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Review Article

Advances in Synthesis and Functional Modification of Nanohydroxyapatite

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Nanohydroxyapatite material has been used to substitute for bone repair materials in clinical therapy in recent years. However, its osteogenesis effects are different due to its morphology, size, calcium phosphate ratio, crystallinity, and other differences. Thus, synthesis methods are continuously being improved to obtain synthetic materials similar to the nanohydroxyapatite in natural bone tissues in terms of biocompatibility and biological activity. Many synthesis methods are available for nanohydroxyapatite, and, among them, biological template biomimetic synthesis is the optimal method for obtaining highly bioactive and biocompatible nanohydroxyapatite, achieved by manipulating the morphology and physical and chemical properties, such as size, calcium phosphorus ratio, and degree of crystallinity. This article reviews the synthesis and functional modification of nanohydroxyapatite.

1. Introduction

Tumors, trauma, congenital developmental deformities, and severe inflammatory diseases often have varying degrees of bone tissue defects [1–4]. Bone allograft immunogenicity cannot prevent trauma and pain of patients undergoing bone transplantation; thus, using apatite as a bone repair replacement material has become one of the important methods of bone repair replacement [5, 6]. With the rapid development of nanotechnology in recent years, nanomaterials are being increasingly used in biomedical science. Some natural or synthetic polymers and inorganic nonmetallic materials have been developed into nanomaterials. The use of passive targeting or the modification of specific molecules on the surfaces of active targets and drug carriers on the target cells provides effective solutions for treating diseases, such as tumors. Hydroxyapatite (HA) is a main inorganic component that is similar to those of natural bone and teeth. The eosinophilic degradation of the nano-HA ensures that it is stable under normal physiological conditions, while it is degraded in low pH environments such as vacuumization, lysosomes, or tumors. Nano-HA has relatively good biocompatibility and biodegradability and thus can be used as an ideal drug carrier material [7, 8]. Given that HA crystals exhibit hexagonal system, their a and c crystals have different properties; thus, the HA particles with a variety of molecular (acidic and alkaline protein) bonding, nano-HA, are used as growth factors, antibodies, and carriers of anticancer drugs in some studies [9, 10]. In addition, Ca^{2+} plays an important role in the inclusion body escape, stable cytoplasm, and nuclei of DNA through nuclear pores, making the nano-HA particle a new generation of nonviral gene-delivery carriers in gene therapy [11, 12]. At present, the application of HA is limited by its low initial fracture and can only be applied to non-load-bearing bone defects or as coatings of high-strength materials, such as stainless steel. Nano-HA has a high specific surface area, which makes it highly bioactive and improves its sintering performance (densification), thereby enhancing its mechanical properties.

2. Physical and Chemical Properties of Nanohydroxyapatite in Natural Bone Tissue

Humans have been using biological materials for a long time. Jade was used as a restorative material in yama until the 1960s. With the rapid developments in materials science and biotechnology, studies on biomedical materials have advanced. Nanoapatite bone repair material is an active research direction in biological materials. Hydroxyapatite (HA) has bone mineral composition. Its theoretical composition is $Ca_{10}(PO4)_6(OH)_2$ with a Ca/P ratio of 1.67. Its crystals have a hexagonal system, and its structure is a hexagonal cylinder. Each unit cell contains 10 Ca^{2+}, 6 PO_4^{3-}, and 2 OH^-. Its theoretical density is high at 3.156 g/cm^3, and its refractive index is 1.64–1.65. Its Mohs hardness is 5; it is slightly soluble in water and weakly alkaline (pH = 7–9), soluble in acid, and difficult to dissolve in alkali. Moreover, HA performance depends on the surface of the structure [13]. Two surface adsorption positions exist. When OH^- is located in the crystal surface, the position is at the connection of the two Ca^{2+} ions in aqueous solution. The surface of the OH^- becomes vacant at one instance because two Ca^{2+} cations are positively charged, thereby forming an adsorption position. Similarly, when the surface Ca^{2+} is vacant at a certain moment, the surface forms another adsorption position, which is negatively charged and can adsorb the group on the cationic and protein molecules, such as Sr^{2+} [14]. Nano-HA essentially lacks Ca, and its calcium and phosphorus ratio is only 1.5 instead of 1.67, which is more closely related to tricalcium phosphate $Ca_3(PO4)_2$ than to hydroxyapatite $Ca_{10}(PO_4)$ $6(OH)_2$. The apatite in human bone tissue is not composed of stoichiometric apatite crystals but contains small amounts of CO_3^{2-}, Na+, and F^- to replace the apatite lattice. As one of the main substitutes, the mass fraction of CO_3^{2-} in the bone mineral is approximately 5% to 8%. CO_3^{2-} can replace the OH^- or PO_4^{3-} in the HA lattice and form type A or B substitution. Moreover, it can occupy two positions at the same time to form type AB substitutions [15, 16]. In fact, in natural bone tissue, a type B substitution occurs, namely, $-PO_4^{3-}$ substitution. The shapes of apatite crystals are irregular, with a thickness of 2–5 nm, width of approximately 20 nm, and length of usually 40–60 nm. Its crystallinity is lower than that of the chemical synthesis of nano-HA [17].

