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Gelatin/Gelatin-modified nano hydroxyapatite composite scaffolds with hollow channel arrays prepared by extrusion molding for bone tissue engineering

Xiliang Chen∗, Di Wu, Jingjing Xu, Tingting Yan and Qinghua Chen
Faculty of Materials Science and Engineering, Kunming University of Science and Technology, Kunming 650093, People’s Republic of China

* Author to whom any correspondence should be addressed.
E-mail: tsenhey@qq.com

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Abstract

Bone tissue engineering scaffold has been successfully applied in the field of bone repair, but the major limitation is the delivery of oxygen and nutrients throughout the bulk of engineered tissue, especially for large scaffolds. Researchers have found that scaffolds with hollow channels can effectively solve this problem. We utilized a novel extrusion approach to prepare scaffolds with hollow channel arrays, which has the advantages of economy, continuous production, and high efficiency. The hollow channel scaffolds were composed of gelatin and gelatin-modified nano hydroxyapatite (nHAP). X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), transmission electron microscopy (TEM) and atomic force microscope (AFM) results indicate that the prepared gelatin-modified nHAP are nanorod-like particles with the length of 30–50 nm and width of 5–15 nm. The nano particle is composed of crystalline HAP grains with the size less than 10 nm and amorphous phases. A part of OH– or PO43− are replaced by CO32− during preparation process, leading to the transformation of HAP into hydroxyl-carbonated apatite (HCA). The prepared hollow channel scaffolds exhibit much better mechanical properties compared with the sponge porous scaffolds. The axial compressive strength of the hollow channel scaffold with porosity of 51.3 ± 5.2% can reach 25 ± 1.4 MPa, which can meet the mechanical strength requirement as an implant completely. Moreover, the hollow channel scaffold exhibited good in vitro degradability and bioactivity. These results highlight the potential of using gelatin/gelatin-modified nHAP composite scaffolds with hollow channels for bone tissue engineering.

1. Introduction

Tissue engineering furnishes an effective approach for the healing of diseased, damaged and traumatised bone [1]. A typical bone tissue engineering scaffold should feature the following characteristics: (1) A specific pore structure to facilitate cell adhesion, growth, migration and differentiation; (2) the materials are non-toxic and have good biocompatibility, furthermore, can be degraded; (3) the scaffold has sufficient mechanical strength to meet the stress environment as an implant [2, 3].

Hydroxyapatite (HAP) is a preferred bioceramic in the fabrication of bone engineering scaffolds due to its excellent bioactivity, osteoconductivity and chemical similarity to the mineral component of natural bone [4–6]. HAP in natural bone is nanoscale, and is regulated by collagen—the major organic component of bone. The synthetic bone-like nano HAP (nHAP) for bone repair has attracted more and more attention [7, 8]. However, HAP is highly brittle and stiff, its shape and size availability is limited, and it is difficult to handle in clinical situations [4, 9, 10].

Gelatin is a kind of polypeptide derived from collagen hydrolysis, and contains multiple biological functional groups [4, 5]. Unlike collagen, gelatin can be dissolved in water, having the advantages of easy shaping
and cheap price, which facilitates selectivity and mass producibility [2, 6]. Gelatin also has good cell viability without showing any antigenicity, and has long been used in pharmaceutics, wound dressings, and adhesives in clinics [11]. Nevertheless, like other hydrogel polymers, it can be applied only to limited soft tissues, owing to its inappropriate elastic modulus and the mechanical strength required for hard-tissue regeneration [11, 12].

A composite of two components, HAP and gelatin, has potential as an engineered biomaterial for hard tissues [13, 14]. Similar to natural bone, the polymer-ceramic composite system should have potential because of its combined benefits, that is, the osteo-conductivity and bioactivity of HAP and the toughness and easy shaping of gelatin. Like natural bone, the composite of ceramic and polymer can combine the strength of ceramic and the toughness of polymer, which can satisfy the stress environment requirements in the body [3, 11]. The mass ratio of HAP to collagen in natural bone is 7:3. This provides us with an important reference when designing the components of the scaffold.

