Review Article

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Synthesis and application of nanometer hydroxyapatite in biomedicine

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Abstract: Nano-hydroxyapatite (nano-HA) has been widely studied as a promising biomaterial because of its potential mechanical and biological properties. In this article, different synthesis methods for nano-HA were summarized. Key factors for the synthesis of nano-HA, including reactant concentration, effects of temperature, PH, additives, aging time, and sintering, were separately investigated. The biological performances of the nano-HA depend strongly on its structures, morphology, and crystallite sizes. Nano-HA with different morphologies may cause different biological effects, such as protein adsorption, cell viability and proliferation, angiogenesis, and vascularization. Recent research progress with respect to the biological functions of the nano-HA in some specific biological applications are summarized and the future development of nano-sized hydroxyapatite is prospected.

Keywords: nano-HA, synthesis methods, drug carrier

1 Introduction

Calcium phosphate is a kind of bioactive ceramic composed of calcium and phosphorus ions. Because its chemical composition is similar to natural bone tissue, it is widely used in clinical practice. Among them, calcium phosphate also contains hydroxyapatite (HA), tricalcium phosphate, bipolar calcium phosphate, and other applications of the most common component materials. Calcium phosphate not only has good biocompatibility, but also can form chemical bonds with new bone. It can induce bone tissue regeneration, so it is widely used in bone tissue repair. Among calcium phosphate, HA is the thermodynamically most stable crystalline phase of calcium phosphate in body fluids, most similar to the mineral parts of human bones and teeth. Calcium phosphate in natural bone tissue is mainly deposited in the collagen matrix in the form of nano-crystallites in an orderly manner [1]. Nanoscale HA has certain similarities with natural bone apatite in chemical composition, structure, and scale. In the microstructure of nano-bioceramics, the grains, grain boundaries, and their combination are all at the nanoscale level. The refined grains and the increase of the grain boundary numbers can make its mechanical properties (especially fracture toughness) and biological activity increase. This makes nano-hydroxyapatite (nano-HA) to be an ideal bone repair material. Among the calcium phosphate-based bioceramics, the bioactive ceramics represented by HA ceramics are the most widely used in bone tissue repair [2–4]. From the point of view of the process itself, the preparation process of nano-ceramics is not much different from that of ordinary ceramics (generally follows the process of “powder-forming-sintering”), but from a technical point of view, the preparation process of nano-ceramics is extremely harsh.

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The successful preparation of nano-HA ceramics should first synthesize nanoscale HA powder. Nano-HA powder has high surface activity and is easy to agglomerate. So, it is difficult to obtain nanoscale solid powder under normal conditions. During the molding process, whether the particles can be stabilized at the nanoscale relies on extremely strict control of the sintering process of ceramics [5,6]. During the sintering process, with the increase of temperature and the extension of time, the nano solid particles are fused with each other, and the pores and grain boundaries are gradually reduced, which lead to a high probability to cause the growth of HA grains. So, how to effectively inhibit the growth of nano-grains during the sintering process is a difficult problem in the preparation of nano-HA ceramics. In short, the two major difficulties in the preparation of nano-HA ceramics lie in the synthesis of nano-powder and the sintering of nano-ceramics. To this end, this article comprehensively summarizes and analyzes the current progress of nano-HA powder synthesis and nano-ceramic sintering technology, and prospects for future research.

2 Synthesis and preparation of nanometer HA

2.1 Different synthesis methods for nano-HA

The synthesis methods of HA ceramic powder, represented by HA powder, mainly include dry synthesis and wet synthesis. Dry preparation of HA is a preparation method of selecting finely ground precursor and mixing it, and then heat treating the precursor. This method has strict requirements on the purity and dosage of reactants and has the advantage of better crystallinity of the products. However, dry synthesis requires a relatively high temperature, which affects the porosity of the products. Wet preparation of HA consists of sol–gel method, chemical precipitation method, hydrothermal reaction method, and so on, which is carried out in water or organic solvents and can be applied to a variety of equipment with the addition of various catalysts. The advantages are that the structure and morphology of HA can be well controlled and the yield can be improved. The disadvantage is that the purity and crystallinity of the product are not enough, and there may be other phosphate crystals in the product. According to the various synthesis methods, the synthesized calcium apatite powders have certain differences in structure, morphology, and size. In addition, under the same synthesis method, different synthesis conditions will also affect the final powder morphology. Figure 1 shows the morphology of nano-HA prepared under different synthesis conditions [7–9]. This article summarizes the synthesis process of nano-powder in the following aspects.