3. Synthesis of Hydroxyapatite

3.1. Chemosynthesis. Many methods exist for preparing HA powder, such as wet and dry methods. Wet methods include precipitation, hydrothermal synthesis, sol-gel, ultrasonic synthesis, and microemulsion [18, 19].

3.1.1. Chemical Precipitation Method. Chemical precipitation is mixed aqueous solution of certain concentration of calcium salt and phosphate salt, by controlling the pH value, produces chemical reaction, generates colloidal hydroxyapatite sediment, and gets its crystalline powder by calcining grinding. This method is simple and easy to prepare, and the powder has high purity, fine particle, and low cost [20]. It is a widely used method for preparing medical HA powder [21, 22].

3.1.2. Hydrothermal Synthesis. The hydrothermal synthesis method is in a closed pressure vessel, using aqueous solution as the reaction medium, heating the reaction vessel, so that the dissolved or insoluble substance is dissolved and recrystallized in normal conditions [23, 24]. The main advantage of this method is that the product has high crystallinity and no need for secondary crystallization treatment, thus reducing the chance of HA particle agglomeration during recrystallization [25]. However, the method also has a complex process, and it is difficult to obtain the HA powder of quantitative Ca/P, and the quality is unstable.

3.1.3. Sol-Gel Method. Sol-gel method is to dissolve alcohol salt in organic solvents and to dissolve, polymerize, and form sol by adding distilled water. Then, with the addition of water, the sol turns to gel, and the gel is processed by low temperature drying and high temperature calcining through vacuum state, which can obtain nanometer powder ceramics. The advantage of this method is that the purity is high, which is beneficial to the preparation of high purity biological ceramics, and the particle size distribution is narrow. However, the application of this method is restricted by the high raw material price, the toxicity of organic solvents, and the easy and rapid aggregation of particles during heat treatment. Sodium- (Na-) doped hydroxyapatite HA powder with crystallite sizes varying from 35 nm to 65 nm and pore sizes that vary between 100 and 300 μm can be prepared by sol-gel method [26].

3.1.4. Dry Method. The dry method is the solid-state reaction method. The process involved mixing solid calcium phosphate and other compounds; when the temperature is higher than 1000°C, reaction will occur with the presence of water vapor and resulted in hydroxyapatite powders. Though dry method prepared hydroxyapatite powder exhibited no lattice contraction and good crystallization performance, impurity phase often exists. Phase pure highly crystalline HA derived from eggshell was reported recently. A flower-like morphology HA (Figure 1) can be obtained via a direct solid-state sintering method after preheat treatment of calcined eggshell and dicalcium phosphate dihydrate at 800°C [27].

3.2. Template Synthesis. Nanometer apatite with crystal structures, orientations, and morphology that are similar to that of human bone tissue is difficult to prepare by traditional chemical methods because crystal morphology and orientation are affected by numerous factors, such as solution pH, temperature, ionic strength, solvents, anionic, ratio of reactants, and crystallization behavior of the material [30–33]. In human bones, apatite is formed with nanometer needle or columnar structure, and the *C* axis along the long axis of the collagen fibers is arranged (Table 1), thus gaining better mechanical properties than single crystals or the chemical synthesis of nano-HA. In the biological activity, the chemical synthesis of nano-HA has certain bone conduction

Table 1: Difference between natural bone and synthetic nanoapatite.

	Morphology	Dimension	Calcium phosphate ratio	Crystallinity	Representative method
Natural bone	Needle	5–10 nm	1.50	low	
	Flaky	20–50 nm			
Chemical synthesis	Needle	130–170 nm	1.640–1.643	high	Hydrothermal
		15–25 nm			Coprecipitation
Organic polymer template synthesis	Needle	70–90 nm	1.35	low	Polyamide
		10–20 nm			Polyacrylic acid
		2–5 nm			Polyethylene glycol
	Spherical	30–50 nm			Polyethyleneimine
		1–5 μm			
Biological template synthesis	Flaky	7–8 nm	1.5–1.66	low	Collagen
	Fusiform	30–40 nm			Chitosan
	Flaky	6 nm			Chondroitin sulfate
		12 nm			

