



The Influence on Microstructure of Hap/Cs-CMC Composite Materials *in vitro* Soaking

Mande Qiu^{1,2*}, Pan Yang¹, Miao Niu¹, Aimei Dai¹ and Yidan Wang¹

¹College of Chemistry and Environmental Science, Hebei University, Baoding, 071002, People's Republic of China.

²Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of Ministry of Education, Hebei University, No.180, Wusi East Road, Baoding, 071002, Republic of China.

Authors' contributions

This work was carried out in collaboration between all authors. Author MQ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors PY and MN managed the analyses of the study. Authors AD and YW managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACSJ/2015/20053

Editor(s):

- (1) Mazeyar Parvinzadeh Gashti, Department of Chemistry, Université Laval, 1045 Avenue of Medicine, Canada.
(2) Mohammad Luqman, Department of Basic Science, College of Applied Sciences, A'Sharqiyah University, Oman.

Reviewers:

- (1) Mona Samir Hashem, National Research Centre, Egypt.
(2) Enobong R. Essien, Bells University of Technology, Nigeria.
(3) Sergey V. Dorozhkin, Kudrinskaja sq, Moscow, Russia.

Complete Peer review History: <http://sciencedomain.org/review-history/11353>

Short Research Article

Received 8th July 2015
Accepted 22nd August 2015
Published 12th September 2015

ABSTRACT

The Hap/Cs-CMC (Hydroxyapatite/Chitosan-carboxymethyl cellulose) composite materials with different mass ratio were firstly prepared by liquid co-precipitation method. According to preliminary results, the mass ratio of 50/50 (Hap/Cs-CMC) composite material was selected as the research object. The microstructure, phase and component of surface of the composite material were investigated *via* soaking in simulated body fluid (SBF) at different time. The purpose was to comparatively investigate these changes based on the degradation behavior and calcium deposition in the physiological solution. The results showed that the phase structure of composite material was relatively stable in SBF solution, and the microstructure of surface had been changed at immersion time period. These changes indicated that the process of ion-exchange, dissolving, deposition, re-crystallization happened between the composite materials and inside of the SBF solution. In addition, the pH value of SBF solution kept basically stable and did not exhibit much variation, and EDS results showed there are some new additional elements (Mg, K, Cl, S, etc) in

*Corresponding author: E-mail: hbuqmd@sina.cn;

composite material after materials soaking in SBF solution. The ion-exchange, dissolving, deposition, re-crystallization process occurred on the surface firstly and these processes tended to equilibrium with the increase of soaking time, which generated the Ca/P ratio of Hap/Cs-CMC composite materials maintained unchanged.

Keywords: Hap/Cs-CMC; simulated body fluid; soaking; microstructure.

1. INTRODUCTION

Hydroxyapatite (Hap) which has good biocompatibility, bone conductivity is the main component of natural bone inorganic salt, and it has been regarded as an ideal material of bone defect repair. However, because of its brittleness and low degradation rate, its application in bone tissue engineering has been limited [1-3]. In order to prepare composite materials with better properties and mechanical strength to improve the performance of bone repair materials, many material were selected, such as titanium alloy, natural polysaccharide, protein, synthetic polymers, and so on [4-6]. However, in accordance with the needs of strength, toughness, biological activity, it was found that the Hap compounded with single material cannot meet demands of bone tissue engineering material. Therefore, improving the performance of HAP compounded with two or more other materials has been a hot spot in the present research.

Chitosan (Cs), which is a natural biodegradable polysaccharide, has good biocompatibility, biological absorbability, stability and blood adhesion, etc. Many chitosan-based materials have been applied in the biomedicine fields [7-9]. Presently, many studies about Hap/Cs composite materials have been reported [10-12]. Unfortunately, some other studies found that there were some disadvantages, such as bad plasticity, mechanical property and poor interface bonding between Hap and Cs. Hence, with the purpose of improving its physical and chemical properties, one good way to modify Hap/Cs composite materials is to add other substance [13-15].