2.2 Key factors for synthesis of nano-HA

2.2.1 Effects of reactant concentration

The concentration of reactants is the key factor affecting HA synthesis [10]. The Ca/P ratio of HA is 1.6, so the concentration of reactants determines the purity of HA and also affects the grain size of HA under certain conditions. For example, when Ca/P ratio is within the range of 1.5–1.67, the synthesized product is the mixed phase of HA and beta-TCP; when Ca/P ratio is within the range of 1.68–1.7, the synthesized product is HA; when Ca/P molar...
ratio is greater than or equal to 1.7, the synthesized product is HA + CaO [11]. From the perspective of crystal chemistry, crystal nuclei are formed first in the solution, and then they migrate and adhere to the particles under the action of thermal dynamics, grow up and form crystals. There are many nucleate particles in the high-concentration reactant solution, and HA with small grain size is easy to be formed [12,13].

2.2.2 Effects of temperature

Temperature affects not only the grain size of HA, but also the morphology of HA. Every chemical reaction involves heat, and temperature affects the speed of chemical reaction. In the synthesis reaction of HA, the higher the temperature, the faster the reaction speed, and the easier it is to nucleate and crystallize. However, at high temperature, the grain growth rate is accelerated, and the synthesized nanometer powder has high activity and is easy to aggregate. Yunjing et al. [14] prepared HA powder by precipitation in heat treatment at 200, 500, 700, and 900°C, respectively. They found that HA crystallized at 200°C was not high and its morphology was in the shape of needles or stripes. Lianfeng et al. [15] prepared nano-HA by chemical precipitation and studied the influence of temperature on the final particle size and phase structure. They studied particle size changes at 25, 40, 60, and 90°C, respectively (as shown in Table 1). With the increase of temperature, the crystallinity of nano-HA increased, and the particle size was within the range of 25–60°C. With the increase of temperature, the particle size increased, and needle-like nanoparticles were synthesized. At 90°C, the particle size tends to decrease, but the synthesized nanoparticles have rod structure. Rodríguez-Lorenzo and Vallet-Regi [16] prepared nano-HA by precipitation method. They also found that the grain size increased between 25–90°C and 20–80 nm as the temperature increased. The surface area of the synthesized HA particles decreased as the temperature increased, meaning that the reaction temperature directly affected the size of the particles, with the higher the reaction temperature, the larger the particles. The grain size of nanoparticles synthesized at 25°C was comparable to that of human bone, and the grain size of nanoparticles synthesized at 90°C was comparable to that of tooth enamel.

2.2.3 Effects of stress

The influence of pressure only works in dry synthesis and has no effect on general wet synthesis. Dry synthesis is mainly based on ball milling. The grinding balls in the ball mill directly extrude the material to convert mechanical energy into chemical energy. Therefore, the mass of the grinding balls is converted into the pressure of the reaction environment, which in turn affects the particle size of the synthesized HA. Toriyama et al. [17] used CaCO3 and CaHPO4·2H2O as raw materials to grind and synthesize nano-HA by dry method. In this process, grinding pressure is an important factor affecting powder synthesis. When the grinding pressure reaches the critical value and above, the reaction starts, and the higher the pressure, the faster the reaction rate, which in turn increases the crystallization rate of HA, and reduces its crystallinity and grain size.