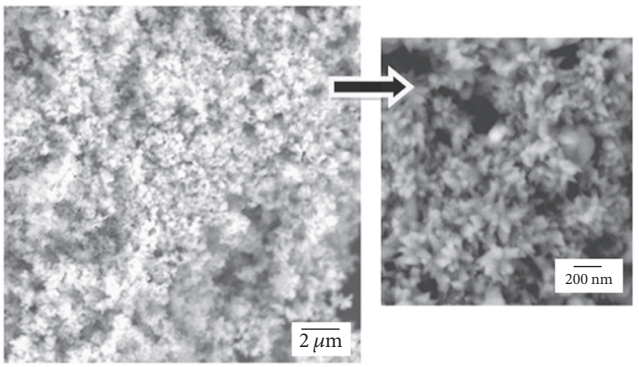
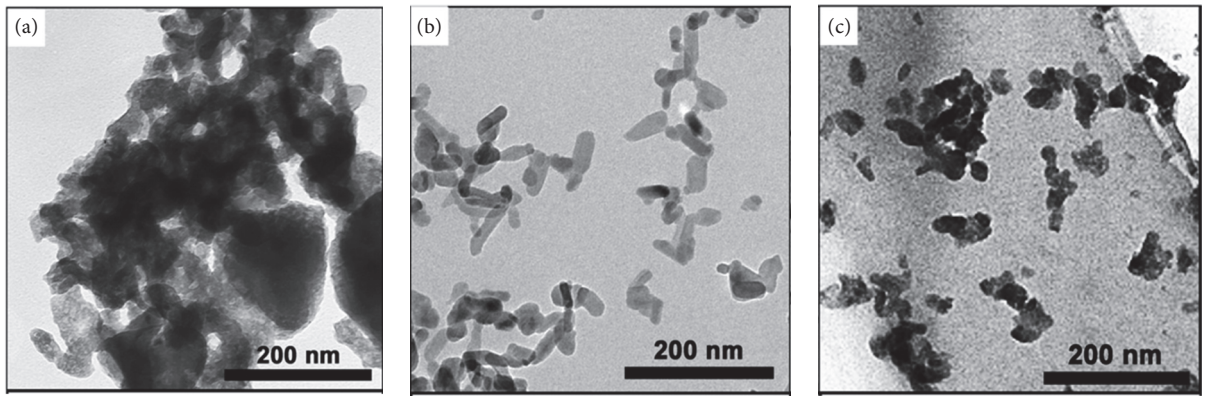


Figure 1: SEM images of the flower-like morphology HA powder derived from eggshell [27].

performance, but its bone induction performance is low, out of the standard requirement to replace the function of the bone tissue [34, 35]. Therefore, measures must be taken for improvement. At present, differences exist among the morphology, size, calcium and phosphorus ratio, and crystallinity of chemically synthesized nanoapatite granules and apatite crystals in natural bone tissue. Therefore, reduction of the calcium and phosphorus ratio is important. Template synthesis occurs under conditions similar to the physical environment, thereby avoiding cumbersome steps and the use of special equipment during chemical synthesis methods. In recent years, the principle and method of synthesis have been introduced into the synthesis of nanohydroxyapatite. An important method of biomimetic synthesis is using a template to control the growth direction of the crystal and to obtain nanoapatite that has a similar structure and components as that in the natural bone. The current popular research template has two kinds: organic polymer and biological templates. Template synthesis yields nanoapatite with relatively low degree of crystallinity and low calcium phosphate ratio [36–38].

3.2.1. Organic Polymer Templates. Polyacrylic acid (PAA) is added to the nanohydroxyapatite synthesis to prepare a HA/PAA composite material; and through molecular thermodynamic simulation of the performance of nano-HA-PA interface, the nHA grows along the c axis of nano-HA under the guidance of polyacrylic acid [39, 40]. Polyethylene glycol (PEG) is an organic matter that is avirulent, without excitants, and has good water solubility. A spherical nanohydroxyapatite with a size of 30 to 50 nm can be obtained by adding 2%–6% PEG to spherical nanometer hydroxyapatite. The nano-HA crystallinity increases with the increase of PEG concentration. The different concentrations of PEG and the interaction between calcium greatly affect the nanometer apatite morphology and growth (Figure 2) [28, 41]. Cross-linked polyethylene imine (PEI) is a template to preparing nHA/PEI in composite materials. The unlinked PEI cannot guide the nucleation and growth of calcium phosphate, and the cross-linked PEI can form a spongy ball with a diameter of 1–5 μm. The inorganic membrane on the ball is made up of tiny, slightly spherical particles that further form larger spheres. The main contents include calcium phosphate and nanoscale hydroxyapatite, and the inorganic phase content accounts for only 10% (Ca/P ratio is 1.35) [42, 43].

3.2.2. Biological Templates. Inspired by oysters, which have strong adsorption ability, dopamine was investigated by polymerization. Polydopamine easily adheres to almost any surface in alkaline solutions without requiring advanced treatment processes for the other surface. The amino and sulfonium groups of polydopamine can further take Schiffer's alkali or Michael addition reactions with other substances to graft other biological molecules with functional activity, thus modifying the surface of biomaterials. Polydopamine can promote the adhesion of cells to multiple surfaces. Given its structure, polydopamine-*o*-phenol diphenol can chelate calcium ions, adsorb phosphate ions, and activate nanoapatite crystal nucleation as a template for nanoapatite synthesis. On the other hand, hydroxyl of phthalates can react to functional groups of polypeptides or proteins, as



(a) (b) (c)

Figure 2: TEM pictures of nanometer apatite morphology using PEG as template. (a) Control, (b) 8% PEG6000, and (c) 16% PEG6000 [28].

the intermediate means of grafting biological molecules. Recently, scholars reported the use of polydopamine-coated nanoapatite, titanium materials, and polyglycolic acid materials for enhancing bone implant material surfaces that improved bone tissue regeneration. Moreover, polydopamine promotes the adhesion function of cells on multiple surfaces. The structure of polydopamine catechol can chelate calcium; thus, further adsorption of phosphate ions is used for the template synthesis of nanoapatite to start the nanoapatite crystal. Catechol hydroxyl can react with peptides or protein amino functional groups, such as in grafting biologically functional molecules [44–46].

