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Preparation, Microanalysis and Performance of Hap/Cs-Cmc Composite Materials

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Abstract The physical and chemical properties of the materials are generally determined by their microstructure and main components. In this paper, the Hap/Cs-Cmc composite materials with different mass ratios were prepared for the first time through liquid co-precipitation method. The microstructure, phase and performance of the composite materials were investigated by FT-IR, XRD, TG-DTA, SEM, and EDS respectively. The purpose of this work is to establish the relationship between material microstructures and properties. The results showed that the composite materials exhibited excellent mechanical performance and thermal stability. The nano-Hap with relatively good crystallinity were dispersed uniformly in the organic phase Cs and Cmc-combined with relatively closely between Hap particles and Cs-Cmc. The particle size of the material is about 50 nm with spherical shape. The mass ratio of Hap and Cs-Cmc directly influenced the crystallization, particle size, and dispersion of Hap. When mass ratio is 50/50, the uniformity, compactness, and thermal stability were the best and the compressive strength is up to 30.5 MPa. EDS analysis showed that the composite material merely contained trace amount of sodium and the ratio of calcium and phosphorus is around 1.85, belonging to the rich calcium type of Hap, and the physical and chemical performance totally met the requirements of bone tissue engineering materials.

Keywords: *nano-hydroxyapatite, chitosan, carboxymethyl cellulose, composite materials, microanalysis*

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1. Introduction

Hydroxyapatite (Hap) is the main component of natural bone inorganic salt which has good biocompatibility, bone conductibility, and is regarded as an ideal material of bone defect repair [1,2]. However, the application of Hap in bone tissue engineering was limited due to its brittleness and low degradation rate [3,4]. In order to improve the performance of bone repair materials, many materials were selected to prepare composites materials with good mechanical strength, such as titanium alloy, natural polysaccharide, protein, synthetic polymers, and so on [5,6,7,8]. Nevertheless, it can be found that the single material compounded with Hap can not meet the requirements of bone tissue engineering material (such as strength, toughness, biological activity, etc) [9,10,11]. Therefore, it is a hotspot for the composite materials combined with two or more other materials to improve the performance of Hap.

The chitosan, as a natural biodegradable polysaccharide, has good biocompatibility, biological absorbability, stability, and blood adhesion, etc [12,13,14], so many chitosan-based materials have been applied in biomedical fields. Recently, many studies have been reported for the Hap/Cs composite materials, which generally exhibit some disadvantages, such as bad plasticity and mechanical property and poor interface bonding between Hap and Cs [15,16,17]. In order to improve its physical and chemical properties, one good way is to add other substance for modifying Hap/Cs composite materials. The carboxymethyl cellulose (Cmc) is a specie of cellulose with non-toxicity, adhesiveness, biodegradability, biocompatibility and containing large number of hydroxyl [18,19]. As the third phase, it can improve the interface combination between Hap and Cs, while endowing the composite material excellent mechanical, chemical and biological properties. In recent years, there were many research reports on Hap/Cs-Cmc composite materials, but these reports mainly focus on their biological activity, biocompatibility, and biological safety evaluation. There are few studies for the relationship between the material preparation, the microstructure, and the performance. In this paper, nano-Hap/Cs-Cmc composite materials with different weight ratios were prepared firstly by a liquid co-precipitation method. The microstructure and performance of composite material were investigated by IR, XRD, TG-DTA, SEM, and EDS respectively. The aim of this work is to establish the relationship between material microstructures and properties.

2. Experimental Section

2.1. Reagent and Instrument

Main reagents: Biomedical grade Chitosan (Cs) with 99 percent degree of the deacetylation; Carboxymethyl cellulose (Cmc); analytical grade $Ca(NO_3)_2•4H_2O$, $(NH_4)_2HPO_4$, Hapc and NaOH. Main instruments: Y-2000 Automatic X-ray diffraction (Dandong Radiative Instrument Group Co. Ltd); JSM-7500F (Field emission scanning electron microscope) with a Thermo Noran X-ray energydispersive spectrometer(EDS); JWE-50 universal testing machine (compression rate of 1mm/min, 5kgf sensors). VERTEX-70 Fourier infrared spectrometer Bruker Company (Germany); Pyris 6 TGA thermo-gravimetric analyzer (Perkin Elmer instruments Shanghai co., LTD., the heating rate of 20 °C/min).

