Hydroxyapatite as a Driver of Osteogenic Differentiation in Mesenchymal Stem Cells: A Review of Molecular Mechanisms, Biomaterial Design, and Translational Prospects

A Review

Amir Hossein Tahmasbi [1] _ Mohammad Velashjerdi [2]*
1: Amol University of special modern technologies
Email: amirhossein.tahmabi@ausmt.ac.ir
2: University of Arak
Email: m-velashjerdi@araku.ac.ir

Abstract

Hydroxyapatite (HA), the calcium phosphate mineral that constitutes the inorganic phase of bone, is among the most widely used bioceramics for guiding mesenchymal stem cell (MSC) commitment toward the osteoblastic lineage. This review synthesizes current understanding of how HA drives osteogenic differentiation, covering the biomolecular mechanisms, ionic dissolution and calcium/phosphate signaling, integrin-mediated focal adhesion, and activation of the Wnt/β-catenin, BMP/Smad, and MAPK (ERK1/2, p38) pathways, alongside the biomaterial design strategies that exploit these mechanisms, including porous and three-dimensionally printed scaffolds, polymer–HA composites, ion-substituted HA (strontium, magnesium, zinc), and HA coatings for metallic implants. Clinical and translational applications in orthopedics, craniofacial reconstruction, and dental implantology are discussed alongside persisting barriers, including scaffold degradation kinetics, vascularization of large defects, and the gap between preclinical efficacy and regulatory approval. The review concludes that continued integration of nanostructural control, ion doping, and additive manufacturing represents the most promising route toward clinically translatable, osteoinductive HA based bone graft substitutes.

Key words*: Hydroxyapatite, Mesenchymal stem cells (MSCs), Osteogenic differentiation, Bone tissue engineering, Biomaterial scaffolds*

1. Introduction

Bone defects arising from trauma, tumor resection, osteoporosis, or congenital malformation remain a substantial clinical burden, and autologous bone grafting while considered the clinical gold standard, is constrained by donor-site morbidity and limited tissue availability[1]. Bone tissue engineering seeks to overcome these limitations by combining osteogenic cells, bioactive scaffolds, and, where appropriate, growth factors to regenerate functional bone[2]. Mesenchymal stem cells (MSCs), whether derived from bone marrow, adipose tissue, or umbilical cord, are the most extensively investigated cell source for this purpose because of their capacity for self-renewal and multilineage differentiation, including toward osteoblasts, chondrocytes, and adipocytes [3].

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is the principal mineral component of vertebrate bone and tooth enamel[4] and has been used clinically as a bone-graft substitute and implant coating for decades because of its excellent biocompatibility, osteoconductivity, and chemical similarity to native bone mineral[4]. Beyond serving as a passive scaffold, however, a substantial body of evidence indicates that HA actively instructs MSC fate: its dissolution products, surface chemistry, and nano/microstructure engage specific cell-surface receptors and intracellular signaling cascades that converge on osteogenic transcription factors such as Runx2 and Osterix [5]. Understanding these mechanisms has, in turn, informed the rational design of HA-based biomaterials, from ion-doped ceramics to three-dimensionally printed composite scaffolds, intended to enhance osteoinduction in clinical bone repair [6].

This review provides a broad overview of both dimensions of this field: (i) the mechanisms and methides that HA can be synthesized. (ii)How exactly HA promotes osteogenic differentiation of MSCs, and (iii) the biomaterial engineering strategies that translate these mechanisms into clinically relevant scaffolds and coatings.

