Research on functional boards and plastic films based on animal skin and hydroxyapatite

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Abstract

Nano-Hydroxyapatite precursors with calcium and phosphorus salts (Ca/P = 1.67) were introduced into a three-dimensional collagen network matrix (3DCM) based on goat skin pretreated with glutaraldehyde for in situ growth, and a functional HAG-3DCM board was obtained. After plasticity compression, a transparent protein plastic film was formed. Response surface methodology based on plasticity pressure conditions was used, and the strength, hardness and water resistance of the HAG-3DCM plastic film was significantly improved. This study demonstrates a new approach for the preparation of animal skin materials with new application value.

1 Introduction

The use of animal skins has a long history, and even 8000 years ago, animal glue from animal skin extracted by cavepeople in the eastern region was used as a home decoration adhesive. Gelatin from animal skin has played an important role in photography, medical accessories, drug packaging, the food industry and so on.¹⁻³ Tanning leather represents the best use of animal skin, and leather products were found in Egyptian pyramids 3,000 years ago.⁴ Animal skin is composed of three parts, namely, the epidermis, dermis and subcutaneous tissue. The main structural component of the dermis is a protein collagen. After the removal of water, interfibrillar substance and fat, collagen accounts for over 95.0% of the remaining material, most of which is type I collagen, and a small amount is type III collagen.⁵

As a natural renewable resource, protein has become a new research focus as an alternative to petroleum as a flaky material in the field of decorative boards and packaging films. Biobased plastics made of protein or "protein plastics" have the characteristics of low cost, nonbiological toxicity, good degradability.⁶ Protein plastics mainly come from soybean protein, cottonseed protein, corn protein and wheat protein.⁶⁻⁸ Because protein generally has inherent defects including poor water resistance, low mechanical properties, poor processability, etc., it is usually used as a filler or additive to blend with ordinary petroleum-based plastic materials, which can reduce the cost of plastic materials and promote plastic decomposition to reduce environmental pollution.^{9,10}

Hydroxyapatite (HA) is slightly soluble in water and weakly alkaline (pH=7.0~9.0), and it has excellent biological activity, biocompatibility and nontoxicity; it is widely used in bone repair, bone transplantation and drug transportation.¹¹ Natural bone is a composite material composed of type I collagen and HA. HA accounts for 70.0% of the backbone mass and 90.0% of the remaining 30.0% organic components are type I collagen. Artificial bone preparation involves adding calcium and phosphate salt solutions to the collagen solution in sequence, and the obtained HA particles are evenly distributed in the collagen matrix or embedded on the surface of the collagen molecule. This type of synthesis method is also called the in situ synthesis law.¹² A large number of research reports have found that HA/collagen composites generally have shortcomings such as low mechanical strength, easy swelling, and poor inducing activity,¹² which limits the application channels. In this paper, HA was introduced into natural three-

dimensional skin collagen pretreated with glutaraldehyde to induce in situ aggregation, crystal growth and collagen tissue mineralization,¹³ which hardened the collagen matrix and improved the thermal stability;^{14,15} the heat insulation and flame retardant capacities of the composites were measured. Further, the preparation of the protein-based plasticity compression film was studied,^{16,17} and the transparency, water resistance and strength of the film samples were measured. Based on the results, new and potential pathways for functional modification of animal skin are presented.

2 Experimental

2.1 Materials

Analytically pure diammonium hydrogen phosphate ((NH₄)₂HPO₄), calcium nitrate (Ca(NO₃)), glycerol, etc., and biological grade hydroxyapatite (HA), were obtained from Chengdu Kelon Chemical Reagent Factory. Industrial grade polyurethane emulsion, was obtained from Xuzhou Hongfeng Polymer Material Company.

Softened goat skin collagen, was obtained from a number of New Zealand salted sheep skins with an area of 0.7~1.0 m², and it was prepared and purified by removing the noncollagen components, including the hair, epidermis, grease, and interfibrillar substances.¹⁸ Finally, the pH of the skin collagen was adjusted to 4.5~5.0 with 2 mol/L ammonium sulfate, and the purified three-dimensional collagen fiber material matrix (3DCM) with a thickness of 0.65±0.05 mm was obtained by 10 kg of centrifugal dehydration.