Gelatin has protein-rich functional groups, such as carboxyl and hydroxyl groups. It is the local hydrolysis product of collagen, which is the main organic composition of the tendon and bone tissue and has a typical three-dimensional spiral structure. It contains numerous specific spiral biometric sites in the molecular chain. Control experimental conditions can cause the nano-HA to nucleate directly on the gelatin fibers, and the c axis is along the direction of the collagen helix. Self-assembly method confirms that the gelatin can regulate nano-HA so that it can be directed to grow along a certain direction [47, 48].

Chitosan (CS) is a rare and positively charged alkaline polysaccharide. It is a product of deethylation (1–4), and its chemical name is 2-amino-2-deoxy-beta-d-glucose. By using chitosan as the template, the nano-HA with class spindle is prepared. When the concentration of CS is 0.5%, the width is approximately 7-8 nm, and the length is between 30 and 40 nm. The CS template changes the appearance of nano-HA [49, 50].

The nano-HA/ChS complex was prepared using chondroitin sulfate (ChS) with high density of negatively charged groups, and the nano-HA, which was a length of 12 nm and a width of 6 nm, was produced after joining ChS. The long axis of ChS was parallel to the *c* axis of nano-HA crystals. Nano-HA nucleation and growth depended on the chemical interaction between nano-HA and ChS. The periodic arrangement of the carboxylic acid group on the ChS can chelate Ca^{2+} format Ca–ChS complex. This complex formed the vug-critical size by absorbing Ca^{2+}, PO_4^{3-}, CO_3^{2-}, and OH^- [51, 52]. The nano-HA crystals were subject to the regulation of the group on the ChS template and the spatial hindrance of nano-HA crystals; thus, a certain size of particles was formed. When no ChS template was present, nano-HA grew naturally without limits [53].

4. Nanoapatite Functional Modification

Combination of growth factors is an important approach for nano-HA functional transformation. According to different applications and application requirements, different kinds of growth factors are loaded on the nano-HA to exert different effects, such as the loading on BMP-2 with nano-HA for bone repair that can effectively induce osteogenesis of mesenchymal cells [54]. Loading of vascular endothelial growth factor nano-HA can effectively promote vascularization when used as a bone implant [55, 56]. The loading of fibroblast growth factor can promote the healing of bone defect and acceleration of bone growth [57, 58]. However, because growth factor is an active protein, it is expensive and its preservation over a prolonged period is difficult, thus greatly restricting its clinical application. Therefore, chemical synthesis method was adopted in vitro according to the active nucleotide sequence of growth factor. The active peptide with the function of growth factor was prepared. This short peptide synthesis is affordable, and its preservation and transportation conditions are not as demanding as those of the growth factor. It is a biological medical material with potential clinical applications. According to the core function sequence of BMP-2, 24 amino acid active peptides were designed and synthesized, further carrying out the activity of short peptide nanoreorganization of HA/collagen/PLA composite artificial bone. In vivo experiments on rats show that the scaffold materials can promote new bone formation and have good biomedical application [59, 60]. Arginine-glycine-aspartate (RGD) peptides modified HA for the promotion of bone integration function. The results showed that