2.2. The Preparation of Materials

The solution of $Ca(NO_3)_2•4H_2O$ and $(NH_4)_2HPO_4$ aqueous solution were prepared with near 1.67 Ca/P stoichiometric ratio. The Cs and 2 wt% Cmc were dissolved in 2 wt% HAc and deionized water respectively. The mass ratio of Cs and Cmc were fixed at 5:1. The $Ca(NO_3)_2$ solution was added into the mixture of Cs and Cmc solution, then the $(NH_4)_2HPO_4$ solution was slowly dropped into the mixture for 6h. At the same time, the pH was adjusted to 10 with NaOH solution. The reaction temperature was maintained at 40 °C. The obtained white slurry was aged for 24 h at room temperature, and then the precipitate was filtered, washed, and then put them into the mould to prepare composite samples of different proportions.

3. Results and Discussion

3.1. XRD Analysis

The XRD pattern of Hap/Cs-Cmc composite materials with different mass ratio were shown Figure 1. The diffraction peaks are identified by standard (PDF file No: 72-1243), and the diffraction peaks were assigned as crystalline Hap at 2 theta 26°, 31.8°, 32.3°, 32.9° and 39°. The diffraction peak at about 20° can be attributed to Cs. It is evident from the results that no characteristic diffraction peak from other phases can be detected. In addition, the Hap diffraction peaks were with line broadening and higher back bottom without annealing treatment. The broadening of the Hap diffraction peaks increased by decreasing Cs and Cmc mass ratio with the presence of poorly crystalline hydroxyapatite as the unique crystalline phase, simultaneously, the relative intensity of the Hap diffraction peaks decreased gradually. The result revealed that the changes of Cs and Cmc amounts affected the crystallite size of Hap in composite materials.

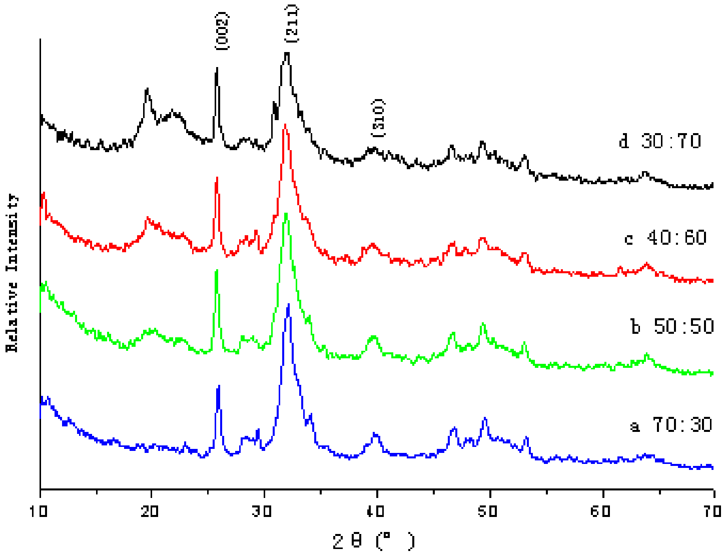

Figure 1. XRD patterns of n-Hap/Cs-Cmc composites materials with different mass ratios

3.2. Microstructure and EDS Analysis

The morphology of the system was investigated by scanning electron microscopy (SEM). Figure 2 shows the image of Hap/Cs-Cmc composite material made by customized mould. Figure 2 (b) shows the SEM image of cross-section of composite material. As can be seen from Figure 2(b), the Hap particles can be homogeneously incorporated with Cs and Cmc matrix with good density, and the inorganic phase Hap bonding with organic phase in composite material with many small pores. It is good for bone cell metabolism of nutrients and moisture transfer.

Figure 2 (c), (d), (e), (f) show the SEM images of different mass ratio of Hap/Cs-Cmc composites materials. It can be seen that the Hap particles are composed of almost spherical aggregates with uniform dispersion, and the particle size is about 50 nm. It can also be seen that the microstrcture have been changed at different mass ratio of Hap/Cs-Cmc. There is a relatively loose combination between Hap and Cs-Cmc when Hap/Cs -Cmc is 70/30. When the content of Cs-Cmc increases, the combination between particles become relatively denser. It could be also observed that the Hap particle size and dispersion are affected by adding different weight percentages of Cs-Cmc, which exhibits that the particle size slightly decreases with increasing Cs-Cmc in composition (Figure 2 (f)). The reasons may be that the Hap crystal growth are influenced by Cs-Cmc addition. It is obvious that the microstrcture is optimal at 50/50 of Hap/Cs-Cmc mass ratio. Furthermore, the chemical composition of Hap/Cs-Cmc composites materials are determined by EDS, a typical spectrum was shown in Figure 3. The result indicates that Ca, P and trace amounts of sodium could be detected in composites materials. The Ca/P ratio is about 1.85, belonging to the rich calcium type of Hap and the existence of sodium is good for natural bone growth.