2.2 Preparation of micronucleus precursor HA in vitro

Then, 1000 mL of deionized water and 200 g of calcium nitrate and diammonium hydrogen phosphate mixture (according to Ca/P=1.67) were added to the three-necked flask. The pH value of the solution was adjusted with ammonia to 11.0, and the solution was stirred at 80°C for 12.0 hours and allowed to stand for 12.0 hours. The solution was centrifuged at 10.0 kg, and the precipitate was dried to a constant weight at 105°C for sample analysis.

2.3 Penetration and growth of precursor HA in 3DCM

3DCM (by dry weight) was weighed and put into a Ø600 drum (Derun Light Industry Machinery Factory, Wuxi, China), and then, 150% (30°C) water and 2.0% glutaraldehyde (by effective content) were added, rotated for 120 min, stopped after 120 min, and rotated again 5 times. Subsequently, the pH was adjusted to 6.0 ~ 6.6 with 10% NaHCO₃ solution within 60 min, and then, the liquid was drained. Water (150%) was added, and then, the solution was rotated for 10 min at 50°C to obtain a 0# sample (3DCM). Based on the 3DCM sample, 5% micronucleus precursor HA and (1~4)×(4.0% Ca(NO₃)₂+2.8%((NH₄)₂PO₄)) were added according to the Ca/P of 1.67 (note 1#~4#). After rotating at 5 r/min for 4.0 hours, the solution was allowed to stand for 48.0 hours, and then, it was dried naturally and stretched to obtain, 1#HAG-3DCM~4#HAG-3DCM samples.

2.4 Optimization of the HAG-3DCM sample preparation

2.4.1 Determination of the HA content and Ca/P in the samples

Certain amounts of the 0#3DCM and 1#HAG-3DCM~4# HAG-3DCM samples were dried at 105°C to a constant weight. The solution was composed of $V(\text{HNO}_3):V(\text{H}_2\text{O}_2)$ at 5.0:2.0. It was digested at 100°C for 2.0 hours and diluted by a certain multiple. Then the contents of Ca and P in the digested samples were measured by ICP (VG PQExCell, TJA Company, USA). The Ca/P ratio and HA content in the samples were calculated.

2.4.2 Moisture and heat stability test of the samples

Shrinkage of collagen is a dynamic process. The shrinkage temperature (T_s) reflects the difference in moisture and heat resistance of treated collagen. The T_s values of the 0#3DCM and 1#HAG-3DCM~4#HAG-3DCM samples were measured with an SMW-YD1 shrink temperature meter.

According to experiments 2.4.1 and 2.4.2, the optimized HAG-3DCM and its preparation process can be determined.

2.5 Characteristics of the optimized HAG-3DCM

2.5.1 HA structure test of HAG-3DCM

The optimized HAG-3DCM sample was calcined in a muffle furnace at 800°C for 2.0 h to remove organic components, and then the calcination residual was washed with water to remove soluble inorganic components. After drying, an X-ray diffractometer (X'PertProMPDY129, Nalytical, Netherlands) was used for the remaining inorganic phases. The construction of outsourced biograde HA and HA in the optimized HAG-3DCM was analyzed and compared. The selected scanning angle step was 0.02°, the scanning range was 10.0 ~ 70.0°, and the scanning speed was 2°/min.

2.5.2 Morphology of precursor HA and HA in HAG-3DCM

To observe the morphology of precursor HA and HA in the optimized HAG-3DCM, the precursor HA and the optimized HAG-3DCM samples were calcined in a muffle furnace at 800°C for 2.0 h, and then, two calcined residuals were washed with water to remove the organic and soluble inorganic components. After drying, TEM analysis was performed on two calcined residuals that were fixed on the sample platform with conductive adhesive, and the surface morphology was observed by a Tecnai G2F20 microscope (S-Twin, FEI Corporation, USA).

2.5.3 Element distribution characteristics in EDS

To understand the penetration and accumulation growth of HA precursors in 3DCM, EDS scanning was performed on the 3DCM cross-section at selected points based on the phosphorus-rich region and

scanning was carried out at a diameter of 0.005 mm by energy dispersive X-ray spectroscopy (EDS, JSM-7500F, Japan Electronics Co., Japan).

2.5.4 Morphology of HAG-3DCM

The morphology of collagen fiber bundles in the optimized HAG-3DCM was observed by scanning electron microscopy (JSM-7500F, JEOL Corporation, Japan). The test surface was sprayed with gold, and the acceleration voltage was 3.00 kV.