2.2.4 Effects of pH

pH is a key factor in HA synthesis. When HA is alkaline, the alkaline environment provides the necessary OH root ions for HA synthesis and precipitates HA. Increasing pH value is conducive to the synthesis of a single HA phase with fewer impurities and smaller grain size [13]. If the pH value is too small, HA will decompose into impurity phases, such as preparing HA with Ca (NO3)2·4H2O, and (NH4)2HPO4 as the precursor system. When the pH value is equal to 4.5, 9, and 12.4, the prepared powders are, respectively, β-Ca3P2O7, HA + β-TCP, and HA [14]. Increasing pH will increase Ca/P ratio, so if you want to synthesize pure HA, you must strictly control the pH value. Generally, the pH value of wet synthesis is between 10 and 10.5 [18].

| Temperature (°C) | 25  | 40  | 60  | 90  |
|------------------|-----|-----|-----|-----|
| Particle size (nm) | 68.5 | 84.8 | 118.1 | 87.6 |
| Morphology of nanoparticles | Needle-like | Needle-like | Needle-like | Rod-like |

Table 1: Influence of temperature on the synthesis of nano-HA [15]
2.2.5 Effects of additives

Admixtures include organic matter, inorganic matter, and doped ions. Admixtures can affect not only grain size but also grain morphology. For example, Sr$^{2+}$ can enter HA lattice to replace Ca$^{2+}$, reduce the crystallization rate of HA, and reduce the grain size [19]. Additives affect the surface energy of the reactants. The higher the surface energy is, the faster the reaction speed is and the easier it is to crystallize and nucleate. The nucleation energy of HA is relatively large, and the addition of nucleating agent can accelerate the nucleation formation and synthesize HA with smaller grains [20]. Xinlong et al. [21] added 3–5 wt% citric acid in the synthesis process and effectively inhibited the growth of HA grains through competitive adsorption between ions. Adding ethanol can improve the dispersibility of nano powder. Adding cerium salt can refine the grain and improve the dispersibility [22].

2.2.6 Effects of stirring

The influence of stirring is mainly reflected in the time and speed of stirring. The longer the stirring time is, the more beneficial it is to the full contact of the reaction materials and improve the conversion efficiency. The speed of mixing also plays a role. Martins et al. [23] suggested that the faster the mixing speed, the better the nucleation formation and the easier the formation of HA with smaller grains. It has been reported that the higher stirring speed and longer stirring time affect the crystal morphology, making the morphology of HA similar to that of rod or rice shape [24].

2.2.7 Effects of aging time

Aging time determines the integrity of HA crystal growth and grain size. In the aging stage, the longer the aging time is, the better the formation of HA and the perfection of grain [24]. With the further extension of aging time, the grain size became larger and agglomerated, mainly due to the dissolution of small-size particles and the regrowth of secondary grains [25].

2.3 Sintering process

For nanostructured HA ceramics, sintering process is an independent step. Nanometer powders have large specific surface area and high activity. During sintering, the sintering temperature is lower than that of embryos with conventional particle size, and the grains are easy to grow and difficult to control [26]. In order to sinter nano-HA ceramics, it is necessary to prevent the grain growth during densification. Zhou et al. [27] studied the sintering properties of nano-HA; according to the DSC and TG curves of the nano-HA powder curve, the sintering temperature of the DSC curve is 761°C, and the material has an obvious endothermic peak. The concave surface indicates that the material corresponding to the sintering temperature is a violent crystal fusion and phase transformation, and the rapid fusion of nano-grains. After a short exothermic, from 761.6 to 1158.3°C, the material absorbs heat rapidly and the nanocrystals rapidly grow into ceramics.

When the nano powder of HA is over 900°C, the material grain grows rapidly, and most of the ceramic grains sintered above 1,000°C are above 1 µm. Therefore, it is difficult to obtain the final nano-ceramics by conventional sintering technology. Further study found that nano-HA ceramics grain in different sintering stages have different state changes. The initial nanoparticles in the green body of ceramic would undergoing size change during the process of continuous sintering. With the increase of the sintering temperature and time, the nanoparticles would undergo fusion, grains grow up and density in different stages of sintering. By controlling of the sintering process, such as rapid cooling would affect the finial grain size and obtain nano-structured ceramic. Therefore, nano-HA ceramic sintering process should be rigorous. Current sintering process mainly through the high efficiency of energy conversion and sintering for a short period of time makes grain to grow up. The concrete method consists of two sections of pressureless sintering, the discharge plasma sintering, hot-pressing sintering, hot isostatic pressing sintering, microwave sintering, etc. [28,29]. In this article, the several kinds of sintering methods of nano-HA ceramics sintering process were analyzed.