2. Physicochemical Properties of Hydroxyapatite Relevant to Bioactivity

Synthetic HA can be produced with a wide range of crystallinities, grain sizes, and Ca/P ratios, all of which influence its solubility, consequently, and its bioactivity. Nanoscale HA (nHA), with grain sizes closer to the ~20–80 nm apatite crystallites found in natural bone, generally exhibits greater surface area, higher protein adsorption capacity, and faster ionic exchange with the surrounding fluid than micron-scale or highly sintered HA [7]. These properties translate into more favorable cell attachment, spreading, and osteogenic gene expression, as nanostructured HA surfaces better mimic the topographical cues of the native extracellular bone matrix [8]. Porosity and pore interconnectivity are equally important, since macropores (typically several hundred micrometers) permit vascular ingrowth and nutrient/waste exchange, while micro- and nanoporosity influence protein adsorption and early cell adhesion.

3. Synthesis of Hydroxyapatite: Hydrothermal and Sol-Gel Methods

The biological performance of HA described throughout this review: its solubility, ion-release kinetics, crystallinity, and surface nano/microstructure, is set largely at the point of synthesis. Among the many routes used to prepare HA (wet chemical precipitation, solid-state reaction, mechanochemical processing, biomimetic mineralization, and others), the hydrothermal and sol-gel methods are two of the most widely used for producing biomedical-grade HA because both allow fine control over crystal morphology, phase purity, and, in the case of sol-gel processing, thin-film deposition onto implant surfaces.

3.1 Hydrothermal Synthesis

Hydrothermal synthesis is a wet-chemical technique in which calcium and phosphate precursors react in aqueous solution inside a sealed autoclave at elevated temperature (commonly 100–200°C) and correspondingly elevated autogenous pressure. Under these conditions, HA nucleates and grows directly from solution, typically without requiring the post-synthesis calcination step needed by many precipitation-based methods, which allows the reaction to yield highly crystalline HA with fewer hydroxyl-site defects than powders produced at ambient pressure [9]. Common precursor pairs include calcium nitrate or calcium hydroxide with diammonium hydrogen phosphate or orthophosphoric acid, and, in some formulations, organic calcium chelates such as calcium lactate pentahydrate, which decompose in situ under hydrothermal conditions to release the calcium needed for apatite formation [10].

The principal appeal of the hydrothermal route is the degree of control it affords over particle morphology. By varying reaction pH, temperature, pressure, reagent concentration, and dwell time, researchers can steer HA crystal growth toward rods, whiskers, hexagonal prisms, or nanorods, morphologies that are difficult to access through simple precipitation [10]. Response-surface and other statistical optimization approaches have been used to systematically map how pH, temperature, and hydrothermal treatment time jointly determine the yield, particle size, and crystallinity of the resulting HA powder, with alkaline pH consistently identified as the dominant factor promoting higher yield and crystallinity [11]. Because the resulting particles are typically well-crystallized and elongated, hydrothermally synthesized HA is frequently selected as a filler for polymer–HA composites and as a feedstock for scaffold fabrication, including the 3D-printed and porous constructs discussed later in this review, where predictable particle geometry and slower, controlled dissolution are advantageous for sustained ion release during MSC culture. The main drawbacks of the method are the need for specialized pressure-rated autoclave equipment, comparatively long reaction times, and limited throughput relative to simpler precipitation routes[8].

3.2 Sol-Gel Synthesis

The sol-gel method proceeds by first preparing a homogeneous liquid "sol" from calcium and phosphorus precursors; typically, calcium nitrate tetrahydrate paired with a phosphorus alkoxide such as triethyl phosphite, or with phosphoric acid. That are dissolved in water or water/ethanol solvent system. Hydrolysis and polycondensation reactions progressively link the precursor species into an inorganic network, transforming the sol into a gel, which is then dried and thermally treated to crystallize the apatite phase. Because reactants are mixed at the molecular level in solution, the sol-gel route achieves intimate, homogeneous contact between calcium and phosphorus species and can produce phase-pure, nanoparticle-

sized HA with narrower size distributions and lower processing temperatures than are typically required for solid-state or plasma-based methods [12].