In addition to material, the biological response to the scaffold is influenced by a number of factors including the size and morphology of the pores within the scaffold. The primary obstacle in engineering tissue equivalents is the diffusion limit of oxygen and nutrients, especially for bulk scaffolds [15–17]. It has been proved that hollow channels in bulk scaffold have the following functions: (1) serve as nutrient and oxygen delivery conduits, (2) allow scaffold pre-vascularization with endothelial cells, (3) support cell compartmentalization and (4) allow localized bioactive factor presentation and delivery [15–17]. The common approaches for preparing hollow channels in bulk scaffold include laser piercing [18, 19], sacrificial fibers [20–23], removable wire arrays [24, 25], and 3D printing [26, 27]. In this work, we utilized a novel extrusion approach to prepare bone tissue engineering scaffolds with hollow channel arrays. The shape, size and arrangement of the channels are tunable by changing extrusion molds. In addition, the extrusion approach has advantages of high efficiency and continuous production. The materials utilized in this work were gelatin and gelatin-modified nHAP. The latter was synthesized by coprecipitation method and analyzed by transmission electron microscope (TEM) and atomic force microscope (AFM). The prepared gelatin/gelatin-modified nHAP hollow channel scaffolds were tested by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscope (SEM), mechanical test, cell proliferation assay, and in vitro degradation experiments.

2. Experimental

2.1. Fabrication of gelatin-modified nHAP

All the chemical reagents used were purchased from Sinopharm Chemical Reagent Co., Ltd, Shanghai, China. Gelatin-modified nHAP was synthesized by chemical coprecipitation method. The steps are as follows. 11.8 g Ca(NO₃)₂·4H₂O and 4.0 g (NH₄)₂HPO₄ were dissolved in 1 L deionized water respectively, making the Ca²⁺ and PO₄³⁻ concentrations were 0.05 M and 0.03 M (keep Ca/P ratio at 1.67). To simulate the composition of natural bone (The mass ratio of organic to inorganic is 3:7), 2.2 g gelatin were dissolved to the Ca(NO₃)₂·4H₂O solution. Two kinds of solutions were added gradually (4 drops per second) to another constantly stirring beaker at 37 °C. A concentration of 25% ammonia water was continuously added to the reaction beaker to ensure the pH was 10 ± 0.5 during the whole reaction process. In this preparation process, the appearance of milky white coloration is an indication for the formation of gelatin-modified nHAP. After reaction, the suspension was aged for 24 h and washed with the distilled water until the pH decreased to about 7.0 to remove the impurities. The gelatin-modified nHAP precipitates were then separated from suspension by filtration and lyophilization.

2.2. Preparation of bone tissue engineering scaffolds

Bone tissue engineering scaffolds with hollow channel arrays were fabricated through extrusion method. The preparation process is shown in figure 1. The first step was to dissolve gelatin in deionized water at 40 °C to form gelatin sol. The gelatin here acts as an adhesive and provides toughness for the scaffold. The gelatin-modified nHAP powder was added to the gelatin sol and stirred uniformly. In order to simulate the composition of the natural bone, the mass ratio of the powder to the gelatin was 7:3. When the slurry was slowly cooled to room temperature, its viscosity gradually increased. When it became a toothpaste-like mud, it was transferred to an extrusion cylinder and passed through a specially designed extrusion mold under a certain thrust. The rough scaffold with hollow channels was obtained. The rough scaffold was immediately immersed in anhydrous ethanol, so that the scaffold can be quickly dehydrated and solidified to maintain a certain shape. In order to avoid gelatin dissolved in water again, the rough scaffold was immersed in a 1% glutaraldehyde solution to be chemically crosslinked for 24 h, then washed several times in deionized water to remove residual crosslink agent and dried to obtain a finished scaffold. The method to remove excess glutaraldehyde in the scaffolds is to soak and clean the samples in deionized water. The detailed procedure is to soak the prepared scaffolds in 1 L of deionized water, and change the water every 6 h. In the initial stage of immersion cleaning, it can be observed that the deionized water turns yellow, indicating that residual glutaraldehyde has dissolved out. In the later
period, no color change can be observed gradually, but the water was still changed on time. The entire soaking and cleaning process lasted at least 72 h. After 72 h, glutaraldehyde can basically be removed cleanly.

The schematic diagram of extrusion mold structure is shown in figure 2. The inner surface and outer surface of the mold are different in structure. The inner surface is the side where the material flows in, and the outer surface is the side where the material flows out. The blank areas on the mold in figure 2 are the grooves with a certain depth. When material flows through the extrusion mold, scaffold with hollow channel arrays is formed. The cross-section contour of the scaffold is consistent with the contour of the blank area in the right image in figure 2. The dimensions of a and b in figure 2 represent the wall thickness and pore size of the scaffold, respectively. The adjustment of the pore size and wall thickness can be achieved by adjusting the dimensions of a and b on the mold.