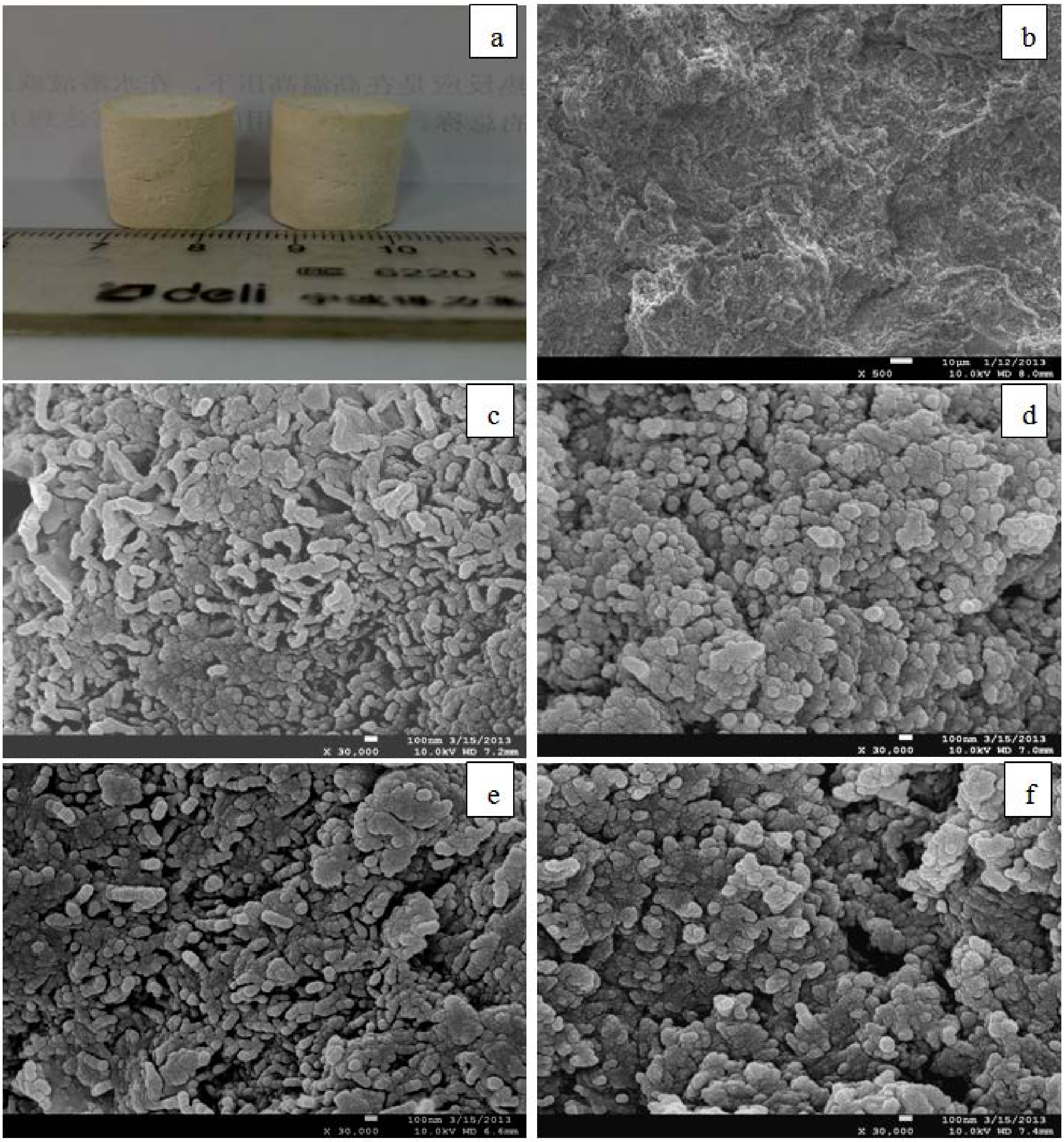

Figure 2. SEM images of n -Hap/Cs- Cmc composites materials (a: actual pictures; b: cross-sectional, c: 70/30; d: 50:50; e: 40:60; f: 30/70)

