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Research Paper

Fabrication and Evaluation of Xeno, Synthetic, and Acellular BCP Powders for Bone Tissue Engineering: A Comprehensive Study on Osteogenic Potential

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ABSTRACT

The development of bioactive calcium phosphate (BCP) powders for bone tissue engineering has attracted significant attention. This study investigates the fabrication and characterization of three BCP powder compositions: Xeno BCP (extracted from cow femoral bone), synthetic BCP (synthesized from $CaHPO_4$ and $CaCO_3$), and acellular BCP (derived through decellularization of human femoral bone). BCP composed mainly of β-tricalcium phosphate (β-TCP) and hydroxyapatite (HAp), is known for its bioactivity and osteoconductive properties. The powders were fabricated using methods aimed at optimizing their structural and biological properties for biomedical use. Characterization was conducted using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Inductively Coupled Plasma (ICP) to analyze functional groups, phase identification, crystallinity, and elemental composition. In vitro assays, including the MTS assay for cell toxicity and biocompatibility, were performed, alongside biodegradation and Alizarin Red S staining to assess mineralization and osteogenic potential. The results revealed that acellular BCP, produced via a non-thermal process, exhibited the highest bioactivity, cell proliferation, and mineralization. Xeno BCP showed superior performance compared to synthetic BCP. These differences are attributed to the non-thermal processing used for acellular BCP, which provided the best results in all assays. In conclusion, acellular BCP demonstrated the highest potential for bone regeneration, followed by Xeno and synthetic BCP, highlighting its promising application in tissue engineering and regenerative medicine.

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1-Introduction:

Biphasic Calcium Phosphate (BCP) materials have a significant history in biomedical applications, dating back to the 1980s, when calcium phosphate ceramics were first investigated for their potential in bone repair. The realization that these materials could closely imitate the mineral phase of natural bone paved the way for their application in orthopedic implants, dental surgeries, and bone grafts. BCP, which includes β-tricalcium phosphate (β-TCP) and hydroxyapatite (HAp), has become a prominent biomaterial because of its unique combination of chemical, physical, and biological properties. Over the years, improvements in the synthesis, characterization, and clinical use of BCP have established its position as one of the most crucial materials in bone tissue engineering. [1].

BCP consists of two main phases: β-TCP and HAp, both of which are naturally occurring calcium phosphates. HAp closely resembles the mineral component of bone, making it highly biocompatible and stable within the body. β-TCP, on the other hand, is more biodegradable, enabling faster resorption and bone tissue remodeling. The ratio of β-TCP to HAp in BCP can be precisely controlled during fabrication, resulting in materials with customizable degradation rates, mechanical strength, and osteoconductivity. [2].

The physical structure of BCP is characterized by high porosity, which enhances its ability to support cell growth and infiltration. The pore size and distribution can be tailored to facilitate cell migration and bone formation. These structural properties, combined with the material's chemical composition, enable BCP to promote cellular activities such as proliferation, differentiation, and mineralization, which are crucial for bone regeneration. [3].

BCP materials closely resemble the natural structure of bone, which is primarily composed of HAp and collagen. While HAp provides the rigidity and structural integrity, the β-TCP phase promotes resorption and bone remodeling. This similarity allows BCP to integrate well with surrounding bone tissue, providing mechanical support while facilitating natural healing. Additionally, the biodegradability of β-TCP enables the material to gradually be replaced by new bone, minimizing the need for surgical removal. [4].

BCP materials have gained significant acceptance in the medical field, with several FDA-approved products available for clinical use. These products are primarily used as bone grafts for orthopedic surgeries, dental implants, and the repair of bone defects. The FDA has recognized BCP as safe and effective for use in bone regeneration, with numerous studies supporting its efficacy in promoting osteointegration and tissue healing. The biocompatibility and resorbable nature of BCP have contributed to its widespread use in both non-load-bearing and load-bearing bone repair applications. Several commercial products based on BCP are currently available for use as bone grafts. These products come in various forms, such as granules, blocks, and putties, and are designed to offer flexibility in treating different types of bone defects. [5].

The primary advantages of BCP as a bone graft material include its osteoconductivity, biocompatibility, and ability to promote natural bone regeneration. The customizable properties of BCP, such as the β-TCP-to-HAp ratio, allow materials to be tailored to specific clinical needs, including adjusting resorption rate and mechanical strength. The high porosity of BCP supports cell infiltration, further aiding in bone healing. [6].

However, there are some challenges and limitations associated with BCP. For instance, while β-TCP is highly resorbable, it can sometimes degrade too quickly for specific applications, leading to a temporary loss of mechanical support. Additionally, BCP performance may be influenced by the formulation and the complexity of the bone defect being treated. Researchers continue to work on improving the material's properties, such as enhancing its mechanical strength and developing composite materials that can offer greater performance in specific clinical scenarios. [7].

BCP’s unique combination of chemical and physical properties, along with its similarity to natural bone, makes it a valuable material in the field of bone tissue engineering. With ongoing advances in its synthesis and clinical applications, BCP continues to play a key role in developing innovative solutions for bone regeneration and repair. [8].

Despite the extensive clinical use of biphasic calcium phosphate (BCP) materials, a systematic understanding of how different fabrication origins and processing routes influence osteogenic performance remains incomplete. In particular, direct comparisons between thermally processed xenogeneic BCP, chemically synthesized BCP, and non-thermally processed acellular human-derived BCP are scarce. Most previous studies have evaluated these materials independently, making it difficult to isolate the role of processing temperature, biological origin, and preservation of native mineral structure on bioactivity and osteogenic potential.