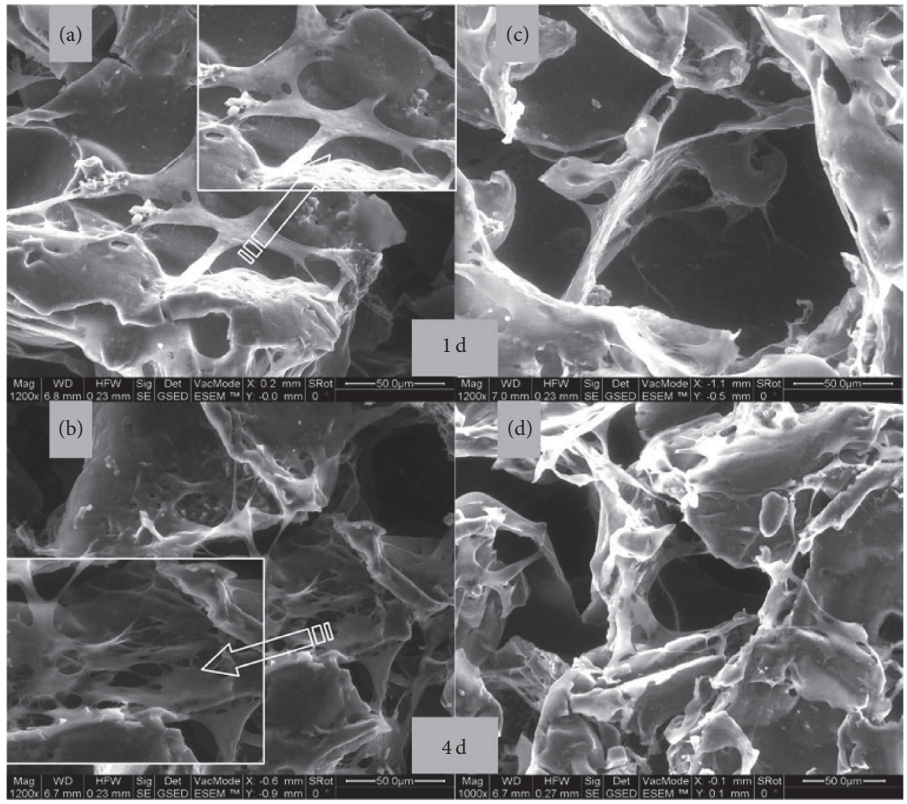


Figure 3: Cell morphology of BMSCs on PLGA/NHA-RGD ((a) and (b)) and PLGA/NHA ((c) and (d)) 3D scaffold surfaces depicted by ESEM after 1- and 4-day cell incubation [29].

HA with RGD peptides has good adsorption capacity for fibronectin, vitronectin, and fibrinogen, which play decisive roles in post-echo and survival of bone mesenchymal stem cells (BMSC) (Figure 3). The newborn bone is directly bound to the HA implant of RGD, but the RGD has a significant inhibitory effect on bone formation [29, 61]. Kasugai et al. [62] grafted six aspartic acid (Asp) molecules to nano-HA by experimental research in mice and demonstrated that the compound material can effectively deliver drugs to the bone tissue. Kim et al. [63] reported for the first time that the bone formation polypeptide is derived from the polypeptide sequence of the BMP-7 nonmature region, which is composed of 15 amino acids. The study confirmed that this peptide can promote BMSCs to differentiate into osteoblasts, and its osteogenic activity is better than that of BMP-7.

5. Conclusion

At present, the physical and chemical properties of the nanometer apatite (crystallinity, calcium phosphate scale, etc.) and induced osteogenesis performance need further improvements. No systematic research has been conducted on the reduction of the nanocalcium phosphorus ratio and crystallinity of apatite and on the improvement of the osteogenic induction activity of the synthesized nanoapatite, which are significant for the regeneration of clinical bone tissue. Therefore, further investigations must be conducted.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

[1] K. Bardsley, A. Kwarciak, C. Freeman, I. Brook, P. Hatton, and A. Crawford, "Repair of bone defects in vivo using tissue engineered hypertrophic cartilage grafts produced from nasal chondrocytes," *Biomaterials*, vol. 112, pp. 313–323, 2017.

[2] M. Emori, M. Kaya, H. Irifune et al., "Vascularised fibular grafts for reconstruction of extremity bone defects after resection of bone and soft-tissue tumours," *The Bone & Joint Journal*, vol. 99B, no. 9, pp. 1237–1243, 2017.

[3] S. N. Pierrie and J. R. Hsu, "Shortening and Angulation Strategies to Address Composite Bone and Soft Tissue Defects," *Journal of Orthopaedic Trauma*, vol. 31, pp. S32–S35, 2017.

[4] D. Borzunov, I. V. Shevtsov, A. I. Mitrofanov et al., "Stimulation of bone tissue regeneration in cavitary defects for management of patients with tumor-like diseases of long bones," 2016.

[5] L. T. Bang, S. Ramesh, J. Purbolaksono et al., "Development of a bone substitute material based on alpha-tricalcium

phosphate scaffold coated with carbonate apatite/poly-epsilon-caprolactone," *Biomedical Materials*, vol. 10, no. 4, Article ID 045011, 2015.

[6] J. Su, L. Cao, B. Yu et al., "Composite scaffolds of mesoporous bioactive glass and polyamide for bone repair," *International Journal of Nanomedicine*, vol. 7, pp. 2547–2555, 2012.

[7] M. Błażewicz, I. Piekarczyk, E. Menaszek, and K. Haberko, "Polymer and carbon fibers with HAp nanopowder: Properties and biocompatibility of degradation products," *European Cells and Materials*, vol. 7, no. 1, p. 47, 2004.

[8] N.-M. Hu, Z. Chen, X. Liu et al., "Mechanical properties and *in vitro* bioactivity of injectable and self-setting calcium sulfate/nano-HA/collagen bone graft substitute," *Journal of the Mechanical Behavior of Biomedical Materials*, vol. 12, pp. 119–128, 2012.

[9] Z. Huang, Y. Liu, and D. Wang, "A study on thermal stabilization of the hydroxyapatite (HAP) synthesized by SolGel process," *Journal of Wuhan Institute of Chemical Technology*, 2002.