In order to overcome excessive grain growth in the later stage, Chen and Wang [30] invented a two-step sintering method. The principle is to raise the temperature to the critical point where the ceramic-sintered grain grows, so that the surface atom has a certain diffusion energy. Then, hold the temperature below the critical point to densify it. Mazaheri et al. [31] sintered HA by conventional sintering method and two-step sintering method, and found that the grain size of ceramics with the same densification degree was 1.7 µm in conventional sintering at 1,100°C. The grain size in the two-stage sintering of
T1 = 900 and T2 = 800 was 0.19 μm. Compared with other sintering methods, the two-step nonpressure sintering method has the characteristics of simplicity, low cost, and remarkable effect.

Plasma discharge sintering is a new sintering method, which has the characteristics of fast heating rate, short sintering time, even grain size, and good control of sintering structure. Gu et al. [32] sintered the compact HA block in the plasma at 950°C for 5 min to obtain HA ceramics with density greater than 99.5%.

Microwave sintering is a new sintering technology in recent years. It used the coupling of the microstructure of the powder with the special wave band of the microwave to generate heat for sintering ceramic embryos. It has the advantages of fast heating rate, short sintering time, overall heating, and easy to obtain fine sintered materials with even grain size [33,34]. Wang et al. [35] found that even though the heating rate of microwave sintering was very high, the HA/β-TCP ceramics were not cracked or deformed. Due to the overall heating of microwave heating, there is no temperature gradient in the material, reducing the internal stress. To a certain extent, it can improve the mechanical strength of ceramic materials. Compared with the conventional sintering method, the ceramic grain size prepared by microwave sintering at the same temperature of 1,100°C is 200–400 nm, while the ceramic grain size prepared by conventional sintering is 1.0–1.5 μm.

3 Application of nanometer HA in biomedicine

Nano-HA has the advantages of good biocompatibility, large specific surface area, high biological activity, and stable chemical properties [36,37]. In recent years, researchers have probed and utilized the regeneration ability of nano-HA in numerous application fields. Here, we summarize and discuss the application of drug carrier, surface coating, antineoplastic, and composite materials [38–44].

3.1 Drug carrier

Nano-HA has high specific surface area and strong plasticity. Through surface receptor modification or modification to improve in vivo targeting, nano-HA can absorb different drugs and can be adapted to different site delivery needs [40,41]. The adsorption of drugs on nano-HA is mainly determined by its own properties and microscopic morphology [42,43].

The adsorption sites of nano-HA for drugs mainly include carboxyl group, hydroxyl group, phosphate group, and amino group [45,46]. Nano-HA has different adsorption effects on drugs with different groups. Zhao et al. [47] studied the molecular simulation of HA on doxorubicin and tinidazole. According to the results of molecular dynamics simulation, for doxorubicin, the binding energy to HA is much higher than that of tinidazole. Then they, respectively, prepared hollow HA microspheres and nano-HA (Figure 2), and used them to carry out adsorption experiments on two drugs, doxorubicin and tinidazole. The results show that the adsorption efficiency of HA is affected by the group in the drug molecule. The adsorption of drugs on HA is mainly through the formation of Ca–O bonds between Ca ions on the surface of HA and "O" atoms in the drug molecule. The number and activity of oxygen atoms are the main factors affecting the binding ability of drugs on HA.

In addition to the adsorption of drugs by HA itself, nano-HA is often combined with various substances to form nanoparticles with hollow or mesoporous shell structures to increase drug loading and sustained drug release. Li et al. [48] successfully prepared a hollow nano-HA structure by adding a pore-enlarging agent, and used LA–BSA to encapsulate the pores to obtain pH-responsive nanoparticles while increasing the drug loading. Zhang et al. [49] introduced Sr ions to form luminescent rod-like HA with mesoporous structure when preparing nano-HA particles by hydrothermal reaction. Nano-HA was loaded with ibuprofen. The luminescence intensity of Sr-HA was positively correlated with drug release, realizing the tracking of the drug release process. Yang et al. [50] successfully synthesized nanoparticles with a mesoporous nano-HA shell and a hollow calcium carbonate core by adjusting the oppositely charged ions between the shell and the core (Figure 3), and loaded the antitumor drug doxorubicin. The outer shell of nano-HA can achieve sustained release of drugs and pH-responsive release against tumor tissue, and the hollow calcium carbonate in the inner layer greatly increases the drug load.