A critical unanswered question is whether non-thermal, acellular processing can better preserve bone-like physicochemical characteristics and osteogenic functionality compared to conventional high-temperature fabrication routes. Thermal treatments, while effective in removing organic components and enhancing crystallinity, may alter

mineral composition, crystal size, and surface chemistry, potentially reducing biological performance. Conversely, acellular processing aims to maintain the native mineral architecture of bone, which may provide improved cellular responses and mineralization behavior.

Therefore, the present study was designed to systematically compare xeno-derived, synthetic, and acellular BCP powders under identical in vitro conditions, with a specific focus on correlating fabrication strategy with crystallinity, elemental composition, degradation behavior, cytocompatibility, and osteogenic potential. By directly addressing these factors, this work seeks to clarify whether a non-thermal acellular approach offers measurable biological advantages over conventional synthetic and xenogeneic BCPs, thereby providing clearer guidance for the rational design of BCP-based bone graft substitutes.

Previous studies have demonstrated that the processing route of bone-derived biomaterials plays a decisive role in determining their physicochemical and biological performance. In particular, thermal treatments, while effective for organic removal and phase stabilization, have been shown to modify crystal size, surface chemistry, and ion substitution patterns, which may negatively affect cellular responses and osteogenic signaling. In contrast, non-thermal or low-temperature processing approaches, including decellularization-based strategies, have been reported to better preserve native bone mineral characteristics and bioactive cues that support osteogenesis and mineralization.

Comparative evaluations between synthetic BCPs and naturally derived bone substitutes have consistently shown superior biological performance for natural-origin materials, attributed to their closer resemblance to native bone mineral composition and structure. However, most available studies focus on binary comparisons, such as synthetic versus xenogeneic or synthetic versus allogeneic grafts, while direct three-way comparisons between synthetic, xenogeneic, and acellular human-derived BCPs under identical experimental conditions remain limited. Moreover, few reports explicitly examine how thermal versus non-thermal processing strategies influence the osteogenic potential of these materials in parallel.

Therefore, the present study addresses a clear gap in the literature by providing a systematic, side-by-side comparison of thermally processed xenogeneic BCP, chemically synthesized BCP, and non-thermally processed acellular human BCP, with an emphasis on correlating fabrication strategy with crystallinity, degradation behavior, cytocompatibility, and in vitro osteogenic performance. This approach enables a more mechanistic understanding of how material origin and processing temperature collectively govern biological outcomes, which is essential for the rational design of next-generation bone graft substitutes.

2- Experimental Procedure:

2-1-Xeno BCP Powder Materials:

BCP powder was obtained from cow femoral bone, which was ethically sourced and cleaned in accordance with approved protocols. All handling and procedures followed the guidelines for animal-derived materials. [9].

2-2-Synthetic BCP Powder Materials:

The Synthetic BCP powder was synthesized using chemical reagents: calcium hydrogen phosphate ($CaHPO_4$) and calcium carbonate ($CaCO_3$), both purchased from Sigma-Aldrich [9].

2-3-Acellular BCP Powder Materials:

Human femoral bone was sourced from a cadaveric donor with ethical approval for medical research. The bone underwent a decellularization process to remove cellular material while preserving the mineral matrix. [5].

2-4-Xeno BCP Powders Fabrication:

Preparation: The femoral bone was cleaned to remove any remaining soft tissues. The bone was then subjected to a three-step thermal treatment in an oven (Exciton oven, IRAN) to ensure complete removal of organic matter and produce a pure BCP powder:

Step 1: Heating to 400°C for 2 hours to remove volatile organic compounds.

Step 2: Heating to 800°C for 4 hours to further remove organic content and initiate partial crystallization.

Step 3: Heating to 1100°C for 6 hours to achieve complete crystallization of the mineral phase, producing β-TCP and HAp.

After the thermal treatment, the bone was ground into a fine powder using a ball mill (Majansanat, IRAN) to achieve a homogenous particle size suitable for further characterization. [10].

2-5-Synthetic BCP Powders Fabrication:

Mixing: The Synthetic BCP powder was synthesized by dry ball milling (Majansanat, IRAN) in 67 rpm for 24h. Calcium hydrogen phosphate ($CaHPO_4$) and calcium carbonate ($CaCO_3$) were combined in a 1:1 molar ratio, and the mixture was ball-milled for 10 hours to ensure uniformity of the powder.

Thermal Treatment: The powder was then subjected to a two-step heat treatment in an oven (Exciton oven, IRAN):

Step 1: Heating to 800°C for 4 hours to initiate the reaction between $CaHPO_4$ and $CaCO_3$.

Step 2: Heating to 1100°C for 6 hours to ensure complete synthesis of the BCP composition (primarily β-TCP and HAp). After cooling, the

synthesized powder was ground into a fine, uniform powder. [11].

2-6-Acellular BCP Powders Fabrication:

Decellularization: Human femoral bone was decellularized using a freeze-thaw method to remove the cellular components while maintaining the mineral matrix. The procedure involved 10 freeze-thaw cycles, where the bone was frozen at -80°C for 12 hours in freezer (KW, Italy), then thawed at room temperature for 12 hours. This process was repeated 10 times to ensure effective decellularization.

Washing: Following the freeze-thaw cycles, the bone was washed extensively with phosphate-buffered saline (PBS) to remove any cellular debris or residual chemicals from the decellularization process.

Grinding: The decellularized bone was freeze-dried to remove residual moisture, then ground to a fine powder in a ball mill for further characterization. [12].