2.6 Functional characterization of HAG-3DCM plate materials

2.6.1 HAG-3DCM thermal insulation test

The thermal conductivity of the optimized HAG-3DCM was characterized by the steady-state method to measure the thermal conductivity of the sample. Using the coefficient of the thermal conductivity tester (DRP-□, Xiangtan Xiangjiang Instrument Co., Ltd.). First, the sample was placed between the upper and lower copper plates and heated to the set temperature. When the temperature of the upper and lower copper plates was stable, the temperatures T_1 and T_2 were recorded. Next, the sample was removed, the copper plate was heated to T_3 (higher than T_2), heating was stopped, and then, the change in temperature of the lower copper plate over time was recorded again.

$$\lambda = -mc \frac{2h_p + R_p}{2h_p + 2R_p} \times \frac{1}{\pi R^2} \times \frac{h}{T_1 - T_2} \times \frac{dT}{dt} \Big|_{T=T_2} \quad (1)$$

where m is the mass of the lower copper plate, g; c is the specific heat capacity of the copper plate, kJ/(K·kg); h_p and R_p are the thickness and radius of the lower copper plate, respectively, mm; h is the sample thickness, mm; and T_1 and T_2 are the temperatures of the upper and lower copper plate, respectively, °C. The basic diagram is shown in Figure 1.

2.6.2 Flame retardant test of HAG-3DCM

The optimized HAG-3DCM was tested according to GB/T2406-1993 (Plastics Combustion Performance Test—Oxygen Index Method). An oxygen index tester (JF-3, Nanjing Jionglai Company) was used to test the limiting oxygen index of the samples. The combustion time was 15.0 s and the combustion length was 5.0 cm.

2.6.3 Test of the physical and mechanical characteristics of HAG-3DCM

Tested samples of the optimized HAG-3DCM were cut and kept at constant temperature (25.0°C) and humidity (65.0% RH) for 24 h. According to the standard QB/T2710-2005 the tensile strength and elongation at break of the samples were measured by using a universal tension machine (AL7000SN, Taiwan High-speed Railway Technology Company). At 25°C, the rising rate of the instrument was 50 mm/min. Each group of samples was measured in 5 parallel tests, and the average value was taken.

2.7 Functional characterization of HAG-3DCM plastic films

2.7.1 Preparation of plasticity pressed films

3DCM and optimized HAG-3DCM samples were cut into small pieces of 10×20 cm and uniformly coated with 100 g/m² glycerin on both sides. The samples were dried completely in a vacuum at 35.0°C, and then kept at constant temperature (25.0°C) and constant humidity (65.0% RH) for 24.0 h. The samples were placed in the die of a hot press, which was designed according to the response surface test. The molding temperature was set at 100, 130 and 160°C, the pressure was set at 8.0, 10.0 and 12.0 MPa, and the molding time was set at 5, 10 and 15 min. The plasticity pressed samples were referred to as 3DCM protein plastic (3DCM-PP) and HAG-3DCM protein plastic (HAG-3DCM-PP).

2.7.2 Hardness test of the 3DCM-PP and HAG-3DCM-PP

According to the GB 2411-1980 method, the 3DCM-PP and HAG-3DCM-PP samples were cut with a sampler. A shore hardness tester (LX-A-1, Wenzhou Weidu Electronics Co., Ltd.) was used to apply 1.0 kg of load and pressure for 30.0 s, and the Shore hardness value of each sample was recorded.

2.7.3 Strength test of the 3DCM-PP and HAG-3DCM-PP

According to the GB/T1040-92 plastic tensile property test method, the 3DCM-PP and HAG-3DCM-PP samples were cut with a sampler. The tensile strength and elongation at break of the samples were measured by universal tensile machine (AL-7000SN, High-speed Rail Technology Co., Ltd.), and the tensile rising rate was 50.0 mm/min. Each group was measured in parallel 3 times, and the average values of the tensile strength and elongation at break of each sample were recorded.

2.7.4 Swelling property test of the 3DCM-PP and HAG-3DCM-PP

3DCM-PP and HAG-3DCM samples of the same amount were placed into Petri dishes and immersed in 50.0 mL of deionized water for 24.0 h at 37.0°C, then, the surface moisture was removed with filter paper, and the samples were weighed. Three sets of parallel values were measured, and the average value was calculated as the swelling degree of the sample. The sample swelling degree is calculated by equation (2):

$$S = \frac{M_s - M}{M} \times 100\% \quad (2)$$

where S is the swelling degree (%), M is the initial mass (g), and M_s is the swelling mass (g).

