 00-014-0693) and 28.08°, 31.77°, 32.75°, 49.29°, 53.24°, 54.26° for Ca_2SiO_4 (ICDD 00-024-0037), respectively, were either markedly diminished after immersion and other disappear. This reduction supports the innovative hydration of silicate phases and their transformation into portlandite and (CSH) gels. A moderate reduction in peaks at 18.16°, 28.72°, 34.19°, 54.47° and 64.43° (ICDD 01-076-0570) was noted, related to to portlandite ($Ca(OH)_2$), due to its consumption during apatite precipitation by exchange with phosphate ions. Likewise, peaks at 32.84°, 40.51° ,47.12° and 53.09° (ICDD 01-076-2478) related to bismuth oxide (Bi_2O_3), a radiopacifier, showing a slight decrease in relative intensity, reflecting either partial surface covering by the new formed apatite layer or minor diffusion during immersion. Significantly, peaks attributable to aluminum-containing phases were below detection limits, in harmony with EDX data that display a significant drop in aluminum content post-immersion. Moreover, peaks at 30.89°, 36.05°,46.84°, 48.19°, 56.69°, 64.36° (ICDD 01-086-2341) connected with calcite ($CaCO_3$), display slight increases, orienting with the rise in carbon content noticed in elemental analysis.

and potentially reflecting carbonation during or after immersion.

From the XRD results, pure MTA display its common hydrated structure, with a broad CSH halo and clear signals for portlandite and silicate. When nCHA is added, the diffractograms beginning to shift—obvious signs of apatite get more noticeable. The broad amorphous hump decreases, the portlandite peaks intensity weaken, and the peaks for apatite around 26° and 31–33° stand out more prominent. This transformation happens gradually from 2% to 3% nCHA, where apatite organization is most noticeable, with sharp, clear peaks indicating that mineral deposition are very crystalline. In 4%, apatite is still common but appears more partly carbonated, resultant the reflection broader and less defined sharp. Throughout, CHA improve hydration–motivated conversion of $Ca(OH)_2$ and promotes surface mineralization, eventually covering the original MTA silicate peaks. Overall, the bioactivity increases from MTA alone through 2% and 3% nCHA, peaks in 4% remains high despite its lower crystallinity.

DISCUSSION

Apatite, as the primary mineral phase of bone and enamel, is fundamental to the bioactive behavior of perforation repair materials[16]. Calcium silicate–based cements promote apatite formation through ion release and alkaline pH in phosphate-rich fluids, enabling calcium phosphate deposition that supports hard tissue regeneration and remineralization[17]. Bioactivity enhances cellular attachment, differentiation, and mineralization[9]. Furthermore, calcium phosphate deposition lends to the occlusion of dentinal tubules and meliorates sealing at the material–dentine interface, essential for the success of vital pulp therapy[18].

FESEM investigation after 21 days of immersion revealed that calcium phosphate mineralization on MTA and nCHA–modified MTA depended on their composition. Unmodified MTA surfaces were covered by porous, spherical layer aggregates with a nanosponge-like morphology attributed to amorphous calcium phosphate (ACP), formed due to Ca^{2+} released from the hydrated calcium silicate parts of the material and local supersaturation in phosphate-rich environments[19]. Plate-like crystallites inform octacalcium phosphate (OCP) as a passing phase preceding apatite, conformable with the ACP→OCP→apatite pathway[20]. Incorporation of 2% nCHA accelerated this transformation, promoting organized lamellar and plate-like apatite structures through heterogeneous nucleation on nCHA surfaces. Raising the nCHA content to 3% produced a uniform surface layer of densely packed nanocrystals with needle- and short plate-like morphologies, indicating increased nucleation density and continued crystal growth. At 4% nCHA, mineralization was most pronounced, with formation of hierarchically organized apatite layer composed of rosette-like assemblies of needle and plate crystallites. Together with the broad, low-intensity apatite XRD peaks, these features are characteristic of nanocrystalline, carbonate-substituted hydroxyapatite typical of bone-like mineral [11, 20, 21].

EDX analysis after 21 days in PBS revealed distinct mineral evolution in unmodified and nCHAp-modified MTA. Pristine MTA initially lacked phosphorus and showed high calcium and silicon levels typical of calcium silicate phases, whereas the appearance of phosphorus after immersion with a concomitant reduction in calcium indicated calcium phosphate precipitation and surface bioactivity[22]. The elevated Ca/P ratio (2.49) further suggests the predominance of amorphous calcium phosphate rather than stoichiometric hydroxyapatite[23].