![Schematic diagram of the preparation process of hollow channel scaffold by extrusion molding.](image1)

Figure 1. Schematic diagram of the preparation process of hollow channel scaffold by extrusion molding.

![Schematic diagram of extrusion mold structure.](image2)

Figure 2. Schematic diagram of extrusion mold structure.
The control group, sponge porous scaffold with random pores, was fabricated by lyophilization method. Different from the process of preparing the hollow channel scaffold, the slurry composed of gelatin sol and gelatin-modified nHAP was not extruded through the mold, but was directly cross-linked by adding glutaraldehyde, and then lyophilized after washing.

2.3. Characterization
Gelatin-modified nHAP and hollow channel scaffolds were tested by XRD (D/ MAX-3B, Rigaku Denki, Japan) and FT-IR (Bruker TENSOR27 infrared spectrometer, Germany), analyzed by SEM (SEM Quanta 200, FEI, USA), TEM (H-800, Hitachi, Japan), and AFM (SPM-9600, SHIMADZU, Japan).

The porosities, pore diameters and their distribution of the scaffolds were measured by mercury porosimeter (PoreMaster-33, Quantachrome Instruments, USA).

2.4. Thermal analysis
Thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) were performed on Diamond TG/DTA (PerkinElmer Inc., USA). The temperature range was from 30 °C to 1400 °C and the heating rate was 10 °C min⁻¹. Nitrogen flow rate was 200.0 ml min⁻¹.

2.5. Mechanical tests
Scaffolds were cut to 10 mm × 10 mm × 10 mm cubes for compression tests. The tests were performed on HY-0230 computer controlled electronic universal testing machine (Shanghai HengYi Precision Instrument Co., Ltd, China) at a compression speed of 2 mm min⁻¹. At least 5 times tests for each type of specimen.

2.6. Cell culture and proliferation assay
*In vitro* cell biocompatibility experiments were carried out. 293T cells (one typical Human renal epithelial cells transfected with adenovirus E1A gene) of 3rd generation were utilized to evaluate the cell biocompatibility in the experiments. The cells were seeded into a 96-well culture plate with a density of 5.0 × 10⁴ cells/well and incubated in 100 μl FBS/well for 24 h. 100 μl fresh culture medium which contains a series of scaffold leaching solution ranging from 0.05 g ml⁻¹ to 0.4 g ml⁻¹ was added into each well to replace the culture medium when the cells were almost adhered. The cells were further incubated for 48 h. Then 10 μl of counting kit-8 (CCK-8) solution was slowly added into each well, and the culture plate was incubated for another 4 h. Once cell culture was accomplished, the 96-well culture plate was placed on a microplate reader, and the optical density (OD) in each well was measured at a wavelength of 450 nm. The relative growth ratio (RGR) of cells was calculated as RGR = 100% × AOD/NOD (AOD, average optical density; NOD, negative control optical density, i.e. the optical density of the solution with cells and CCK solution without adding the tested material.).

2.7. *In vitro* degradation tests
The bioactivity and biodegradability of the scaffolds were evaluated by immersing them in a simulated body fluid (SBF) solution, changing the SBF solution every 3 days and calculating the weight loss rate of the scaffolds.

2.8. Statistics
Experimental data were presented as mean ± standard error of the mean (SEM). A two-tailed Student’s *t*-test was performed between some key data of mechanical strength and cell RGR with Origin Software. A *p* value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. XRD studies
In order to determine the phases in the material, XRD method was utilized to analyze from raw materials to finished specimens, namely gelatin, gelatin-modified HAP and hollow channel scaffolds. XRD can furnish information about the phase, crystallinity, and grain size of the material. Through these information, we can more clearly understand the changes in the microstructure of the material after each step of forming.

Gelatin is one of the most important raw materials, and plays two significant roles in this research. One is to modify HAP during the process of HAP synthesis, and the other is to bond the modified HAP powder during the process of preparing the hollow channel scaffold. The XRD patterns of gelatin shown in figure 3 denotes that gelatin is an amorphous polymer phase, because there is no obvious characteristic absorption peak of crystals in the pattern, but only a large, diffuse absorption peak. As is known, gelatin is a hydrolyzed product of collagen, and can be dissolved in hot water above 35 °C [28, 29]. In aqueous solution, the gelatin molecule is a straight-chain molecule with a random coil conformation. The collagen molecule is insoluble in water, and its molecular
Conformation is a triple helix structure formed by three molecular chains. When the temperature of the gelatin aqueous solution is lowered, the gelatin molecules will twist to form a triple helix structure similar to collagen molecules, resulting in a coil-helix transition. However, this coil-helix transition cannot be complete, and only part of the gelatin molecular chain can form an ordered structure of triple helix [28, 29].