2.7.5 Light transmittance test of the 3DCM-PP and HAG-3DCM-PP

The 3DCM-PP and HAG-3DCM samples were cut to a size of 2.0×5.0 cm. The transmittance of the samples in three different locations was measured by a light transmittance tester (LS116, Shenzhen

Lianhuicheng Technology Co., Ltd.) in the length direction, and the average values were obtained.

2.7.6 Flexural resistance of the 3DCM-PP and HAG-3DCM-PP

According to the standard of leather torsion fastness (QB/T 2714-2018), constant temperature (25.0°C) and constant humidity (65.0% RH) conditions were applied for 24.0 h, and then, the 3DCM-PP or HAG-3DCM-PP films were placed in a torsion fastness tester (XK-3014, Jiangsu Xiangke Instrument Company, China). The film size was 70×45 mm and the winding angle was 22.5°. The maximum torsion fastness was observed by 5 cycles until cracks were observed under 5× magnification.

3 Results And Discussion

3.1 Optimized HAG-3DCM

The analysis results of the complex formed by HA growth in the 3DCM sample are shown in Table 1. Ca and P determined by ICP after digestion of HAG-3DCM and $w(\text{HA})/\%$ were calculated according to a Ca/P of 1.67. The results show that 0#3DCM contains negligible amounts of HA and 1#~4#HAG-3DCM are close to the $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ structure in 3DCM. The Ca/P ratios in 1#~4#HAG-3DCM are all higher than 1.67 because there was a small amount of Ca in 3DCM, which was derived from the preparation process of 3DCM. Table 1 shows that the addition of $\text{Ca}(\text{NO}_3)_2$ and $(\text{NH}_4)_2\text{PO}_4$ in 4#HAG-3DCM compared with 3#HAG-3DCM increases by 25%, although the effective binding $w(\text{HA})/\%$ increases by 0.08%. The Ts values of 1#~4# HAG-3DCM are mainly from glutaraldehyde tanning; thus, the difference among them is small, but the 3# is much closer to the 4# Ts. Therefore, 3#HAG-3DCM can be considered to have reached the stabilization effects, turning point and represents the optimized process for the preparation of 3DCM in this experiment. Hereafter, this variant is called HAG-3DCM in the experimental samples.

Table 1 Analysis of HA in HAG-3DCM

EXPNO	Ca/P	$w(\text{HA})/\%$	Ts/°C
0#	-	0.02	57.8
1#	1.68	3.90	78.1
2#	1.72	6.08	78.7
3#	1.73	7.96	79.4
4#	1.73	8.04	79.5

3.2 Element distribution characteristics in HAG-3DCM

Since almost no P is observed in 3DCM, it represents the aggregation point of HA in the phosphorus-rich region. Four HA growth points were identified by scanning from the 3DCM surface in an approximate range of 0.7 mm. The elemental analysis is shown in Figure 2. According to the analysis of the surface

scanning results, the Ca/P in HA-3DCM was 1.71~1.80, which was larger than that of standard HA. When the pH is higher than the pl of 3DCM, the penetration and combination of Ca^{2+} in 3DCM is easier while that of PO_4^{3-} is impeded; thus, Ca/P can not meeting the theoretical value of 1.67.

3.3 Morphology of the HA precursor and HA in HAG-3DCM

3.3.1 HA morphology in 3DCM

The two calcination residuals from the precursor HA and HA in the HAG-3DCM sample were observed by TEM, as shown in Figure 3. Compared with the HA from the precursor, the HA from HAG-3DCM was similar to the precursor HA in a columnar shape, although the diameter of the precursor HA was less than 100 nm, and HA particles in the HA-3DCM sample were more bonded and slightly larger in volume. Therefore, crystal growth occurs in the 3DCM sample, as shown in Figure 3.

3.3.2 HA structure in HAG-3DCM

The XRD spectra of standard HA¹⁹ and inorganic phase samples in the HAG-3DCM sample were compared, as shown in Figure 4. The main diffraction peaks of the inorganic phase in the body correspond to the characteristic diffraction peaks of the standard HA in Figure 4, in which the characteristic peaks (002), (210), (211), (300) and (213) appear in turn. It was further confirmed that the inorganic phase in the HA-3DCM sample was HA.