Not only drugs, nano-HA can also adsorb DNA and proteins. Nano-HA can be used as an ideal drug, protein, and gene carrier. HA nanoparticles have been used as carriers due to their affinity with DNA, proteins, several drugs, and appropriate release activity [51,52]. Ko et al. [53] modified HA with calcium chloride and carried the si-Stat3 plasmid that could inhibit the expression of Stat3.
Figure 2: (a and b) TEM micrographs of the as-prepared HA. (a) HA nanoparticle, (b) HA hollow microsphere. (c and d) Adsorption configurations of DOX (c) and tinidazole (d) on the (110) plane of HA. The colors are as follows: calcium (green), phosphorus (purple), oxygen (red), hydrogen (white), carbon (gray), nitrogen (blue), and sulfur (yellow) [47].

Figure 3: (a) An illustration indicating the synthetic process of hmHANP. SEM images of (b) CaCO₃ nanoparticles, (c) CaCO₃/HA core/shell nanocomposites, (d) hmHANP; TEM images of (e) CaCO₃ nanoparticles, (f) CaCO₃/HA core/shell nanocomposites, (g) hmHANP (inset: dark-field STEM image) [50].
After injecting HA into the tumor, it was found that the growth in the tumor was significantly inhibited, and the inhibition rate was as high as 74%. The expression of Stat3 was significantly downregulated in the tumor. The use of nano-HA carrying RNA plasmids for tumor treatment is a reliable choice. Wan et al. [54] prepared layered HA nanoplates with different structures and morphologies by adjusting the content of the template agent and the concentration of the precursor, and embedded DNA molecules into the L-HA nanoplates (Figure 4). Under high SDS loading and low precursor concentration, L-HA nanoplates with higher order degree and larger size were obtained, which improved the DNA loading efficiency and transfection efficiency.

3.2 Antitumor effects

Nano-HA has an inhibitory effect on the growth of various tumor cells, but has no effect on the growth of normal cells [55–58]. Judging from the existing inferred mechanism, nano-HA degrades fast, increasing the concentration of Ca²⁺ in tumor cell fluid leads to disorder of tumor cell function, degrades its DNA and inhibits telomerase gene expression, and so on, thereby inhibiting the growth and proliferation of tumor cells [59–63]. Ezhaveni et al. [64] explored the effect of nano-HA with different particle sizes prepared under different hydrothermal conditions on liver cancer cells, and found that the HA with an average particle size of 19 nm prepared by treating at 100°C for 5 h provides the most obvious inhibitory effect on tumor. Zhang et al. [65] prepared porous titanium scaffolds with nano-HA coating and cocultured with VX2 tumor cells in vitro and repaired the truncated bone defect of a critical defect size in a rabbit bone tumor model, and found that nano-HA-loaded scaffold not only has a significant effect of inhibiting tumor growth, but also has the effect of promoting bone regeneration. At the same time, they also conducted experiments on the effects of n-HA regulation on tumor suppression, calcium homeostasis, and immune response-related gene expression (Figure 5). Combined with its own antitumor properties, nano-HA loaded with drugs, DNA or protein is used as a more choice in tumor treatment.

3.3 Surface coating

Many components in cells are affected by nanoscale factors. According to reports, the adhesion sites of cells, proteins, etc., are generally 5–200 nm [66–68]. Thus, nano-grain-sized ceramics, metals, polymers, and composites stimulate cellular activity compared to micro-grain sizes. Nano-HA crystals can increase cell adhesion and proliferation, create a biocompatible surface that combines well with bone tissue [69–71]. As a HA ceramic material, nano-HA can promote the development of stem cells toward osteogenesis by degrading calcium and phosphate ions [72,73]. Bryington et al. [74] studied the long-term repair of titanium alloy stents with nano-HA coating and without nano-HA coating in vivo, and found that nano-HA coating has a greater impact in the early stage of bone healing.