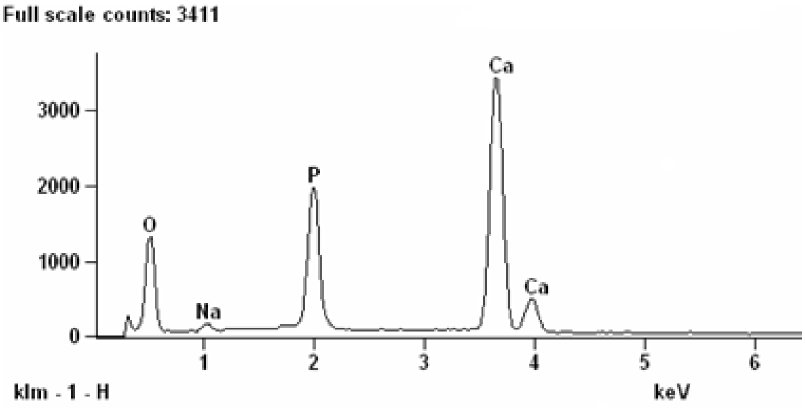

Figure 3. EDS spectrum of n-Hap/Cs -Cmc composites materials

3.3. FT-IR Analysis

The FT-IR spectra of pure n-Hap, Cs, Cmc, and Hap/Cs-Cmc composites are shown in Figure 4. Figure 4 (a) shows the IR spectra of Cmc. The absorption bands at 3443 cm^{-1} for free -OH stretching vibration peak, and the peaks at 1605 and 1428 cm^{-1} are assigned to the stretching and antisymmetric stretching vibration peak of COO-. Figure 4 (b) shows the FT-IR spectrum of Cs. The bands at 1656 cm^{-1} and 1587 cm^{-1} correspond to amide I and amide II band characteristic peaks. Figure 4 (d) is the FT-IR spectrum of pure Hap. The bands at 1039, 604, 565 cm^{-1} are for characteristic PO_4^{3-}, and the bands at 3450 cm^{-1} and 1640 cm^{-1} are assigned to Hap-OH. Figure 4 (c) is the FT-IR spectrum of Hap/Cs-Cmc composite material. Compared with Figure 4(a),(b),(d), the position and intensity of primary absorption peaks have been changed, the amide I (1656) cm^{-1} and amide Ⅱ (1587 cm^{-1}) bands move to low wave number band group at 1648 cm^{-1} and 1564 cm^{-1}. The reasons may be that $-NH_2$ of chitosan interacts with –OH and Ca^{2+} of Hap in synthesis process, indicating the chemical bond interactions between Hap and Cs-Cmc.