3.3.3 Formation process of HAG-3DCM

According to the preparation process of HAG-3DCM and the morphology and structure of HA, the crystal growth process of HA in 3DCM is shown in Figure 5.

3.4 Structure and function of HAG-3DCM

3.4.1 HAG-3DCM fiber bundle braiding

By comparing the SEM images of the cross-section and longitudinal section of raw material 3DCM and compound HAG-3DCM. As shown in Figure 6, the formation of HA in the 3DCM caused the HAG-3DCM fiber bundles to separate from the aggregates, which is consistent with the mechanism of the separation and fixation of skin collagen tissues by tanning agents in the tanning chemistry. Thus, HAG-3DCM can be considered a new type of chrome-free tanned leather.

3.4.2 Heat insulation of HAG-3DCM

The thermal conductivity of 3DCM and the HAG-3DCM film was measured by the steady state method at 60°C, as shown in Table 2. The thermal conductivity of HAG-3DCM is decreased from 0.25 W/K·m to 0.12 W/K·m compared with that of 3DCM. The decrease in thermal conductivity is caused by cavitation (see Figure 5) of the collagen matrix after 3DCM is treated with HA, and the increase in porosity leads to a decrease in thermal conductivity.²⁰

Table 2 Thermal conductivity of HAG-3DCM

Samples	3DCM	HAG-3DCM
$\lambda/W/K \cdot m$	0.25	0.12
LOI/%	20.6	26.5

3.4.3 HAG-3DCM flame retardancy

In the combustion process, a carbonization layer is formed on the surface of the 3DCM and HAG-3DCM samples. The many internal pores in HAG-3DCM can store the generated ammonia nitrogen and block the oxygen and external heat sources to prevent further combustion.²¹ Table 2 shows that HAG-3DCM has a relatively high limiting oxygen index of 26.5%.

3.4.4 Physical and mechanical characteristics of HAG-3DCM

Table 3 shows the physical and mechanical values of the HAG-3DCM. Due to the lack of a standard comparison, the sample was used as a type of leather for reference in this experiment. Compared with the national standard of chrome-free upper leather (≤ 1.0 mm), it can be seen that except for the bursting force, the other indexes such as the tensile strength, tearing strength, and load elongation all meet the requirements.

Table 3 Physical and mechanical properties of chrome-free leather (≤ 1.0 mm)

Samples and Standards	bursting /(N/mm)	tearing /(N/mm)	tensile /MPa	elongation (10 kg)/%
HAG-3DCM	359	28.1	10.0	73.2
QB/T 1873-2010(≤ 1.0 mm)	≥ 400	≥ 21	≥ 8.0	≤ 35

3.5 Plasticity pressing and HAG-3DCM film

3.5.1 Optimization of plasticity pressing

The 3DCM and HAG-3DCM samples were plastically pressed to prepare the 3DCM-PP and HA-3DCM-PP protein plastic films. Taking the tensile strength of the plastic film as the performance index of the sample tested, the influence of different molding pressures, molding times and molding temperatures on the tensile strength of the protein plastic film sample was evaluated.

Tensile strength is related not only to the strength of a single fiber, but also to the number of fibers per unit cross section. The greater the strength of a single fiber and the greater the number of fibers per unit cross section, the greater the tensile strength. For a protein-based structure, as the density increases, the binding between molecules and molecular chains is strengthened until reaching a limit.

A response surface analysis was performed on the tensile strength test results of the sample with a thickness of 1.0 ± 0.12 mm, as shown in Figure 7. When the molding time was 11.0 min, the tensile strength of the protein plastics increased with increasing pressure. When the molding time was 11.0 min, the increase in temperature had little effect on the tensile strength of the sample.

According to the results shown in Figure 7, Table 4 integrates the analysis of pressure, temperature and time factors and obtains the order of influence as follows: pressure > temperature $\approx$ time.

Table 4 Effect of the factors on the sample tensile strength

Test factor	<i>F</i> (HAG-3DCM-PP)	<i>F</i> (3DCM-PP)
A-pressure	63.8	64.9
B-temperature	9.26	20.5
C-time	11.7	12.4

For 3DCM, in addition to the pressure factor, the influence of the time factor is greater than that of the temperature because when the temperature is higher than 100°C, the 3DCM is subjected to thermal denaturation and gelation, which easily results in a rapid increase in the density of the 3DCM-PP and a change in the tensile strength because the rheological velocity becomes a major factor.