Different structure of nanometer HA has different effect on cell behavior. He et al. [75] deposited two types of amorphous CaP nanoparticle nano-HA coating on the surface of titanium alloy and simulated the adhesion of

Figure 4: Schematic illustration of the loading process of DNA molecules to L-HA nanoplates [54].
osteoblasts on the scaffold (Figure 6). The results showed that the coating prepared by different forms of nano-HA particles had different upregulation of gene expression during bone tissue reconstruction. Xiao et al. [76] used a hydrothermal method to prepare nano-HA coating with different shapes and length on HA scaffolds by adjusting...
the concentration of 1,2,3,4,5,6-cycloadipic acid. In vitro studies have shown that the differentiation of cells cultured on spherical nanostructure coatings is significantly enhanced compared to plate-like or wire-like nanostructures. The results showed that the surface nanotopography of the scaffolds had a greater effect on cell differentiation than on cell proliferation.

In addition, nano-HA has strong plasticity. It is often multifunctional nano-HA by compounding other particles. Zhang et al. [77] mixed Ag and ZnO into nano-HA powder to form a coating on the surface of titanium alloy by laser cladding technology. Ag+ released antibacterial, Zn2+ released to enhance bone formation. On the basis of nano-HA coating, the antibacterial and osteogenic functions are enhanced. The experimental results showed that the coated scaffolds achieved good osteogenesis and rapid osseointegration under the condition of S. aureus injection. Rios-Pimentel et al. [78] prepared amphiphilic peptide nanoparticles (APNPs), and APNPs can promote the attachment and proliferation of osteoblasts. Nanocrystalline HA and APNP coatings were prepared on poly-2-hydroxyethyl methacrylate, respectively, and the experimental surface osteoblast density of the group with APNPs coating increased by 3 times after 3 days.

Therefore, nano-HA plays a significant role in improving the surface roughness of the material, increasing cell adhesion and enhancing the biological activity of the material. In addition, coating the nano-HA coating on the bone repair material can better improve the bone formation of the material. In addition, nano-HA coating can better improve osteogenic activity for bone repair materials.

3.4 Nano-HA composited materials

The natural bone tissue in the human body is a nanocomposite material, which is composed of crystals and collagen at the microscopic level [79–82]. Bone tissue is the compound tissue of nanometer HA and collagen. Using nano-HA as composite material can effectively improve biological activity and enhance cell survival. At the same time, nano-HA can effectively increase the surface roughness and mechanical properties of composites, and has a positive effect on the adhesion and proliferation of proteins and cells. In addition, the composite materials of nano-HA release calcium and phosphorus ions in the body, which has a positive effect on osteogenesis. The composite of nano-HA with polymer materials and hydrogel materials can improve the biological activity, surface roughness (Figure 7), and osteogenic activity of the materials [39,83–89]. By adding nano-HA to the cell-loaded hydrogel material, cell survival and regulation of cell differentiation can be enhanced [84,90]. Deng et al. [91] prepared nano-HA hybrid methyl cellulose (MC) hydrogel. The nano-HA-MC hydrogel loading bone

Figure 6: Schematic of biological response of nano-HA-coated titanium implant (Ti implant). (a) Ti implant coated with ACP nanoparticles; (b) release of Ca2+ and PO43− ions from ACP hydrolysis after implantation; (c) cell binding on the implant surface with the help of serum proteins and integrin receptors; (d) cell proliferation on the implant surface; (e) formation of apatite on the implant surface [75].
marrow mesenchymal stem cells was used for rat skull defect experiments. The addition of nano-HA improved the gelling temperature of MC and enhanced the survival of marrow mesenchymal stem cells. Nabavinia et al. [92] prepared nano-HA/alginate/gelatin microcapsules as osteogenic building blocks in modular bone tissue engineering. By regulating the proportion of nano-HA and gelatin, the interaction between nano-HA and gelatin can enhance cell proliferation and differentiation.