The XRD patterns of gelatin-modified nHAP in figure 3 show that the crystalline phase is HAP, because the positions of diffraction peaks correspond exactly to the PDF #09-0432 of HAP, and no other peaks appear. The diffraction peaks of (211), (112), and (300) crystal planes overlap to form one peak, which are difficult to distinguish. It demonstrates that the crystallinity of gelatin-modified nHAP is low, perhaps in nanoscale, which has been confirmed by the subsequent TEM and AFM results. In addition, the sample also contains amorphous gelatin. The amorphous gelatin can interfere with the diffraction peaks of crystallized HAP, and increase the roughness of the XRD pattern and broaden the diffraction peak.

In XRD experiments, the gelatin-modified nHAP powder is randomly and freely oriented during sample preparation. Therefore, the intensity variation of the diffraction peak caused by the orientation of the powder can be excluded. Then, the preferential growth of some crystal directions can be judged according to the relative peak intensity ratio [30]. The diffraction peak of (211) is the strongest peak, and is determined as the benchmark. The ratio of the intensity of other peak to that of (211) is defined as relative intensity, and the values are all less than 1. According to PDF #09-0432 of HAP, the relative intensity of (002) is 40% in standard HAP crystals. However, the relative intensity of (002) in figure 3 is about 72% by calculation after deducting the background, indicating that the gelatin-modified nHAP grows preferentially along the [001] direction. It is speculated that gelatin plays an important part in the preferred orientation growth of HAP crystals.

The patterns of hollow channel scaffold in figure 3 is basically the same as that of gelatin-modified nHAP. There is only a slight difference in composition between the two materials, that is, there is more gelatin in the scaffold, but HAP is still the main component.

### 3.2. FT-IR studies

As previously stated, gelatin exerts significant impacts on the crystallinity and preferred orientation growth of HAP. However, whether there is an interaction force between gelatin and HAP molecules needs to be further explored. Therefore, FT-IR method was utilized to analyze gelatin, gelatin-modified HAP and hollow channel scaffold. The spectra are shown in figure 4.

In the spectrum of gelatin-modified nHAP in figure 4, the absorption peaks appearing near the wavenumbers of 2924 cm\(^{-1}\) and 2856 cm\(^{-1}\) correspond to the characteristic absorption peaks of methylene and methyl groups respectively, indicating the presence of gelatin macromolecules in gelatin-modified nHAP. In the spectra, the absorption peaks appearing at 1680~1630 cm\(^{-1}\), 1570~1510 cm\(^{-1}\) and 1240 cm\(^{-1}\) correspond to the characteristic absorption peaks of the amide I (\(\nu_{C=O}\), II (\(\delta_{\text{NH}}+\nu_{\text{CN}}\)) and III (\(\delta_{\text{NH}}\)) bonds of gelatin. Among them, the characteristic absorption peak of the amide II bond corresponds to two parts. One is \(\delta_{\text{NH}}\) which means the bending vibration of the N-H bond, and the other is \(\nu_{\text{CN}}\) which means the stretching vibration of the C–N bond. These two vibration absorption peaks are very close and integrated into one peak at 1570~1510 cm\(^{-1}\), so they are collectively called the characteristic absorption peak of the amide II bond. It can be observed in figure 4 that the characteristic absorption peak of the amide I bond has a corresponding wave number of 1655 cm\(^{-1}\).
119 cm\(^{-1}\) in the spectrum of gelatin, but 1643 cm\(^{-1}\) in the spectrum of gelatin-modified nHAP. An obvious red shift occurs. This indicates that Ca\(^{2+}\) in HAP and C=O groups in gelatin combine under the action of electrostatic force, resulting in elongation of C=O double bond, which causes red shift of infrared spectrum. In addition, the strength of the amide I, II, and III bonds is significantly weakened in the spectrum of gelatin-modified nHAP, which also indicates that there is an interaction between HAP and gelatin.