[10] J. H. Lee, *Tensegrity-Inspired Nanocomposite Structures*, Georgia Institute of Technology, 2012.

[11] C. M. Curtin, E. G. Tierney, K. Mcsorley, S.-A. Cryan, G. P. Duffy, and F. J. O'Brien, "Combinatorial gene therapy accelerates bone regeneration: Non-viral dual delivery of VEGF and BMP2 in a collagen-nanohydroxyapatite scaffold," *Advanced Healthcare Materials*, vol. 4, no. 2, pp. 223–227, 2015.

[12] G. Li, L. Ye, J. Pan et al., "Antitumoural hydroxyapatite nanoparticles-mediated hepatoma-targeted trans-arterial embolization gene therapy: In vitro and in vivo studies," *Liver International*, vol. 32, no. 6, pp. 998–1007, 2012.

[13] J. W. L. Wilson, P. G. Werness, and L. H. Smith, "Inhibitors of crystal growth of hydroxyapatite: A constant composition approach," *The Journal of Urology*, vol. 134, no. 6, pp. 1255–1258, 1985.

[14] P. L. Koch, A. N. Halliday, L. M. Walter, R. F. Stearley, T. J. Huston, and G. R. Smith, "Sr isotopic composition of hydroxyapatite from recent and fossil salmon: the record of lifetime migration and diagenesis," *Earth and Planetary Science Letters*, vol. 108, no. 4, pp. 277–287, 1992.

[15] W. Bonfield and I. R. Gibson, "Novel synthesis and characterization of an AB-type carbonate-substituted hydroxyapatite," *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol. 59, no. 4, pp. 697–708, 2002.

[16] J. P. Lafon, E. Champion, and D. Bernache-Assollant, "Processing of AB-type carbonated hydroxyapatite Ca10-x(PO4)6-x(CO3)x(OH)2-x-2y(CO3)y ceramics with controlled composition," *Journal of the European Ceramic Society*, vol. 28, no. 1, pp. 139–147, 2008.

[17] S. Joschek, B. Nies, R. Krotz, and A. Göpferich, "Chemical and physicochemical characterization of porous hydroxyapatite ceramics made of natural bone," *Biomaterials*, vol. 21, no. 16, pp. 1645–1658, 2000.

[18] S. S. A. Abidi and Q. Murtaza, "Synthesis and characterization of nano-hydroxyapatite powder using wet chemical precipitation reaction," *Journal of Materials Science and Technology*, vol. 30, no. 4, pp. 307–310, 2014.

[19] H. Eslami, M. Solati-Hashjin, and M. Tahriri, "Synthesis and characterization of hydroxyapatite nanocrystals via chemical precipitation technique," *Iranian Journal of Pharmaceutical Sciences*, 2008.

[20] S. Gao, K. Sun, A. Li, and H. Wang, "Synthesis and characterization of hydroxyapatite nanofiber by chemical precipitation method using surfactants," *Materials Research Bulletin*, vol. 48, no. 3, pp. 1003–1006, 2013.

[21] H. Wang and Q. Sun, "Preparation of High Quality Nano-HA Ceramics by Chemical Precipitation Method," *China Ceramics*, 2014.

[22] N. Monmaturapoj and K. Luang, "Nano-size Hydroxyapatite Powders Preparation by Wet-Chemical Precipitation Route," *Journal of Metals*, 2017.

[23] X. Chen, Y. Liu, J. Yang et al., "The synthesis of hydroxyapatite with different crystallinities by controlling the concentration of recombinant CEMP1 for biological application," *Materials Science and Engineering C: Materials for Biological Applications*, vol. 59, pp. 384–389, 2016.

[24] M. Wang, J. Gao, C. Shi, Y. Zhu, Y. Zeng, and D. Wang, "Facile one-pot synthesis of oriented pure hydroxyapatite with hierarchical architecture by topotactic conversion," *Crystal Growth and Design*, vol. 14, no. 12, pp. 6459–6466, 2014.

[25] W. Lai, C. Chen, X. Ren, I.-S. Lee, G. Jiang, and X. Kong, "Hydrothermal fabrication of porous hollow hydroxyapatite microspheres for a drug delivery system," *Materials Science and Engineering C: Materials for Biological Applications*, vol. 62, pp. 166–172, 2016.

[26] I. Sopyan, E. Pusparini, S. Ramesh et al., "Influence of sodium on the properties of sol-gel derived hydroxyapatite powder and porous scaffolds," *Ceramics International*, vol. 43, no. 15, pp. 12263–12269, 2017.

[27] S. Ramesh, A. N. Natasha, C. Y. Tan et al., "Direct conversion of eggshell to hydroxyapatite ceramic by a sintering method," *Ceramics International*, vol. 42, no. 6, pp. 7824–7829, 2016.

[28] H. Zhang, M. Liu, H. Fan, and X. Zhang, "An efficient method to synthesize carbonated nano hydroxyapatite assisted by poly(ethylene glycol)," *Materials Letters*, vol. 75, pp. 26–28, 2012.