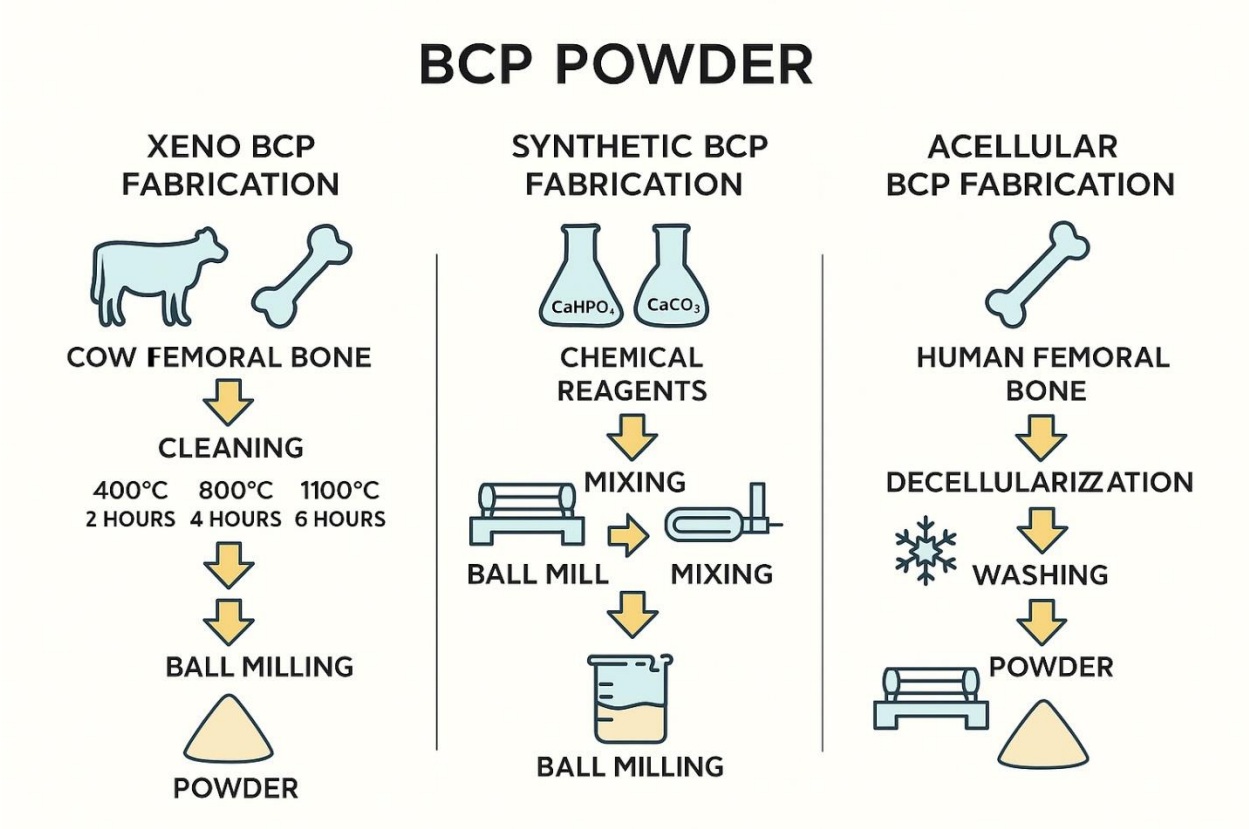


Fig. 1. Fabrication of Synthetic-BCP, Xeno-BCP and Acellular-BCP Powders.

2-7-Characterization of BCP Powders:

2-7-1-Fourier Transform Infrared Spectroscopy (FTIR):

FTIR analysis (Thermo Scientific™ Nicolet) was performed to determine the chemical composition and functional groups of the BCP powders. The powders were analyzed in the range of 4000–400 cm^{-1}. The spectra were compared with standard reference spectra for HAp and β-TCP to identify characteristic peaks. [13].

2-7-2-X-ray Diffraction (XRD):

XRD analysis using a Cu Kα radiation source (λ = 1.5406 Å) on an XRD system (Anton Paar, XRDynamic 500) by (PANalytical X'Pert PRO) was conducted to examine the crystallinity and phase composition of the BCP powders. The diffraction patterns were recorded over 20°–50° in 2θ with a scanning rate of 2°/min. The obtained patterns were compared with reference standards for HAp and β-TCP to confirm the presence of these phases. [14].

2-7-3-Inductively Coupled Plasma (ICP):

The elemental composition of the BCP powders was quantified using ICP (PerkinElmer Optima series, USA). The powders were dissolved in a mixture of concentrated nitric acid, and the resulting solutions were analyzed for calcium and phosphate content by ICP-OES. [15].

2-7-4-Biodegradation Testing:

Biodegradation of the BCP powders was evaluated by immersing the powders in simulated body fluid (SBF) at 37°C. pH changes of the SBF solution (SBF powder, Merk, Germany) with solution ratio: 1

mg/mL were recorded at weekly intervals over a period of 1 to 7 weeks. The pH was measured using a calibrated pH meter. [16].

2-7-5-MTS Assay:

The MTS assay was employed to assess the cytotoxicity and metabolic activity of the BCP powders. Human osteoblast-like cells (MG-63) were cultured in a 96-well plate and exposed to BCP powders for 24, 48, and 72 hours. MG-63 osteoblast-like cells were seeded at a density of 1×10^4 cells per well in 96-well plates. BCP powders were applied at a concentration of 100 μg/mL, and cell viability was evaluated after 24, 48, and 72 h using the MTS assay according to the manufacturer's protocol. Absorbance was measured at 490 nm using a microplate reader (Varioskan LUX, multimode microplate reader). [17].

2-7-6-Alizarin Red Staining:

Osteogenic differentiation of MG-63 cells was assessed using alizarin red staining to detect calcium deposition. The cells were cultured in osteogenic medium for 21 days, with media changes every 3 days. At the end of the culture period, the cells were fixed in 4% paraformaldehyde and stained with 2% (w/v) Alizarin Red S solution (pH 4.2) (Merk, Germany) for 20 min at room temperature to visualize the mineralized nodules they formed. [18].