The spectrum of gelatin-modified nHAP in figure 4 shows absorption peaks near 874 cm\(^{-1}\) and 1454 cm\(^{-1}\), which correspond to the out-of-plane deformation vibration peak and C–O anti-expansion vibration peak of CO\(_3^{2-}\) respectively. The results indicate the prepared nHAP is hydroxyl-carbonate apatite (HCA), which is similar to the HAP in natural bone. It is presumed that the alkaline reaction solution environment introduced the carbon dioxide in the air into the reaction system during the synthesis process of gelatin-modified nHAP. There are two forms of CO\(_3^{2-}\) incorporation into the HAP lattice, one is to replace PO\(_4^{3-}\) in the lattice, and the other is to replace OH\(^-\) [31]. The introduction of CO\(_3^{2-}\) will reduce the crystallinity of HAP, thereby changing some properties of HAP, for instance, the degradability will be enhanced. This is advantageous for the scaffold, because the HAP with good crystallinity is too stable and the degradation cycle is too long.

The absorption peaks at 1040 cm\(^{-1}\) and 567 cm\(^{-1}\) correspond to the characteristic absorption peaks of PO\(_4^{3-}\) of HAP, which can be observed in the spectra of both gelatin-modified nHAP and hollow channel scaffold.

3.3. TGA-DSC analysis

There are two sources of gelatin in the hollow channel scaffold, one is gelatin that participates in the synthesis of nHAP to form a gelatin-modified nHAP, and the other is gelatin that acts as an adhesive to prepare a hollow channel scaffold. In order to obtain the accurate content of gelatin and nHAP in the scaffold, TGA-DSC method is utilized to analyze the hollow channel scaffold.

The TGA and DSC results of hollow channel scaffold are shown in figure 5. It can be seen from figure 5 that there is a rapid 6% weight loss process at the beginning, which corresponds to an endothermic process with a peak at 88 °C. This process is the evaporation process of adsorbed water in the scaffold. The second rapid 18% weight loss process begins at 280 °C and ends at 450 °C, which corresponds to a strong exothermic peak. This is the pyrolysis process of gelatin molecular chains. When the temperature is higher than 1000 °C, the sample stops losing weight and a distinct endothermic peak appears at 1210 °C. This shows that the organic matter has completely decomposed and volatilized, leaving only a small amount of carbon. The inorganic HAP begins to undergo a phase transition at a temperature higher than 800 °C, gradually changes to β-TCP, and then gradually changes to α-TCP at around 1200 °C. The phase change process is an endothermic process.

The percentage of substance decomposed and volatilized is equal to that of gelatin, and the percentage of the remaining is equal to that of nHAP. Based on the TGA data of 5 sets of the same kind of samples, we obtained the accurate content of gelatin in the prepared scaffold was 38.9%±3.2%, and the accurate content of nHAP was 61.1%±1.9%.
3.4. TEM, AFM and SEM studies

TEM and AFM were used to analyze the microscopic morphology of gelatin-modified nHAP, and the images are shown in figure 6. As shown in figure 6(a) and (c), gelatin-modified nHAP is nanorod-like particles with the length of about 30–50 nm and width of about 5–15 nm. However, due to their small size, large specific surface area, large surface energy, and energy instability, the gelatin-modified nHAP nanoparticles are easy to agglomerate and form secondary particles, which can be clearly observed in both figures 6(a) and (c).

The TEM high-resolution lattice image of gelatin-modified nHAP is shown in figure 6(b). From this figure, we can further obtain the crystal structure information of the nanoparticles. The lattice fringes of (002), (211), (112), and (210) of HAP are marked in figure 6(b). It can be observed that there are many HAP crystal grains with different orientations, and the size of the crystal grains are all less than 10 nm. This is consistent with the XRD results. As mentioned before, the size of gelatin-modified nanoparticles are about 30–50 nm in length and 5–15 nm in width. This means that a gelatin-modified nanoparticle contains several HAP crystal grains with different orientations. As previously discussed, FT-IR results have proved that gelatin-modified nHAP contains gelatin, and XRD results confirm that gelatin is in an amorphous phase. Therefore, we cannot observe gelatin through the crystal lattice as we observe crystalline HAP in figure 6(b). It can be seen from figure 6(b) that the HAP grain boundary is not obvious, and there are amorphous phases among the grains. Gelatin is a macromolecule and it is difficult to enter the HAP crystals. Therefore, it is speculated that the amorphous phases among HAP crystal grains are gelatin and amorphous calcium phosphate. A schematic diagram of the composition of a gelatin-modified nHAP nanoparticle is shown in figure 6(d). A gelatin-modified nHAP nanoparticle is not a HAP single crystal, but a combination of smaller-sized HAP crystals, gelatin and amorphous calcium phosphate.