For the HAG-3DCM-PP, the temperature factor is greater than the time factor. Although the entry and growth of HA greatly improves the gelling resistance and inhibits the rheological rate after gelation of HAG-3DCM, the gelling degree of the HAG-3DCM-PP is greatly affected when the temperature is over 100°C.

The optimal test conditions for the 3DCM-PP and HAG-3DCM-PP and the tensile strength of the samples are analyzed according to the response surface, as shown in Table 5. The tensile strength of the HAG-3DCM-PP is better than that of the 3DCM-PP and exceeds 41.0 MPa. This indicates that HA aggregates and grows in 3DCM, which can mineralize collagen fibers well, stabilize the structure of 3DCM, and significantly increase the tensile strength of the plastic film. For the two kinds of films, the optimum conditions that have common significance are a pressure of 10.5 MPa, temperature 135°C and time of 11.0 min.

Table 5 Optimum conditions and sample tensile strength

Test factor	3DCM-PP	HAG-3DCM-PP
Pressure/MPa	10.5	10.7
Temperature/°C	135.0	136.0
Time/min	11.8	11.4
Tensile strength/MPa	26.0	67.0

3.5.2 HAG-3DCM plastic film function

The 3DCM-PP film and HAG-3DCM-PP film were prepared under optimized plastic pressure conditions (pressure 10.5 MPa, temperature 135°C and time 11.0 min). The tensile strength, elongation at break, flexural resistance, hardness, swelling rate and light transmittance of the sample were tested, and the results are shown in Table 6.

Table 6 Physical performance of the two protein plastic films

Indicators	3DCM-PP	HAG-3DCM-PP
Tensile strength/MPa	26.0	67.0
Elongation at break/%	18.0	11.0
Flexural resistance/times	0~5	45~50
Shaw hardness	95.0	96.0
Swelling rate/%	55.0	12.0
Light transmittance/%	71.0	55.0

After HA enters 3DCM to form HAG-3DCM, a special stabilization effect or mineralization effect occurs, under which tensile strength and hardness are increased and the water swelling resistance is greatly improved. Although the transparency of 3DCM is higher than that of HAG-3DCM (as shown in Figure 8), the pores between the fibers in HAG-3DCM can be removed by plasticity pressure to satisfactorily improve its transparency. According to the comparison of the flexural resistance in Table 6, without HA support, glycerol could not improve the tortuous resistance of 3DCM. The data shown in Table 6 show that 3DCM has potential application value.

4 Conclusions

When nano-HA precursors with calcium and phosphorus salts (Ca/P=1.67) were infiltrated into the three-dimensional collagen network matrix (3DCM) based on goat skin pretreated with glutaraldehyde for in-situ growth, an ecological animal skin function board called HAG-3DCM was obtained. The collagen tissue was fixed or mineralized in the form of dispersion or cavitation. After plasticity compression a

transparent protein plastic film (HAG-3DCM-PP) was formed. For the optimization of plasticity pressure conditions, the strength, hardness and water resistance of HAG-3DCM were significantly improved compared with the 3DCM (without HA). This study demonstrates a new approach to the preparation of ecological materials from animal skins with new application value.

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Figures

Figure 1

Principle of the heat conduction test

E	<i>weight%</i> in four points			
	①	②	③	④
<i>C</i>	48.7	41.7	43.4	47.7
<i>N</i>	21.3	14.4	18.2	20.1
<i>O</i>	26.1	22.7	30.5	29.3
<i>Ca</i>	2.20	14.9	5.60	1.60
<i>P</i>	1.00	6.50	2.40	0.70
<i>Cl</i>	0.40	0.60	0.40	0.40
<i>Na</i>	0.30	0.20	0.50	0.20

Figure 2

Elemental ratio of HA growth point

Figure 3

TEM morphology of the precursor HA (left) and in vivo HA (right)

Figure 4

XRD of standard HA and in vivo HA

Figure 5

In vivo growth process of HA

Figure 6

SEM image of the collagen matrix before and after HA treatment

Figure 7

3D diagram of response surface analysis of plasticity compression for 3DCM-PP

Figure 8

Appearance of two kinds of protein plastic films

Supplementary Files

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