The reduction in silicon content likely reflects silicate leaching and silanol formation that favor apatite nucleation, while minor carbon enrichment and decreased bismuth and aluminum suggest surface carbonation and elemental redistribution[24, 25]. Elevated calcium levels think over the conjunctive effects of continued $Ca(OH)_2$ release and dissolution of nCHAp, both contributive to supersaturation and later apatite formation. While decreased bismuth signal is assigned to occlusion by newly deposited mineral layers, rather than its dissolution [27]. When nCHAp content increase from 2% to 4%, the Ca and P level increase, while Ca/P ratio declining from 1.80 to 1.70, toward the natural ratio of hydroxyapatite (1.67), points to the formation of more mature and bioactive apatite. At the same time, silicon content dropped from 3.13% to 0.40%, meditates silicate ion leaching or integration into the structure of apatite, chemical action connected to enhanced bioactivity and osteogenic stimulation in silicate-based biomaterials[28]. The increase in carbon content in the modified groups reinforced the formation of carbonated hydroxyapatite, which more closely mimics bone mineral and exhibits better biologically[29]. Stable level of bismuth, mark its function as a radiopacifier without participation in mineralization. On the other hand, lower aluminum levels in the modified groups might be a good action since aluminum concerns to the cell cytotoxicity[30]. The maturity and biological compatibility of calcium phosphate on biomaterials evaluated by Ca/P ratio. Normally, hydroxyapatite has a Ca/P ratio close to 1.67, whereas biological apatites can vary because of ionic substitutions such as carbonate[31]. Unmodified MTA had upraised Ca/P ratio (2.49), imply there are calcium-rich phases such as portlandite, unreacted calcium silicates, or amorphous calcium phosphate[24]. Quantitative range in 2.0–2.5 can initiate nucleation but also inform supersaturation with calcium oxide phases, which have been coupled to increased oxidative stress, and reduced osteoblast viability [9, 32].

Unlike the pure MTA, the nCHAp-modified type with 2–4% exhibited lower Ca/P ratios, about 1.80 to 1.70, approaching to the ratio in natural bone-like carbonated apatite, which imply best phosphate integration and more bone-like mineral formation[26]. Fundamentally, nCHAp do two roles — it supply phosphate and regarded as a starting point for mineral to form. This shift calcium-rich intermediates into well-structured apatite. Also, the carbonate groups in nCHAp speed up the nucleation process [33]. Integration of nCHAp boosts MTA bioactivity, due to its high surface area and chemical similarity to natural bone minerals, nCHAp

serve as an efficient scaffold and phosphate origin, favoring fabrication of more stoichiometric, bone-like hydroxyapatite with Ca/P ratios close to about 1.67 [11]. This controlled ion exchange comparing with unmodified MTA, has calcium-rich, amorphous phases supports improvement of even, stable, and biologically mineral layers [8]. Ca^{2+} and OH^- ions released from hydration of calcium silicates in MTA, make an alkaline, and calcium-rich environment that, in the lack of adequate phosphate or nucleation sites, favour formation of amorphous calcium-rich phases and raised Ca/P ratios, as dictated in the control group[35]. In comparison, nCHAp integration give phosphate, carbonate and supply effective nucleation, promoting the more crystalline, carbonated apatite with Ca/P ratios closer to the native bone[36]. These mineral phases enhance ion balance and chemical stability encouraging cell adhesion, osteoconductivity, and mineral integration compared with unmodified MTA[37].

SEM shows the form of the apatite deposits, however, for the exact mineral phase, XRD were necessary. Later on 21 days in PBS, the unmodified MTA displayed distinct hydroxyapatite peaks, which strengthen that HA formed in these phosphate-rich conditions. The higher Ca/P ratio in the control group hints that the mineral phases might be more amorphous or poorly crystalline calcium phosphate, acting as transformation minerals[38]. The noticeable intensity of phosphate-related XRD peaks in nCHAp-modified groups, after soaking in PBS pointing to faster surface mineralization. It believably starts with quick-forming calcium phosphate hydrate layers that mature rapidly into carbonated hydroxyapatite [39]. The drop in the peaks for C_3S, C_2S, and portlandite display that hydration is still take place and their consumption in apatite formation[40]. Diminished bismuth and aluminum signals are assigned to surface coverage by newly formed apatite, while minor calcite peaks inform limited carbonation[41]. Overall, these changes sustain enhanced and more quickly apatite formation, supporting the better bioactivity of modified MTA.

CONCLUSION

Adding nCHAp to MTA actually rise its ability to activate bone growth. It assist the material form bone-like apatite quicker and in a more controlled way. The modified mixes show better balance of ions, with calcium to phosphorus ratios closer that is present in the body. They also develop more mature, crystalline mineral phases compared to plain MTA. These improvements propose that the material is best at starting mineralization, has more accessible phosphate, and mimics natural bone formation more closely. Overall, it's more stable chemically and more related biologically. Collectively, nCHAp-modified MTA demonstrates improved potential for clinical applications requiring effective hard tissue regeneration and long-term interfacial stability.

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