[29] Y. Huang, J. Ren, T. Ren et al., "Bone marrow stromal cells cultured on poly (lactide-co-glycolide)/nano- hydroxyapatite composites with chemical immobilization of Arg-Gly-Asp peptide and preliminary bone regeneration of mandibular defect thereof," *Journal of Biomedical Materials Research Part A*, vol. 95, no. 4, pp. 993–1003, 2010.

[30] F. Chen, Z.-C. Wang, and C.-J. Lin, "Preparation and characterization of nano-sized hydroxyapatite particles and hydroxyapatite/chitosan nano-composite for use in biomedical materials," *Materials Letters*, vol. 57, no. 4, pp. 858–861, 2002.

[31] Y. C. Chai, A. Carlier, J. Bolander et al., "Current views on calcium phosphate osteogenicity and the translation into effective bone regeneration strategies," *Acta Biomaterialia*, vol. 8, no. 11, pp. 3876–3887, 2012.

[32] T. Kawasaki, K. Ikeda, S. Takahashi, and Y. Kuboki, "Further study of hydroxyapatite high-performance liquid chromatography using both proteins and nucleic acids, and a new technique to increase chromatographic efficiency," *European Journal of Biochemistry*, vol. 155, no. 2, pp. 249–257, 1986.

[33] M. H. Fathi, A. Hanifi, and V. Mortazavi, "Preparation and bioactivity evaluation of bone-like hydroxyapatite nanopowder," *Journal of Materials Processing Technology*, vol. 202, no. 1-3, pp. 536–542, 2008.

[34] S. Koutsopoulos, "Synthesis and characterization of hydroxyapatite crystals: a review study on the analytical methods," *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol. 62, no. 4, pp. 600–612, 2002.

[35] A. K. Nayak, "Hydroxyapatite synthesis methodologies: an overview," *International Journal of ChemTech Research*, vol. 2, no. 2, pp. 903–907, 2010.

[36] S. Kannan, S. I. Vieira, S. M. Olhero et al., "Synthesis, mechanical and biological characterization of ionic doped carbonated hydroxyapatite/β-tricalcium phosphate mixtures," *Acta Biomaterialia*, vol. 7, no. 4, pp. 1835–1843, 2011.

[37] M. Stefanic, K. Krnel, I. Pribosic, and T. Kosmac, "Rapid biomimetic deposition of octacalcium phosphate coatings on zirconia ceramics (Y-TZP) for dental implant applications," *Applied Surface Science*, vol. 258, no. 10, pp. 4649–4656, 2012.

[38] R. Bhowmik, K. S. Katti, and D. Katti, "Molecular dynamics simulation of hydroxyapatite-polyacrylic acid interfaces," *Polymer Journal*, vol. 48, no. 2, pp. 664–674, 2007.

[39] L. Jing, L. Chen, H. Peng, M. Ji, Y. Xiong, and G. Lv, "Employing the cyclophosphate to accelerate the degradation of nano-hydroxyapatite/poly(amino acid) (n-HA/PAA) composite materials," *Journal of Biomaterials Science, Polymer Edition*, pp. 1–17, 2017.

[40] Z. Dai, J. Li, and Z. Zhao, "Biological safety of nano-hydroxyapatite and poly-amino acid compound," *Acta Academiae Medicinae Militaris Tertiae*, 2010.

[41] C. Qiu, X. Xiao, and R. Liu, "Biomimetic synthesis of spherical nano-hydroxyapatite in the presence of polyethylene glycol," *Ceramics International*, vol. 34, no. 7, pp. 1747–1751, 2008.

[42] A. Shkilnyy, R. Gräf, B. Hiebl et al., "Unprecedented, low cytotoxicity of spongelike calcium phosphate/poly(ethylene imine) hydrogel composites," *Macromolecular Bioscience*, vol. 9, no. 2, pp. 179–186, 2009.

[43] C. Shuai, P. Feng, Y. Nie, H. Hu, J. Liu, and S. Peng, "Nano-hydroxyapatite improves the properties of β-tricalcium phosphate bone scaffolds," *International Journal of Applied Ceramic Technology*, vol. 10, no. 6, pp. 1003–1013, 2013.

[44] A. Ficai, E. Andronescu, G. Voicu et al., "Self-assembled collagen/hydroxyapatite composite materials," *Chemical Engineering Journal*, vol. 160, no. 2, pp. 794–800, 2010.

[45] S.-H. Rhee and J. Tanaka, "Effect of chondroitin sulfate on the crystal growth of hydroxyapatite," *Journal of the American Ceramic Society*, vol. 83, no. 8, pp. 2100–2102, 2000.

[46] H. Lee, S. M. Dellatore, W. M. Miller, and P. B. Messersmith, "Mussel-inspired surface chemistry for multifunctional coatings," *Science*, vol. 318, no. 5849, pp. 426–430, 2007.