2-7-7- Quantitative Biodegradation Measurement

To quantitatively evaluate biodegradation behavior, the mass loss of BCP powders during immersion in simulated body fluid (SBF) was determined. Briefly, 20 mg of each BCP powder was immersed in 20 mL of SBF and incubated at 37 °C for up to 6 weeks. At predetermined time points (1, 3, and 6 weeks), samples were collected, rinsed thoroughly with deionized water to remove residual ions, and dried at 60 °C for 24 h. The remaining dry mass was recorded using an analytical balance. The percentage mass loss (degradation percentage) was calculated using the following equation:

$$\text{Degradation (\%)} = \frac{W_0 - W_t}{W_0} \times 100$$

where $W_0$is the initial dry weight and $W_t$is the dry weight after immersion time t.

All experiments were performed in triplicate (n = 3) unless otherwise stated. Quantitative data are presented as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p < 0.05$ considered statistically significant.

3-Results and Discussion

3-1-FTIR Analysis

The Fourier Transform Infrared Spectroscopy (FTIR) spectra of Xeno, Synthetic, and Acellular BCP powders were recorded to characterize their chemical composition and functional groups. The FTIR spectra of all three groups exhibited characteristic peaks corresponding to the functional groups of hydroxyapatites (HAp) and β-tricalcium phosphate (β-TCP), the primary components of BCP. The peaks around 1010 cm^{-1} and 570 cm^{-1} were observed, which are attributed to the phosphate (PO_4^{3-}) group in HAp and β-TCP. Additionally, the Xeno and Acellular BCP powders exhibited slight differences in the hydroxyl (OH) stretch band around 3570 cm^{-1}, suggesting a higher presence of hydroxyapatite in these groups compared to Syntethic BCP, bands around 470 cm^{-1} indicate phosphate (PO_4^{3-}) bending vibrations, as these compounds often exhibit low-frequency vibrations in this range, where the characteristic peaks were slightly shifted, indicating a lower degree of hydroxyapatite content.

The FTIR analysis confirms the presence of key functional groups indicative of the successful fabrication of BCP powders. Acellular BCP showed the strongest OH band, which is associated with better crystallinity and a higher content of hydroxyapatite, reflecting its superior osteoconductivity and compatibility for bone graft applications. Xeno BCP exhibited a similar but slightly less intense OH stretch, suggesting a lower but still significant hydroxyapatite presence. In contrast, Synthetic BCP showed less pronounced hydroxyl stretching, indicating a less structured composition, consistent with other studies showing that synthetic BCP typically has lower bioactivity and osteoinductivity than natural BCPs [19].

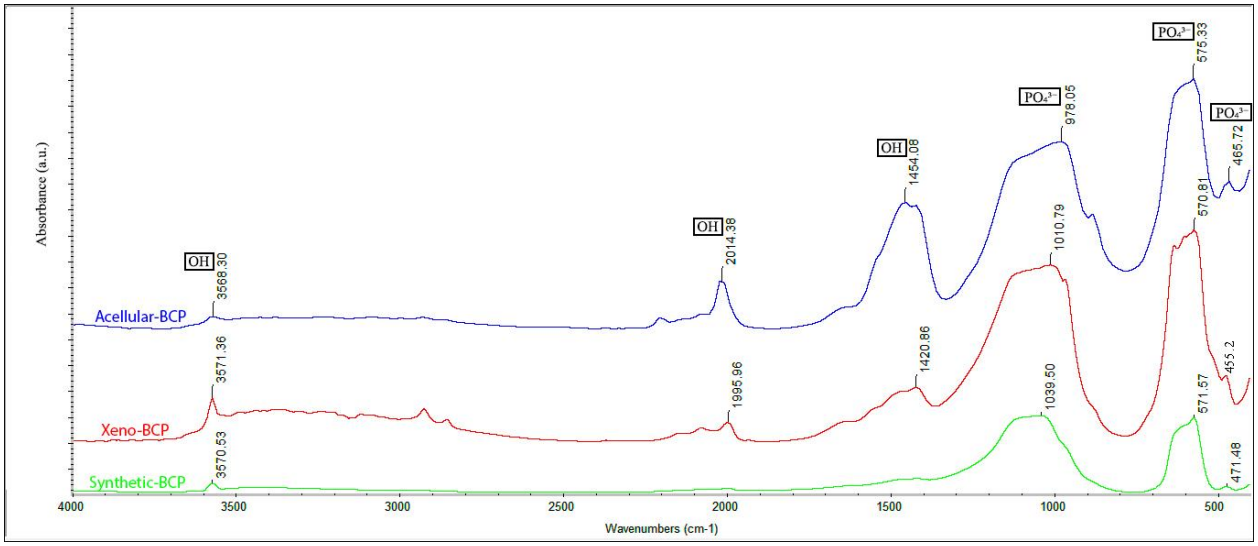


Fig. 2. PO_4^{3-} and OH Group in FTIR Spectra of Synthetic-BCP, Xeno-BCP, and Acellular-BCP Powders.

3-2-XRD Analysis

X-ray Diffraction (XRD) patterns were used to analyze the crystalline phases of the BCP powders. Acellular BCP demonstrated sharp peaks corresponding to the β-TCP and HAp phases, with a higher degree of crystallinity compared to Xeno BCP. Acellular BCP displayed firm diffraction peaks at 2θ values of 26.4° and 31.8°, corresponding to the HAp (Hydroxyapatite) and β-TCP (β-tricalcium phosphate) phases, respectively. These peaks were sharp and well-defined, indicating better crystallinity. The distinct diffraction patterns observed in the Acellular BCP samples reflect a more ordered crystalline structure, which is essential for enhancing bioactivity and osteoinductive potential. The HAp phase at 26.4° (peaks pattern analyzed based on JCPDS Card No. 74-0566) and the β-TCP phase at 31.8° (peaks pattern analyzed based on JCPDS Card No. 09-0169) are critical for bone integration, further emphasizing the superior bone regeneration capacity of Acellular BCP.