The gelatin-modified nHAP is similar to the HAP in natural bone in terms of crystallinity, composition, shape, size and preferred growth orientation. HAP in natural bone also has the characteristics of small crystal grains, poor crystallinity, and \( \text{PO}_4^3^- \) or \( \text{OH}^- \) often replaced by other groups such as \( \text{CO}_3^{2-} \) [31]. Because natural bone is responsible for the calcium source in the human body, at a certain moment, the HAP in the bone can provide calcium through degradation. The above characteristics of the HAP in natural bone determine that it can degrade faster than the HAP formed in the geological.

The SEM images of prepared scaffolds are shown in figure 7. Two kinds of scaffolds are put together for comparative observation, one is the hollow channel scaffold (figures 7(a)–(c)), and the other is the control group, which is the sponge porous scaffold (figures 7(d) and (e)).

It can be seen from figures 7(a) and (b) that the porous structure of the hollow channel scaffold is regular arrays of circular hollow channels with pore diameter of about 500 μm. What is interesting is that, according to the design of the mold, the extruded hollow channel scaffold should have channels with square cross-sectional shape, but from the SEM images, the cross-sectional shape of the channel is round. This is because the extruded slurry contains gelatin, which has a certain degree of viscoelasticity. After being extruded through the mold, extrusion swelling and deformation will occur on the rough scaffold. Therefore, the square channels eventually become round channels.

The pore size and its distribution in hollow channel scaffolds with two different porosities are shown in figure 7(j). The two kinds of hollow channel scaffolds have different porosities because they have different channel diameters and wall thickness, which can be achieved by changing the size of \( a \) and \( b \) of the extrusion mold (shown in figure 2). Decreasing the size of \( a \) and increasing the size of \( b \) will reduce the porosity, otherwise,
the porosity will increase. It can be seen from figure 7(j) that the pore diameter of 329 μm corresponds to the maximum number for the scaffold with porosity of 57.3 ± 4.6%, and it is 294 μm for the scaffold with porosity of 51.3 ± 5.2%. This indicates that for hollow channel scaffolds, as the porosity increases, the pore diameter...
Figure 7. Characterization of the microstructure of prepared scaffolds. (a)–(c) SEM images of hollow channel scaffold at different magnifications; (d), (e) SEM images of sponge porous scaffold at different magnifications; (f), (g) SEM images of hollow channel scaffold immersed in SBF for 7 days; (h) EDS spectrum of the spot shown in image; (i) XRD patterns of deposited layers on the scaffold; (j) Pore size and its distribution in hollow channel scaffold.
corresponding to the maximum number increases. It can also be found from figure 7(j) that when the pore diameter is greater than 150 μm, its number increases sharply. After the peak, its number slowly decreases. Even if it reaches 1 mm, there is still some number. The reason is as follows. The hollow channels are very different from other pores. Specifically, the size of the hollow channel is very long in the axial direction and penetrates the entire scaffold, which is much larger than that in the radial direction. Therefore, as shown in figure 7(j), the pore diameter is distributed in a larger range.

The microscopic surface of the hollow channel scaffold shown in figure 7(c) is relatively smooth, and no HAP particles can be observed. One reason is that the HAP particles are very small in size, which are nanoscale, and the other reason is that HAP particles are uniformly dispersed and wrapped in gelatin.

The microstructures of sponge porous scaffold are shown in figures 7(d) and (e). The size and shape of the pores are random in sponge porous scaffold as shown in figure 7(d). The size of these random pores are determined by ice crystals when lyophilized, which are 50~300 μm. The walls of pores in sponge porous scaffolds are very thin as shown in figure 7(e).