Solvent choice and processing parameters strongly influence the outcome: ethanol-based sol-gel routes tend to favor formation of a thermally stable, stoichiometric HA phase, whereas purely water-based systems more often yield calcium-deficient apatite, and the mixture pH exerts a dominant influence on which calcium phosphate phase nucleates first. A particular strength of the sol-gel approach is its suitability for thin-film deposition directly onto complex or heat-sensitive substrates, most notably titanium and titanium-alloy implants. Because sol-gel HA films can be crystallized at substantially lower temperatures than plasma-sprayed coatings, they avoid the oxidation, phase transformation, and thermal-mismatch defects that high-temperature deposition methods can introduce into the underlying metal, while still producing uniform, well-adhered, and bioactive HA coatings suitable for enhancing osseointegration [13]. Ion substitution (e.g., fluoride, strontium) and composite reinforcement can also be readily incorporated at the sol stage, offering a convenient route to the ion-doped HA formulations discussed later in this review. The principal limitations of sol-gel synthesis are its sensitivity to process parameters, precursor concentration, solvent ratio, pH, and aging time all require careful optimization and the risk of cracking or delamination in coatings if drying and sintering are not carefully controlled.

Taken together, the hydrothermal and sol-gel methods occupy complementary niches within HA biomaterial fabrication: hydrothermal synthesis is generally favored when highly crystalline, morphologically controlled bulk powders or particulate fillers are required, while sol-gel processing is favored when homogeneous nanoscale powders or thin, conformal implant coatings are the desired end product. Both routes ultimately feed into the mechanistic and biomaterial design considerations discussed in the remainder of this review, since the crystallinity, particle morphology, and surface characteristics established during synthesis directly determine the ionic dissolution behavior, protein adsorption, and topographical cues that drive MSC osteogenic differentiation.

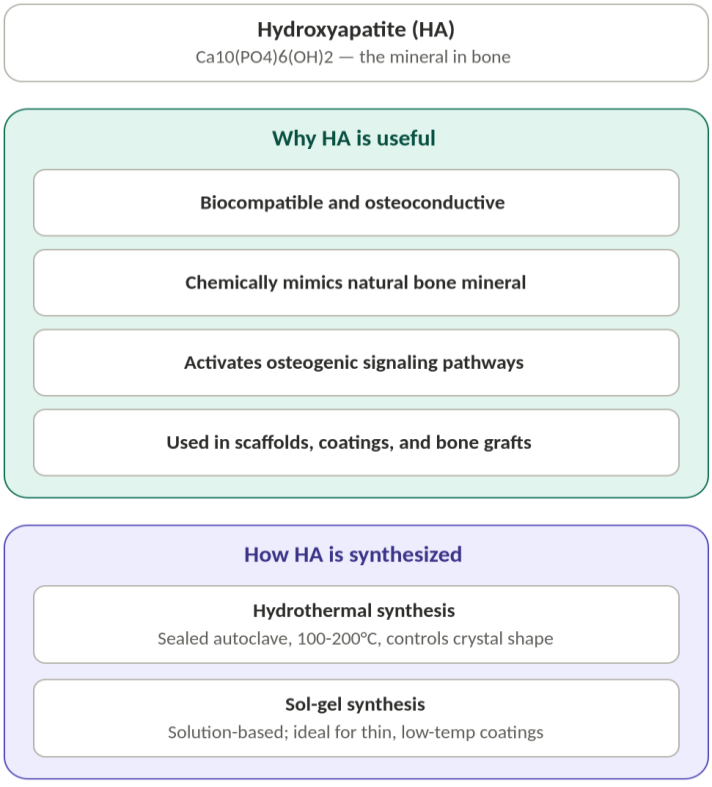


Figure 1. Summary of hydroxyapatite's key bioactive properties and its two principal synthesis routes.