In contrast, Xeno BCP, while showing similar peaks at 26.4° and 31.8°, exhibited slightly lower intensity. This reflects a less crystalline structure and suggests that the naturally derived Xeno BCP powder possesses reduced crystallinity compared to the Acellular BCP. This lower intensity of the diffraction peaks indicates a less ordered structure, consistent with the generally observed lower bioactivity and mechanical properties in natural-derived BCP powders.

Synthetic BCP had the broadest peaks, indicating poor crystallinity and a predominantly amorphous material. This broadness in the peaks suggests that synthetic BCP lacks the well-defined crystalline structure observed in Acellular BCP, which correlates with its reduced bioactivity and mechanical properties. The XRD results indicate that Acellular BCP has superior crystallinity and a more defined structure than Xeno BCP, contributing to its higher bioactivity and osteoinductive potential. Xeno BCP showed relatively lower crystallinity, consistent with other studies indicating that naturally derived BCP powders, such as Xeno, often exhibit lower crystallinity and bioactivity than acellular bone materials. Synthetic BCP, owing to its artificial origin, showed the lowest crystallinity, which correlates with its reduced bioactivity and mechanical properties. All major diffraction peaks were indexed and labeled in Figure 3 according to their corresponding HAp and β-TCP phases by Miller indices. [20].

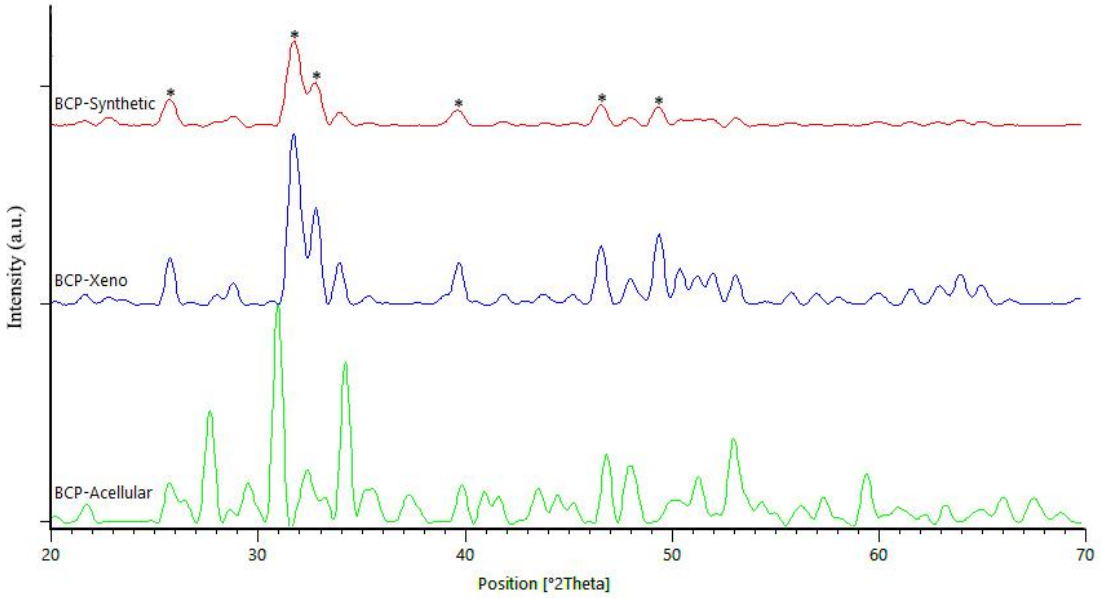
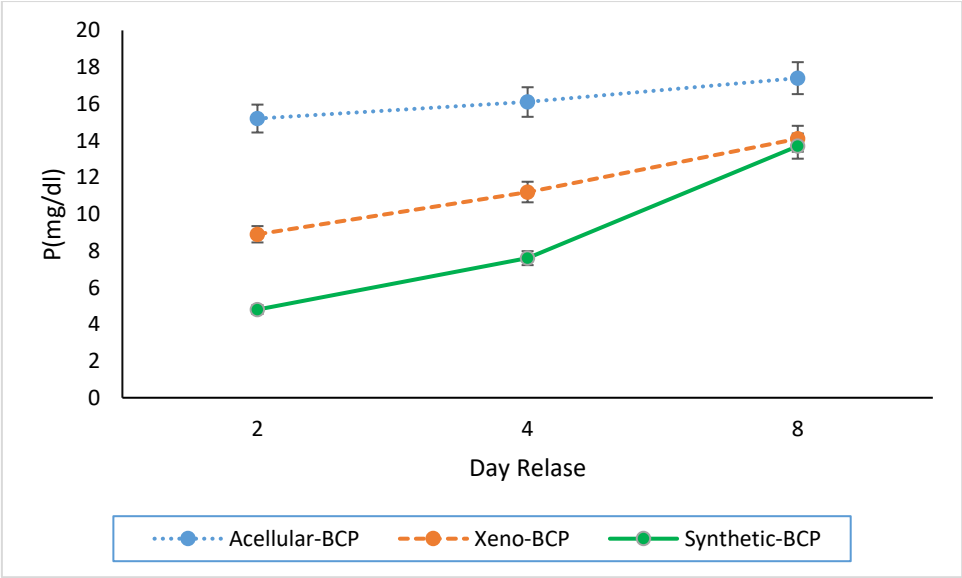


Fig. 3. Major diffraction peaks were indexed by * in XRD patterns of Synthetic-BCP, Xeno-BCP, and Acellular-BCP Powders.