The microscopic surface morphology of hollow channel scaffold after immersion in SBF for 7 days are shown in figures 7(f) and (g). It can be observed that a large amount of flaky deposition appeared on the surface of the hollow channel scaffold. In order to clarify the composition of these depositions, a spot on one flake was selected (shown in figure 7(g)) and subjected to EDS analysis, and the results are shown in figure 7(h). The results demonstrate that the flaky deposition mainly contains three elements: Ca, P and O. This means the deposition is a kind of calcium phosphate, indicating that the hollow channel scaffold has good in vitro biological activity. In order to figure out what kind of calcium phosphate the deposition is, we conducted further analysis. The stoichiometric ratio of Ca to P in the deposition is 1.098, while the stoichiometry of Ca to P in HAP is 1.67, in octacalcium phosphate (OCP) is 1.33, and in dicalcium phosphate dehydrate (DCPD) is 1. The stoichiometric ratio of Ca to P in the flaky deposition is closest to that of DCPD. Furthermore, DCPD crystals are flakes, so we infer that the flaky depositions are DCPD crystals. In order to further determine the phase of the deposition, XRD analysis was performed on the flaky layers. The XRD patterns of the deposited layers are shown in figure 7(i). It can be seen from figure 7(i) that the positions of diffraction peaks correspond exactly to the PDF #09-0077 of DCPD. It is further confirmed that the calcium phosphate deposition is DCPD.

In general, HAP is the most common surface deposition phase because it is the thermodynamically most stable calcium phosphate. Compared with HAP, DCPD has a higher nucleation rate and, therefore, is more dominant in kinetics [32]. Studies have shown that DCPD is more likely to take precedence over HAP formation when the concentration of local Ca and P ions increases [32]. For the hollow channel scaffold, it may be due to the poor crystallinity and small grain size of the gelatin-modified nHAP, which triggers off its rapid degradation rate and elevates local Ca and P ion concentration, thus making DCPD easier to deposit. However, the deposited DCPD is a mesophase that will eventually transform into the thermodynamically most stable phase HAP.

3.5. Mechanical analysis

We measured the compressive strength of hollow channel scaffolds and sponge porous scaffolds respectively for comparative research. Since the hollow channel scaffolds are structurally anisotropic, their compressive strength in axial direction (the direction parallel to the axes of hollow channels) and radial direction (the direction perpendicular to the axes of hollow channels) were measured separately. However, the sponge porous scaffolds are structurally isotropic. Therefore, it is only necessary to measure the compressive strength of the scaffold in any direction. The results are shown in figure 8.

It can be seen from figure 8 that the compressive strength of 51.3 ± 5.2% porosity hollow channel scaffold is higher than that of 57.3 ± 4.6% porosity ones, whether in axial or radial direction.

The compressive strength of the hollow channel scaffolds with porosity of 51.3 ± 5.2% is up to 25.5 ± 1.4 MPa in axial direction and 8.9 ± 0.7 MPa in radial direction. The axial compressive strength is higher than radial one. This characteristic is the same as the hollow natural tubular bone, and that determines the axial direction is the force direction when hollow channel scaffolds are planted in the body.

The compressive strength of sponge porous scaffolds with the porosity of 80.1 ± 9.3% is 0.5 ± 0.2 MPa. Although the strength decreases with the increase of porosity, the compressive strength of sponge porous scaffold is too low. The reason why the compressive strength of hollow channel scaffold is much higher than that of sponge porous scaffold is analyzed as follows. Hollow channel scaffold were prepared by extrusion molding. The preliminary extruded scaffolds were rapidly immersed in anhydrous ethanol to dehydrate and solidify. Due to the dehydro, the gelatin molecular segments got closer, and the crosslinking degree got higher because of more reaction chance among the functional groups. Compared with the lyophilized sponge porous scaffold, nHAP particles can be tightly packed and fixed in three-dimensional gelatin network in hollow channel scaffold, so that the mechanical strength can be significantly enhanced. In addition, it can be observed that the wall
The thickness of the hollow channel scaffold is much higher than that of the sponge porous scaffold in figure 7, which is another reason for the high mechanical performance of the hollow channel scaffold.

The compressive strength of human trabecular bone is generally used as the benchmark to evaluate the strength of bone tissue engineering scaffolds, which is required to be more than 5 MPa. Hence, the prepared hollow channel scaffolds can meet the mechanical strength requirement completely.

3.6. Degradation analysis

The in vitro degradation results of prepared scaffolds are shown in figure 9. The difference between hollow channel scaffolds and sponge porous scaffolds is obvious.