4. Mechanisms of HA-Induced Osteogenic Differentiation of MSCs

4.1 Ion Release and Calcium/Phosphate Signaling

As HA degrades or exchanges ions with the culture microenvironment, it releases calcium (Ca^{2+}) and phosphate ($PO4^{3-}$) ions that act as direct signaling cues rather than merely structural building blocks. Extracellular calcium modulates gene transcription of bone morphogenetic proteins, including BMP2 and BMP6, in human MSCs, and calcium-phosphate substrates have been shown to upregulate osteoblast-specific genes even in the absence of exogenous osteogenic supplements, implicating a calcium-sensing-receptor-mediated signaling route that intersects with downstream MAPK and JAK2/STAT3 cascades. Released phosphate ions independently influence osteopontin transcription through glucocorticoid-receptor-linked pathways, indicating that the two principal constituent ions of HA act on at least partially distinct regulatory nodes.

4.2 Integrin-Mediated Adhesion and Focal Adhesion Signaling

MSC attachment to HA surfaces is mediated principally by integrin receptors that bind adsorbed extracellular matrix proteins (fibronectin, vitronectin) rather than the ceramic directly. Engagement of $\alpha_5\beta_1$ and $\alpha v\beta_3$ integrin subunits nucleates focal adhesion complexes containing talin, vinculin, and focal adhesion kinase (FAK), which couple mechanical and biochemical cues from the HA surface to downstream cytoskeletal remodeling and transcriptional programs governing osteoblastic commitment. Calcium phosphate substrates have been reported to increase the mobility and turnover of focal adhesions relative to tissue-culture plastic, consistent with a functionally active adhesion state that favors osteogenic rather than proliferative signaling. Notably, distinct HA surface architectures preferentially activate different integrin subunits, suggesting that surface engineering can be used to bias the specific adhesion-signaling route engaged by MSCs.

4.3 Wnt/β-Catenin and BMP/Smad Pathways

The canonical Wnt/β-catenin pathway is a central regulator of osteoblast differentiation, acting through stabilization and nuclear translocation of β-catenin to promote Runx2 and Osterix expression. HA-gold nanocomposites and other functionalized HA surfaces increase β-catenin, Runx2, osteocalcin, and osteopontin expression in cultured MSCs in a manner dependent on Wnt/β-catenin signaling, while pharmacological inhibition of this pathway attenuates HA-induced mineralization[14]. In parallel, HA and HA-composite surfaces activate BMP/Smad signaling: nano-HA coatings on porous calcium phosphate ceramics upregulate BMP receptor type I (BMPRI) together with Smad1, Smad4, and Smad5, driving osteogenic gene expression and ectopic bone formation in vivo[15]. Because Wnt and BMP signaling regulate osteoblast differentiation simultaneously and interact at multiple nodes, HA-induced activation of one pathway frequently potentiates the other, contributing to the overall osteoinductive effect of the material.

4.4 MAPK Signaling (ERK1/2 and p38)

Mitogen-activated protein kinase cascades, particularly ERK1/2 and p38, act downstream of both integrin engagement and calcium-sensing receptor activation to phosphorylate Runx2 and enhance its transcriptional activity. Integrated transcriptomic, proteomic, and microRNA analyses of MSCs cultured on natural HA implicate ERK1/2 and JNK MAPK signaling, together with microRNA-mediated repression of pro-adipogenic targets, in tipping the balance of MSC differentiation away from the adipogenic lineage and toward the osteogenic lineage, illustrating that HA-induced osteogenesis is achieved partly through active suppression of a competing differentiation program rather than osteogenic activation alone.

4.5 Surface Topography and Micro-/Nanostructure Effects

Independent of chemical composition, the physical topography of HA surfaces is itself a potent regulator of MSC fate. Hydrothermally engineered micro/nano-hybrid HA bioceramics activate integrin

expression, BMP2 signaling, and connexin-43-mediated gap-junction communication through distinct mechanisms depending on whether micro- or nano-scale features dominate, producing a synergistic osteogenic effect when both are combined [8]. At larger length scales, hierarchical micro-/nanostructured HA scaffolds have been reported to promote osteogenic lineage commitment in osteoporotic bone marrow-derived MSCs through activation of Hedgehog and HIF-1α signaling, restoring osteogenic capacity that is otherwise impaired under osteoporotic conditions. These findings collectively establish that both nanoscale roughness and hierarchical micro/nano patterning function as mechanotransductive and biochemical cues that can be engineered independently of bulk material chemistry.