3-3-ICP Analysis

Inductively Coupled Plasma (ICP) analysis was used to assess the elemental composition of the BCP powders, specifically calcium (Ca) and phosphorus (P) concentrations. Acellular BCP exhibited the highest calcium-to-phosphorus ratio (Ca/P), which is typical of naturally derived BCPs and is essential for optimal bone bonding. Xeno BCP also had a high Ca/P ratio, but it was slightly lower than Acellular BCP. Synthetic BCP showed the lowest Ca/P ratio, suggesting that the synthesis method might not ideally mimic the natural composition of bone mineral. ICP results reveal that Acellular BCP has a composition that is closer to that of natural bone, making it the most favorable for bone regeneration. Xeno BCP also demonstrates a favorable Ca/P ratio but is slightly less optimized for bone mineralization compared to Acellular BCP. Synthetic BCPs with lower Ca/P ratios correlate with lower bioactivity, as commonly observed in synthetic BCPs that do not closely replicate natural bone composition. [21].



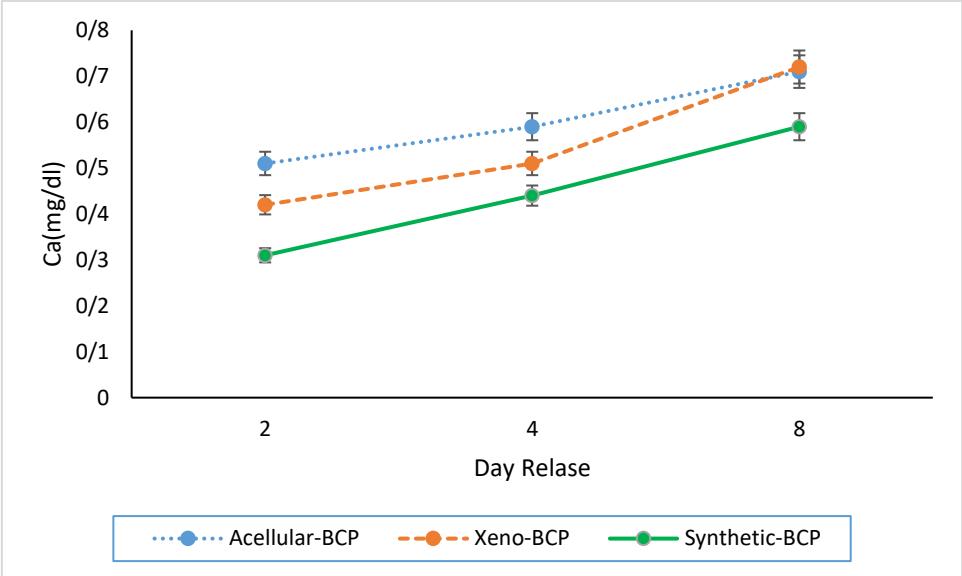


Fig. 4. Calcium (Ca) and Phosphorus (P) Concentrations Release of Synthetic-BCP, Xeno-BCP, and Acellular-BCP Powders.

3-4-Biodegradation

The biodegradation behavior of the BCP powders was assessed by monitoring pH changes in Simulated Body Fluid (SBF) over 6 weeks. Acellular BCP exhibited a slow degradation rate and minimal pH fluctuations, indicating good stability and controlled degradation, which are essential for its application in bone grafting. Xeno BCP showed slightly higher degradation, with a more pronounced decrease in pH, while Synthetic BCP had the highest degradation rate, resulting in a significant drop in pH.

The drop in pH during the first three weeks of degradation can be attributed to the initial dissolution of calcium phosphate phases, such as HAp and β-TCP, which release ions into the surrounding fluid. This release leads to an acidic environment in the early stages, as documented in various studies. In the case of synthetic materials, this process is often accelerated by their more amorphous nature, leading to higher degradation rates than those of naturally derived BCPs.

After the initial pH drop, the pH gradually rises, indicating a stabilization phase. This behavior is typically due to the formation of a protective layer or to the neutralization of the acidic environment as degradation slows. Studies suggest that as the material continues to degrade, the acidic byproducts are either consumed in further degradation reactions or precipitate out, leading to a more neutral or basic pH environment as the system approaches equilibrium. In Acellular BCP, this stabilization is more pronounced due to its slower, more controlled degradation rate, as the decellularization process typically preserves the material's structural integrity longer.

The degradation behavior of Acellular BCP is consistent with other studies, which show that decellularized BCP materials tend to degrade more slowly and maintain their structural integrity longer than synthetic BCPs. Xeno BCP's degradation rate, though slower than Synthetic BCP, was higher than Acellular BCP, suggesting a lower stability in biological environments. The accelerated degradation of Synthetic BCP is consistent with synthetic materials, which tend to degrade more rapidly under physiological conditions.[22].

Quantitative biodegradation analysis revealed distinct degradation behaviors among the three BCP groups (Figure X). Synthetic-BCP exhibited the highest degradation rate, with a mass loss of approximately 18–22% after 6 weeks, reflecting its lower crystallinity and higher solubility. Xeno-BCP showed a moderate degradation profile, with a mass loss of 10–14%, indicating improved structural stability compared to Synthetic-BCP.

In contrast, Acellular-BCP demonstrated the lowest degradation rate, with only 5–8% mass loss over the same period. This reduced degradation is attributed to its preserved native mineral architecture and higher crystallinity, which slow dissolution in physiological environments. The quantitative mass loss results correlate well with the observed pH stabilization trends, confirming that Acellular-BCP undergoes a more controlled and gradual degradation process.