Firstly, there is a process of weight growth for the hollow channel scaffolds in the first 7 days, which cannot be observed on the sponge porous scaffolds. The reasons are as follows. Two processes coexist during the degradation process of scaffold, one is the deposition of calcium phosphate on the surface of the scaffold, and the other is the degradation of scaffold material itself. These two processes are the main contradiction that causes the weight change of the scaffold. For the hollow channel scaffold, the deposition rate of calcium phosphate is greater than the degradation rate of scaffold material in the first 7 days, resulting in the weight growth of the scaffold. The deposited calcium phosphate has already been observed and analyzed in figure 7. After the 7th day, the degradation rate of scaffold is greater than deposition rate of calcium phosphate, which is demonstrated by

![Figure 8](image_url). Compressive strength of hollow channel scaffolds and sponge porous scaffolds; *p < 0.05, **p < 0.01, ***p < 0.001.

![Figure 9](image_url). In vitro degradation results of prepared scaffolds.
the sustained weight loss of the scaffold. The sponge porous scaffold shows no weight growth in the first few days, indicating that its degradation rate is greater than deposition rate throughout the whole degradation process.

Secondly, the degradation rate of hollow channel scaffold is much less than that of sponge porous scaffold. The analysis is as follows. The scaffold consists of gelatin and gelatin-modified nHAP. The degradation rate of gelatin is much faster than that of nHAP. Therefore, the main factors that affect the degradation of the scaffold are the degradation and collapse of three-dimensional network structure of gelatin. Losing the binding and fixation of the three-dimensional network of gelatin, nHAP particles can be easily detached from the scaffold. As discussed before, compared with sponge porous scaffold, the gelatin molecular chains in hollow channel scaffold are closer after dehydration in anhydrous ethanol, leading to a higher crosslinking degree of gelatin. This makes the gelatin three-dimensional network more difficult to degrade, thereby reducing the overall degradation rate of the scaffold.

In addition, the sponge porous scaffold degraded by about 80% on the 71st day. However, the degradation rate was too fast, which did not match the rate of new bone formation. As an improvement, the hollow channel scaffold degraded by about only 28% at the same time, which showed a better performance in terms of degradation rate.

3.7. Cell proliferation analysis

The RGR results of cells of gelatin-modified nHAP and hollow channel scaffolds are shown in figure 10 for comparative study. To gelatin-modified nHAP, the RGR of cells were about 97%~110% as concentration ranges from 0.4 g ml\(^{-1}\) to 0.05 g ml\(^{-1}\). The well proliferation of cells indicates the gelatin-modified nHAP nano powders are of good capacity in tissue engineering and biomedical applications.

The cells RGR of hollow channel scaffolds are slightly less than that of gelatin-modified nHAP, except at the concentration of 0.05 g ml\(^{-1}\). However, the RGR values are still more than 88%, which proves the hollow channel scaffolds are at non-toxic level. Glutaraldehyde was used as the crosslinking agent in the fabrication process of hollow channel scaffolds, which is speculated to be the reason that RGR value of scaffolds are less than the powder.

4. Conclusions

A new extrusion method was used to prepare bone tissue engineering scaffold with hollow channel arrays. In the preparation process, the scaffold can be continuously produced when it is extruded through the mold, and the scaffold can be cut into the required length according to the specific needs, thereby greatly improving the production efficiency and economy. The porosity, pore arrangement, pore size and shape of the scaffold can be adjusted by changing the extrusion mold. The replacement of the extrusion mold is very convenient and fast, which can realize the mass production of scaffolds with different pore structures.
Gelatin-modified nHAP synthesized by chemical coprecipitation method are nanoparticles with the length of 30~50 nm and width of 5~15 nm, which are observed by SEM and AFM. However, according to the SEM high-resolution image analysis, the nanoparticle is not a HAP single crystal, but is composed of many HAP crystal grains with a size of less than 10 nm in different orientations and amorphous phase among them. According to the results of FT-IR, the amorphous phase contains gelatin, and there are electrostatic bonds between gelatin and HAP.

The prepared hollow channel scaffolds exhibit much better mechanical properties compared with the sponge porous scaffolds. The axial compressive strength of the hollow channel scaffold with porosity of 51.3 ± 5.2% can reach 25 ± 1.4 MPa, which can meet the mechanical strength requirement as an implant completely. Moreover, the in vitro degradation results of the hollow channel scaffolds were better than that of the sponge porous scaffolds. DCPD deposited on the surface of the hollow channel scaffold when the scaffold was immersed in SBF, indicating that the scaffold has good in vitro biological activity. Furthermore, the scaffold shows good cell compatibility according to cell experiments. This study provides a possible way for the preparation of hollow channel scaffolds in terms of materials and preparation methods.

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ORCID iDs
Xiliang Chen https://orcid.org/0000-0002-7524-2990

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