5. Biomaterial Design Strategies Exploiting HA Osteoinduction

5.1 Porous and 3D-Printed HA Scaffolds

Additive manufacturing allows fabrication of patient-specific HA scaffolds with precisely controlled pore geometry, interconnectivity, and mechanical properties. Three-dimensionally printed HA scaffolds, including biomimetic calcium-phosphate-coated variants, support attachment, proliferation, and osteogenic differentiation of both bone-marrow and umbilical-cord-derived MSCs, with performance influenced by pore size, surface coating, and cell source [16]. Perfusion-based dynamic culture within macroporous HA scaffolds further enhances osteogenic marker expression relative to static culture by improving nutrient transport and applying physiologically relevant mechanical stimulation, underscoring that scaffold architecture and culture conditions act synergistically with the intrinsic bioactivity of HA.

5.2 Polymer–HA Composite Scaffolds

Because sintered HA is intrinsically brittle, it is frequently combined with natural or synthetic polymers; collagen, chitosan, alginate, polylactic acid (PLA), and poly (lactic-co-glycolic acid) (PLGA); to improve toughness, degradability, and handling while retaining osteoconductivity. Trimodal nano-HA/chitosan/PLGA scaffolds have been shown to outperform simpler formulations in supporting attachment, proliferation, and osteogenic marker expression of umbilical-cord MSCs, and chitosan–nano-HA composites more broadly show consistent in vivo bone-regenerative capacity across a range of defect models [17]. Collagen–HA and HA–mineralized-collagen composites similarly promote osteogenic gene expression profiles beyond what either component achieves alone, reflecting a complementary relationship between the organic scaffold, which supports cell adhesion and remodeling, and the inorganic HA phase, which supplies osteoinductive ionic and topographical cues.

5.3 Ion-Substituted Hydroxyapatite

Doping HA with therapeutically relevant trace ions is a widely used strategy to augment its intrinsic osteoinductivity. Strontium substitution is the most extensively studied example: strontium-doped HA (Sr-HA) increases HA solubility and provides sustained Sr^{2+} release, which promotes osteogenic differentiation of bone-marrow MSCs while simultaneously suppressing osteoclast formation and activity, an effect mediated in part through calcium-sensing-receptor–JAK2/STAT3 signaling and shown to be effective even in osteoporotic animal models [18, 19]. Sr-doped HA embedded in polysaccharide or alginate-hydrogel matrices similarly supports osteoblastic differentiation of human MSCs in vitro and induces ectopic osteoid and vascular tissue formation in vivo, without evidence of cytotoxicity across a range of substitution levels [20]. Magnesium and zinc substitution have been explored analogously, generally reported to enhance early cell adhesion and osteogenic marker expression, and to confer secondary benefits such as antibacterial activity, although the optimal doping concentration is compound- and application-specific and excessive substitution can compromise crystallinity or induce cytotoxicity.

5.4 HA Coatings on Metallic Implants

Titanium and titanium-alloy implants remain the standard for load-bearing dental and orthopedic fixtures because of their mechanical strength and corrosion resistance, but bare titanium is only weakly

osteoconductive. Coating titanium surfaces with HA; via plasma spraying, detonation spraying, atomic layer deposition, or dip-coating, substantially improves osseointegration by promoting osteoblastic and MSC adhesion and reducing the time to functional bone-implant contact. Systematic reviews of nano-HA-coated titanium report consistent improvements in osseointegration outcomes across the majority of included studies, and combination coatings pairing HA with chitosan or other polymers, or with therapeutic ions such as magnesium, further improve interfacial bonding and bioactivity relative to HA alone [21].