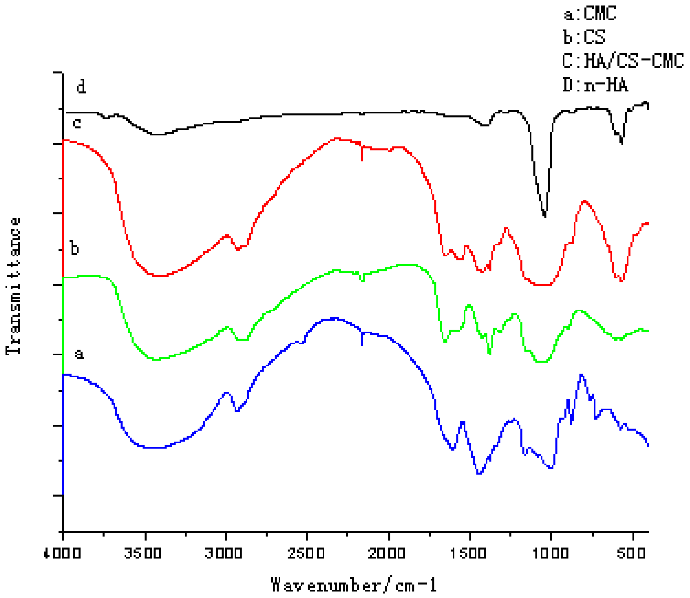

Figure 4. IR spectra of Cmc, Cs, n-Hap/Cs/Cmc and n-Hap

3.4. Thermal Analysis

In order to evaluate the thermal stability of the composite materials, the TGA analysis was performed in the range 50 to 800 °C for the weight rate 50:50 sample (Figure 5). As can be seen from the TGA curve, the initial weight loss from 50 to 280 °C is about 8%, which may be due to the evaporation of surface adsorbed water molecules and hydroxylation of Hap. The main weight loss is observed about 30% from 280 to 650 °C. The weight loss is possibly due to decomposing of organic phase (Cs-Cmc) in composite materials. When the temperature was higher than 650 °C, there was no obviously weight loss for the composite material. The result indicates that the composite materials exhibit good thermal stability and meet the requirements of tissue engineering materials.

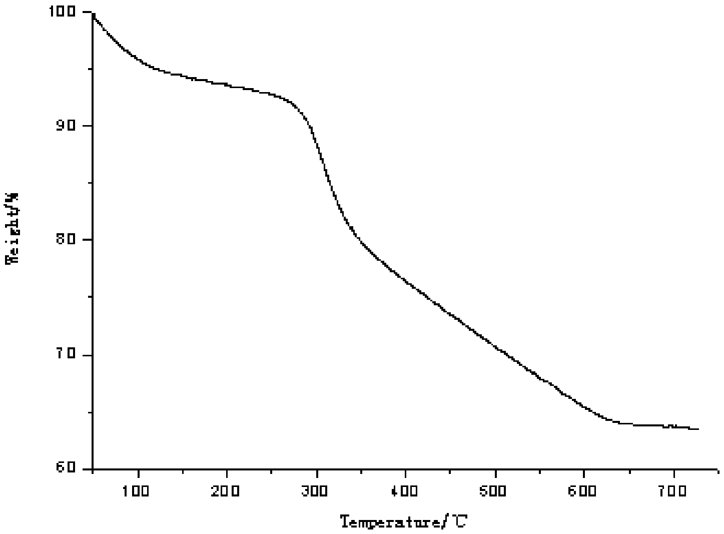

Figure 5. TGA curves of n - Hap/Cs - Cmc Composites

3.5. Mechanical Testing

In order to determine compressive strength of composite materials, Hap/Cs-Cmc composite materials were cut into column (1.0cm×3.0cm). The compressive strength was measured using a computer-controlled Universal Testing Machine (JWE-50). Each group samples were tested five times, and calculating the average error. The results are shown in Table 1.

Table 1. changes of compressive strengths with composite materials with different weight ratios

Hap/Cs-Cmc		compressive strength (MPa)
Hap	Cs-Cmc	
70	30	14.2±0.5
50	50	30.5±0.2
40	60	17.2±0.3
30	70	22.5±0.25

It can be seen from Table 1 that these Hap/Cs-Cmc composite materials show quite good mechanical properties for tissue engineering. The maximum compressive strength can reach 30.5 MPa, and the amount of Cs and Cmc directly affects the mechanical properties of composite materials. The observed results can be attributed to the cohesive strength between the Hap particles and organic phase Cs-Cmc. When the proportion of the Hap particles increases, it could lead to non-homogeneous distribution, aggregation and poor adhesion to the matrix then result in a decrease in the compressive strength. On the contrary, the proportion of the Cs-Cmc is too high, and makes the mechanical properties of the composites decrease which is caused by its high toughness and water-soluble. The results are consistent with the analysis results of microstructure.

4. Conclusions

The Hap/Cs-Cmc composite materials with different mass ratio were prepared through liquid co-precipitation method. The composite materials exhibit excellent mechanical performance and thermal stability. The nano-Hap were dispersed uniformly in the organic phase Cs and Cmc with relatively good crystallinity, combined with relatively closely between Hap particles and Cs-Cmc. The particle size of Hap is about 50 nm with a spherical shape. The change of mass ratio between Hap and Cs-Cmc directly influences the crystallization, particle size, and dispersion of Hap. When mass ratio is 50/50, the uniformity, compactness, and thermal stability are optimal and the compressive strength is up to 30.5MPa. EDS analysis shows that the composite material merely contains trace amount of sodium and the ratio of calcium and phosphorus is around 1.85 which is belonged to the rich calcium type of Hap. The physical and chemical performance of the as-synthesized composite materials totally meet the requirements of bone tissue engineering material.

Acknowledgements

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