[47] Y. Liu, Y. Lu, X. Tian et al., "Segmental bone regeneration using an rhBMP-2-loaded gelatin/nanohydroxyapatite/fibrin scaffold in a rabbit model," *Biomaterials*, vol. 30, no. 31, pp. 6276–6285, 2009.

[48] M. Zandi, H. Mirzadeh, C. Mayer et al., "Biocompatibility evaluation of nano-rod hydroxyapatite/gelatin coated with nano-HAp as a novel scaffold using mesenchymal stem cells," *Journal of Biomedical Materials Research Part A*, vol. 92, no. 4, pp. 1244–1255, 2010.

[49] L. Kong, Y. Gao, W. Cao, Y. Gong, N. Zhao, and X. Zhang, "Preparation and characterization of nano-hydroxyapatite/chitosan composite scaffolds," *Journal of Biomedical Materials Research Part A*, vol. 75, no. 2, pp. 275–282, 2005.

[50] B. H. Atak, B. Buyuk, M. Huysal et al., "Preparation and characterization of amine functional nano-hydroxyapatite/chitosan bionanocomposite for bone tissue engineering applications," *Carbohydrate Polymers*, vol. 164, pp. 200–213, 2017.

[51] D. He, X. Xiao, F. Liu, and R. Liu, "Chondroitin sulfate template-mediated biomimetic synthesis of nano-flake hydroxyapatite," *Applied Surface Science*, vol. 255, no. 2, pp. 361–364, 2008.

[52] X.-F. Xiao, D. He, F. Liu, D.-C. Zhu, and R.-F. Liu, "Template induced biomimetic synthesis of nano flake-like hydroxyapatite by chondroitin sulfate," *Rengong Jingti Xuebao/Journal of Synthetic Crystals*, vol. 37, no. 2, pp. 388–393, 2008.

[53] T. Abdullah, N. Ibrahim, and M. Warsi, "Chondroitin sulfate-chitosan nanoparticles for ocular delivery of bromfenac sodium: Improved permeation, retention, and penetration," *International Journal of Pharmaceutical Investigation*, vol. 6, no. 2, p. 96, 2016.

[54] F. Z. Notodihardjo, N. Kakudo, S. Kushida, K. Suzuki, and K. Kusumoto, "Bone regeneration with BMP-2 and hydroxyapatite in critical-size calvarial defects in rats," *Journal of Cranio-Maxillo-Facial Surgery*, vol. 40, no. 3, pp. 287–291, 2012.

[55] S. Xin-Ping U, G. Huang, X. Hui U et al., "Effect of Buyang Huanwu decoction on expression of vascular endothelial growth factor in a rabbit model of cerebrospinal fluid leak," *Journal of Traditional Chinese Medicine*, 2017.

[56] M. J. Feito, M. C. Serrano, M. Oñaderra et al., "Effects of immobilized VEGF on endothelial progenitor cells cultured on silicon substituted and nanocrystalline hydroxyapatites," *RSC Advances*, vol. 6, no. 95, pp. 92586–92595, 2016.

[57] D. Qu, J. Li, Y. Li et al., "Angiogenesis and osteogenesis enhanced by bFGF ex vivo gene therapy for bone tissue engineering in reconstruction of calvarial defects," *Journal of Biomedical Materials Research Part A*, vol. 96, no. 3, pp. 543–551, 2011.

[58] H. Tsurushima, A. Marushima, K. Suzuki et al., "Enhanced bone formation using hydroxyapatite ceramic coated with fibroblast growth factor-2," *Acta Biomaterialia*, vol. 6, no. 7, pp. 2751–2759, 2010.

[59] T. Sun, Y. Qu, W. Cui et al., "Evaluation of osteogenic inductivity of a novel BMP2-mimicking peptide P28 and P28-containing bone composite," *Journal of Biomedical Materials Research Part A*, vol. 106, no. 1, pp. 210–220, 2018.

[60] X. Zhang, W. Guo G, H. Cui et al., "In vitro and in vivo enhancement of osteogenic capacity in a synthetic BMP-2 derived peptide-coated mineralized collagen composite," *Journal of Tissue Engineering & Regenerative Medicine*, vol. 10, no. 2, p. 99, 2016.

[61] K. M. Hennessy, W. C. Clem, M. C. Phipps, A. A. Sawyer, F. M. Shaikh, and S. L. Bellis, "The effect of RGD peptides on osseointegration of hydroxyapatite biomaterials," *Biomaterials*, vol. 29, no. 21, pp. 3075–3083, 2008.

[62] S. Kasugai, R. Fujisawa, Y. Waki, K.-I. Miyamoto, and K. Ohya, "Selective drug delivery system to bone: Small peptide $(Asp)_6$ conjugation," *Journal of Bone and Mineral Research*, vol. 15, no. 5, pp. 936–943, 2000.

[63] H. K. Kim, J. H. Kim, D. S. Park et al., "Osteogenesis induced by a bone forming peptide from the prodomain region of BMP-7," *Biomaterials*, vol. 33, no. 29, pp. 7057–7063, 2012.