6. Clinical and Translational Applications

HA-based materials are already established in clinical practice as bone-void fillers, as coatings on cementless orthopedic and dental implants, and, increasingly as scaffolds for craniofacial reconstruction. Custom three-dimensionally printed HA scaffolds, in some cases augmented with recombinant BMP-2 or small-molecule osteogenic agents, have demonstrated bone regeneration in large, critical-sized cranial defects in translational large-animal models, representing a direct bridge toward human craniofacial applications. In implant dentistry, HA and HA-chitosan coated titanium fixtures are associated with improved osseointegration outcomes relative to uncoated implants, supporting continued clinical use of HA surface modification. Nonetheless, the translational pathway from bench to bedside for MSC-seeded HA scaffolds specifically, as opposed to acellular HA grafts, remains comparatively immature, constrained by regulatory requirements around cell-based therapies, scalability of MSC expansion, and the need for robust vascularization once scaffolds exceed a few millimeters in thickness.

7. Challenges and Future Directions

Several obstacles continue to limit the clinical translation of HA-MSC bone tissue engineering strategies. First, the mismatch between the slow degradation rate of highly crystalline HA and the pace of new bone deposition can leave residual, non-remodeled ceramic within regenerated tissue; more soluble or ion-substituted HA formulations partially address this but at some cost to mechanical strength. Second, achieving adequate vascularization within the interior of large scaffolds remains a persistent bottleneck, since MSC survival and osteogenic activity decline rapidly under the hypoxic and nutrient-poor conditions typical of scaffold cores. Third, although the individual signaling pathways engaged by HA, ionic, integrin-mediated, Wnt/BMP, and MAPK, are increasingly well characterized in isolation, their temporal integration and relative contribution under clinically relevant, load-bearing, and inflammatory conditions remain incompletely understood. Finally, batch-to-batch variability in HA crystallinity, particle size, and ion content, combined with heterogeneity in MSC source and donor characteristics, complicates reproducibility across preclinical studies and represents a practical barrier to regulatory approval. Future work integrating precise nanostructural control, rational ion doping, and patient-specific additive manufacturing, ideally validated in large-animal models that recapitulate human bone biology, is likely to be most productive in closing the gap between mechanistic understanding and clinical deployment.

8. Conclusion

Hydroxyapatite promotes osteogenic differentiation of mesenchymal stem cells through a convergent set of mechanisms encompassing ionic signaling, integrin-mediated adhesion, canonical Wnt and BMP/Smad pathway activation, MAPK signaling, and topographical mechanotransduction. These mechanistic insights have directly informed the development of increasingly sophisticated HA-based biomaterials, including three-dimensionally printed scaffolds, polymer–HA composites, ion-substituted ceramics, and functionalized implant coatings, several of which have already achieved clinical or near-clinical use. Continued progress will depend on better integrating mechanistic understanding across

signaling pathways and length scales, and on addressing the persistent translational challenges of degradation kinetics, vascularization, and manufacturing reproducibility.

References:

1. *Ielo, I., et al., Recent advances in hydroxyapatite-based biocomposites for bone tissue regeneration in orthopedics. International journal of molecular sciences, 2022.* ***23****(17): p. 9721.*
2. *Selim, M., et al., Innovative designs of 3D scaffolds for bone tissue regeneration: Understanding principles and addressing challenges. European Polymer Journal, 2024.* ***215****: p. 113251.*
3. *Dalle Carbonare, L., et al., The bone microenvironment: new insights into the role of stem cells and cell communication in bone regeneration. Stem cell research & therapy, 2025.* ***16****(1): p. 169.*
4. *Kumar, R. and S. Mohanty, Hydroxyapatite: a versatile bioceramic for tissue engineering application. Journal of Inorganic and Organometallic Polymers and Materials, 2022.* ***32****(12): p. 4461-4477.*
5. *Xu, H.-J., et al., Runx2 overexpression promotes bone repair of osteonecrosis of the femoral head (ONFH). Molecular Biology Reports, 2023.* ***50****(6): p. 4769-4779.*
6. *Monte, J.P., et al., Recent advances in hydroxyapatite/polymer/silver nanoparticles scaffolds with antimicrobial activity for bone regeneration. Materials Letters, 2023.* ***338****: p. 134027.*
7. *Farazin, A. and S. Mahjoubi, Dual-functional Hydroxyapatite scaffolds for bone regeneration and precision drug delivery. Journal of the Mechanical Behavior of Biomedical Materials, 2024.* ***157****: p. 106661.*
8. *Zhao, C., et al., The role of the micro-pattern and nano-topography of hydroxyapatite bioceramics on stimulating osteogenic differentiation of mesenchymal stem cells. Acta Biomaterialia, 2018.* ***73****: p. 509-521.*
9. *Feng, P., et al., Hydrothermal synthesis of hydroxyapatite nanorods and their use in PCL bone scaffold. Ceramics International, 2022.* ***48****(22): p. 33682-33692.*
10. *Szterner, P. and M. Biernat, The synthesis of hydroxyapatite by hydrothermal process with calcium lactate pentahydrate: the effect of reagent concentrations, pH, temperature, and pressure. Bioinorganic Chemistry and Applications, 2022.* ***2022****(1): p. 3481677.*
11. *Ebrahimi, S., C. Stephen Sipaut@ Mohd Nasri, and S.E. Bin Arshad, Hydrothermal synthesis of hydroxyapatite powders using Response Surface Methodology (RSM). PLOS ONE, 2021.* ***16****(5): p. e0251009.*
12. *Devesa, S., et al., Sol-gel synthesis of hydroxyapatite: applications, methods, and a case study. 2025.*
13. *Jaafar, A., et al., Sol-gel derived hydroxyapatite coatings for titanium implants: a review. Bioengineering 7, 1–23 (2020).*
14. *Khotib, J., et al., Signaling pathway and transcriptional regulation in osteoblasts during bone healing: direct involvement of hydroxyapatite as a biomaterial. Pharmaceuticals, 2021.* ***14****(7): p. 615.*
15. *Wang, J., et al., Nano-hydroxyapatite coating promotes porous calcium phosphate ceramic-induced osteogenesis via BMP/Smad signaling pathway. International journal of nanomedicine, 2019: p. 7987-8000.*

16. *Meesuk, L., et al., Osteogenic differentiation and proliferation potentials of human bone marrow and umbilical cord-derived mesenchymal stem cells on the 3D-printed hydroxyapatite scaffolds. Scientific Reports, 2022.* ***12****(1): p. 19509.*
17. *Piszko, P.J., et al., Bone regeneration capabilities of scaffolds containing chitosan and nanometric hydroxyapatite—Systematic review based on in vivo examinations. Biomimetics, 2024.* ***9****(8): p. 503.*
18. *Ran, L., et al., Strontium-doped hydroxyapatite and its role in osteogenesis and angiogenesis. The International Journal of Developmental Biology, 2023.* ***67****(4): p. 137-146.*
19. *Ren, W.-H., et al., Strontium-doped hydroxyapatite promotes osteogenic differentiation of bone marrow mesenchymal stem cells in osteoporotic rats through the CaSR-JAK2/STAT3 signaling pathway. Advanced NanoBiomed Research, 2022.* ***2****(9): p. 2200018.*
20. *Ehret, C., et al., Strontium-doped hydroxyapatite polysaccharide materials effect on ectopic bone formation. PLoS One, 2017.* ***12****(9): p. e0184663.*
21. *Duraisamy, R., D. Ganapathy, and L. Thangavelu, Systematic review on hydroxyapatite and chitosan combination-coated titanium implants on osseointegration. World Journal of Dentistry, 2024.* ***15****(1): p. 79-86.*