Overall, the degradation rate followed the order: Synthetic-BCP > Xeno-BCP > Acellular-BC

This controlled biodegradation behavior is desirable for bone graft applications, as it allows sufficient time for new bone formation while maintaining temporary mechanical support.

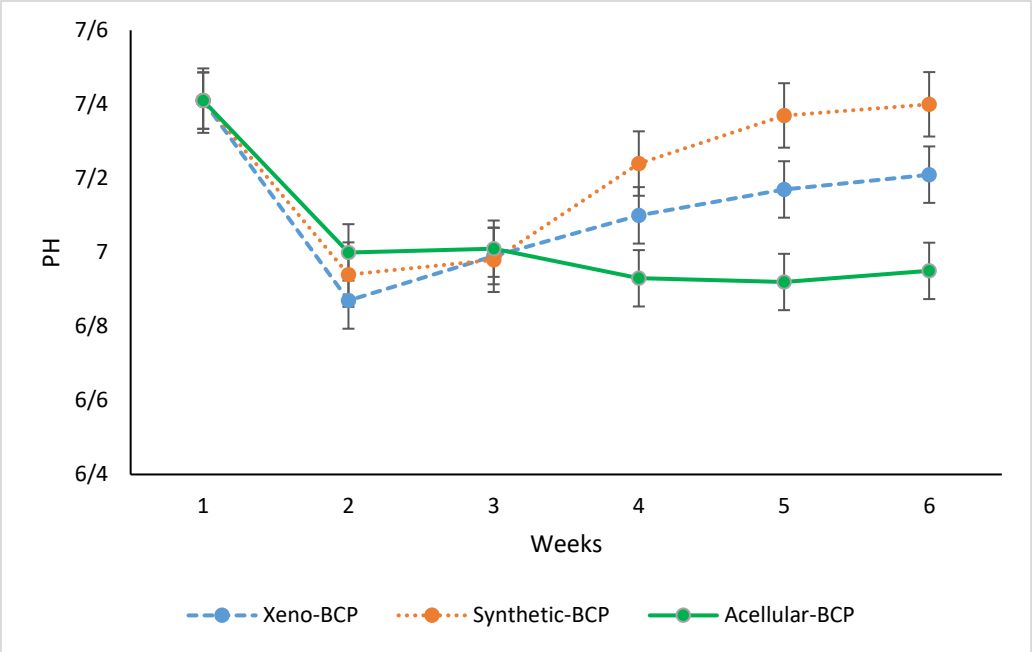


Fig. 5. PH Change in Synthetic-BCP, Xeno-BCP, and Acellular-BCP Powders.

3-5-MTS Assay

The MTS assay was conducted to evaluate cell metabolic activity and cytotoxicity. Acellular BCP demonstrated the highest cell viability and metabolic activity, followed by Xeno BCP, with Synthetic BCP exhibiting the lowest activity. Acellular BCP promoted cell proliferation and metabolic activity, suggesting excellent biocompatibility. Xeno BCP, while still supportive of cell growth, showed slightly lower metabolic activity than Acellular BCP. Synthetic BCP showed the lowest support for cell metabolism, suggesting potential cytotoxicity. The MTS assay results indicated that Acellular BCP, with its closer composition to natural bone, exhibited superior cell viability and metabolic activity compared to Xeno BCP. These findings align with other studies, which have shown that acellular materials derived from natural bone tend to support better cellular activity and proliferation than synthetic alternatives. Synthetic BCP's poor performance can be attributed to its lower crystallinity and less favorable composition for osteoblast growth, as synthetic materials often lack the biological signals present in natural bone. [23].

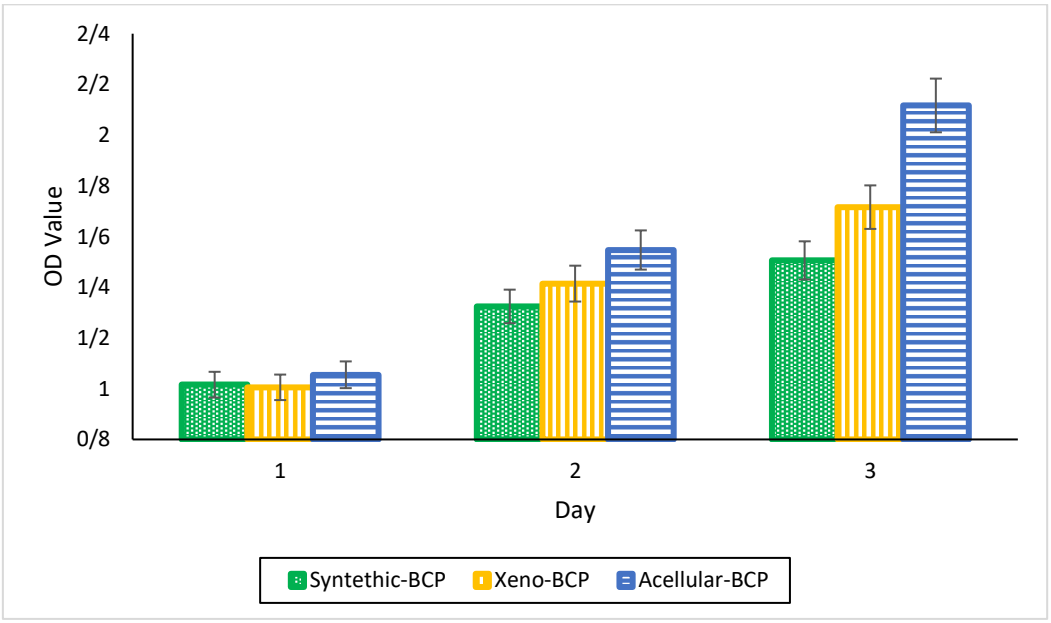


Fig. 6. MTS Assay in Synthetic-BCP, Xeno-BCP, and Acellular-BCP Powders.