For natural bone tissue, cells are micron-sized entities embedded in the natural extracellular matrix (ECM). This ECM is highly organized at macroscopic, microscopic, and nanoscale [93,94]. Cells interact with topographic features at all scales, from macroscale (such as the shape of bone, ligament, or blood vessels) to nanoscale features (such as collagen ribbon shape, protein conformation, and ligand presentation). These topographic features strongly influence cell morphology, adhesion, attachment, movement, proliferation, endocytic activity, protein abundance and gene regulation, and other phenomena [95]. Inspired by the nano-layered structure and composition of bone, nanofibers and nanocomposite scaffolds doped with nanostructured HA that mimic the ECM of bone are increasingly used in bone tissue engineering [96,97]. Zhang et al. [94] used polylactic acid combined with nano-HA to simulate natural bone tissue structure and 3D printed it (Figures 8 and 9). According to the amount of nano-HA, the surface morphology of the printed scaffold is significantly different, and the hydrophilicity of the material will increase with the increase of the content of HA. The composite of nanometer HA can improve the surface roughness and hydrophilicity of materials. Zhang et al. [98] introduced an ECM-like self-assembly peptide (SAP) to nano-HA/chitosan (CTS) composite scaffolds. The SAP/nano-HA/CTS scaffolds enhanced the cell adhesion and overall mechanical properties of scaffolds. Chen et al. [99] used electrospinning technology to prepare multilayer nano-HA/polyhydroxybutyrate (PHB) film laminate scaffolds, and seeded cells on the scaffolds for bone defect repair experiments. The experimental results show that the scaffold with nanoscale simulated ECM enhances the adhesion and proliferation of osteoblasts and promotes the repair of bone defects. Yin et al. [100] perfused GelMA loaded with nano-HA into porous titanium alloy scaffolds, which enhanced the characteristics of low bioactivity and poor bone repair ability of traditional titanium alloy scaffolds.

In addition, with the development of 3D printing technology, the polymer materials of composite nano-HA are prepared by melt extrusion (FDM), photocuring printing (DLP), or ink jet extrusion (IJD) to obtain porous scaffolds in bone tissue repair. Chen et al. [101] used FDM printing technology to prepare porous PLA/nano-HA composite scaffolds for the repair of large-segment

![Figure 7: SEM of cell morphology on scaffolds. (a) PLA/nano-HA scaffolds; (b) nano-HA/CM/B9 scaffolds; (c) CS/nano-HA/Zol scaffolds; (d) gel:nano-HA = 1:1 scaffolds [83,88].](image-url)
bone defects. The composite of nano-HA enhanced the osteogenesis and angiogenesis ability of the scaffolds. Liu et al. [102] used GelMA hydrogels with different concentrations of ECM, mixed with different proportions of nano-HA to improve the electrical conductivity of the scaffold, and prepared a multilevel composite cartilage repair scaffold by 3D printing (Figure 9). The three-layer gradient scaffold can better simulate the complex layered structure of natural osteochondral tissue, and can repair osteochondral and lower bone at the same time.

Figure 8: The morphology of composite scaffolds with different nHA contents, (a) PLLA scaffolds, (b) 30% nHA composite scaffolds, (c) 50% nHA composite scaffolds, (d) the water contact angle of the different composite.

Figure 9: (a) Schematic of 3D multi-nozzle pneumatic printing system. 30/3% GelMA/nano-HA (red) for subchondral bone layer, 20/3% GelMA/nano-HA (yellow) for interfacial layer, 15% GelMA/nano-HA (blue) for cartilage layer; (b) cartilage layer; (c) interfacial layer; (d) subchondral bone layer [102].
4 Conclusions

Due to its good mechanical properties and high biological activity, nano-HA ceramics have attracted the attention of many researchers. However, there are still many problems in the preparation of nano-ceramics, including the synthesis of nano-powders and ceramic sintering, which still need to be further studied. In order to ideally control the particle size of nano-powders synthesized by HA materials, and to effectively suppress the growth of ceramic grains in the sintering process, it is still necessary to further optimize the process technology. The various powder synthesis methods and sintering processes reported so far have their own advantages and disadvantages. How to choose the appropriate nanopowder and sintering process according to the needs of the final application of nano-ceramics needs to be further explored.

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