3-6-Alizarin Red Staining

Alizarin Red staining was performed to assess mineralization and the ability of BCP powders to induce osteogenic differentiation. Acellular BCP showed the highest degree of mineralization, with dense calcium deposits, indicating superior osteoinductive potential. Xeno BCP showed moderate mineralization, while Synthetic BCP exhibited the least mineralization, suggesting that it has the lowest osteogenic potential.

The Alizarin Red staining results support the findings that Acellular BCP is the most effective in promoting osteogenic differentiation, a crucial factor for bone regeneration. This finding is consistent with studies indicating that decellularized bone materials are more osteoinductive than both synthetic and natural BCPs, owing to their ability to retain the organic matrix and biological cues that promote bone formation. Xeno BCP, while still capable of inducing some degree of mineralization, was less effective than Acellular BCP, which can be attributed to the absence of decellularized bone matrix in Xeno. Synthetic BCPs' lower mineralization aligns with their inferior biological performance and lower potential for bone healing. [24, 25].

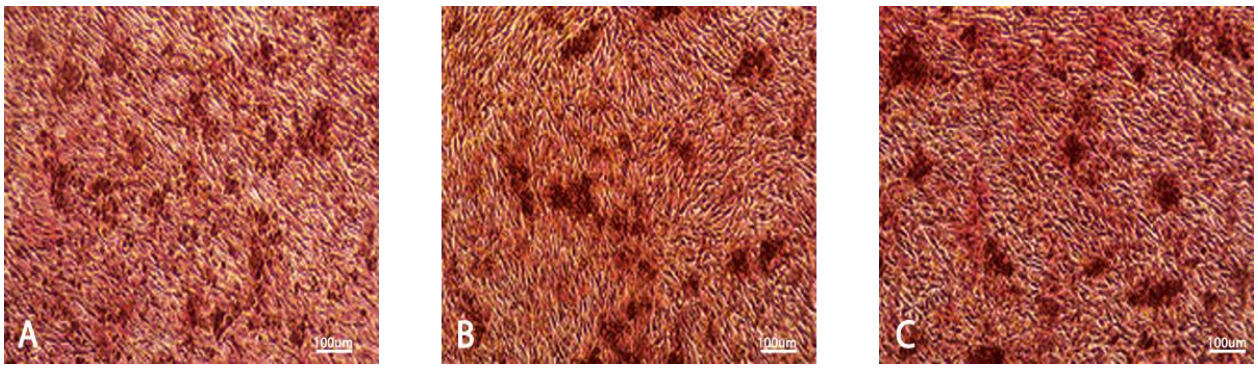


Fig. 7. Alizarin Red staining in A: Synthetic-BCP, B: Xeno-BCP, and C: Acellular-BCP Powders.

4-Conclusion:

In summary, the results of this study clearly indicate that Acellular BCP outperforms both Xeno BCP and Synthetic BCP in most of the analyzed tests, including crystallinity, chemical composition, degradation rate, cell viability, and osteoinductive potential. Acellular BCP, produced through a non-thermal process, demonstrated the highest osteoinductive potential and cell viability, making it the most promising material for bone regeneration. The non-thermal process contributes to superior bioactivity, significantly enhanced osteogenic potential, and slower degradation, positioning Acellular BCP as an ideal candidate for bone tissue engineering applications.

The present study systematically compared xeno-derived, synthetic, and acellular biphasic calcium phosphate (BCP) powders fabricated using different processing strategies. Based on the physicochemical characterization and in vitro biological evaluations, the key findings can be summarized as follows:

1. Acellular BCP exhibited the highest osteogenic performance, demonstrating superior cell viability, metabolic activity, and mineralization compared to Xeno-BCP and Synthetic-BCP. This behavior is attributed to its non-thermal processing route, which better preserves native bone-like mineral characteristics.

2. Xeno-BCP showed intermediate performance, with favorable crystallinity and chemical composition resembling natural bone. While its biological activity was lower than that of Acellular-BCP, it remained superior to Synthetic-BCP, indicating its suitability for applications requiring balanced stability and bioactivity.

3. Synthetic-BCP displayed the lowest crystallinity and bioactivity, as evidenced by broader XRD peaks, reduced Ca/P ratio, faster degradation, and lower cell metabolic activity. These results suggest that purely synthetic fabrication routes may lack essential biological cues required for optimal osteogenic response.

4. Quantitative biodegradation analysis revealed controlled degradation behavior, following the order Synthetic-BCP > Xeno-BCP > Acellular-BCP. The slower degradation of Acellular-BCP is advantageous for maintaining structural integrity during bone regeneration.

5. Overall, fabrication strategy and processing temperature were identified as critical determinants of BCP performance, with non-thermal acellular processing offering clear advantages in preserving bioactivity and osteogenic potential.

In conclusion, Acellular-BCP emerges as the most promising candidate for bone tissue engineering applications, while Xeno-BCP may be suitable for scenarios requiring higher crystallinity and controlled resorption. Further in vivo investigations

are warranted to validate these findings and assess long-term performance in clinical settings.

Further investigation, including in vivo testing, is necessary to confirm these findings and evaluate the long-term performance and stability of these materials in clinical applications. Ultimately, this study highlights the importance of selecting an appropriate BCP composition tailored to specific clinical needs, with Acellular BCP emerging as the most promising material for bone regeneration.

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