Carboxymethyl cellulose (CMC) is a kind of cellulose with non-toxicity, adhesiveness, biodegradability, biocompatibility and containing large number of hydroxyl [16]. As a third phase, it can not only improve the interface combination between Hap and Cs, but also enhance the mechanical, chemical and biological properties of the composite material. Though many efforts have been devoted to Hap/Cs-CMC composite materials in recent years [17-20], the relationship

between the material preparation, the microstructure and the performance was rarely reported. In this study, the Hap/Cs-CMC composite materials with 50/50 mass ratios were prepared for the first time through a liquid co-precipitation method [21]. The microstructure, phase and component of the composite material were investigated *via* soaking in simulated body fluid (SBF) at different times. The purpose is to comparatively investigate these changes based on the degradation behavior and calcium deposition in the physiological solution.

2. EXPERIMENTAL PART

2.1 Materials

Biomedical grade Chitosan (Cs) with 99 percent degree of the deacetylation; Biomedical grade Carboxymethyl cellulose (CMC); analytical grade $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{HPO}_4$, CH_3COOH (HAC) and NaOH chemicals were purchased from Baoding Huaxin Reagent and Apparatus Co. Ltd. All the materials were used as received without further purification. Deionized water was used throughout.

2.2 The Preparation of Materials

The solution of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4$ aqueous solution was prepared with near 1.67 Ca/P stoichiometric ratio. The Cs and CMC were dissolved in 2 wt% HAC and deionized water respectively. The mass ratio of Cs and CMC were fixed at 5:1. The $\text{Ca}(\text{NO}_3)_2$ solution was added into the mixture of Cs and CMC solution, and then the $(\text{NH}_4)_2\text{HPO}_4$ solution was slowly dropped into the mixture for 6 h. 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 and washed. Finally, put them into the homemade mould to prepare composite samples of different proportions.

2.3 Characterization

The microstructure was characterized by FE-SEM (Field Emission Scanning electron

Microscopy, model: JEOL-7500F, Japan). The surface compositions of the materials were analyzed by X-ray energy dispersive spectroscopy (EDS) using a Thermo Norman System 7 spectrometer. X-ray diffraction (XRD) patterns were acquired on a Y-2000 X-ray diffractometer with Cu Ka radiation (Dandong Radiative Instrument Group Co. Ltd).

2.4 SBF Immersion

To evaluate the degradation behavior and calcium deposition of the composite scaffolds in vitro, the composite materials were cut into 6 mm (diameter)×2 mm (thickness) plate-like sample. To explore its bioresorbability, the samples were incubated in SBF at a temperature of 37°C under agitation. the prescription of SBF referring to the No. 19th reference. The SBF solution kept fresh in a period of one week with adjustable pH at 7.4. After soaking in SBF at different times (1, 2, 3weeks) respectively, the samples were moved from the SBF solution at each time point, then cleaned with ethanol, and dried in air.

3. RESULTS AND DISCUSSION

3.1 The XRD of Soaked Material

Fig. 1 showed the XRD pattern of Hap/Cs-CMC composite material before and after soaking in SBF at different time. The broad peak that appeared around 20° was assigned to Cs (20.305°, 21.290°), and the sharp diffraction characteristic peaks that appeared at around 31.8° and 26° correspond to the peaks of HAP (32.3°, 32.9°, 39°. PDF#72-1243). It is evident from the observed results that no new characteristic diffraction peaks from other phases are detected *via* soaking in SBF at different time. The results also showed that composite materials immersed in SBF were relatively stable. In addition, the Hap diffraction peaks of composite materials were with line broadening and higher back bottom, which revealed the presence of poorly crystalline hydroxyapatite as the unique crystalline phase.

3.2 The Microstructure of Soaked Material

Fig. 2 is the surface microstructure of the composite materials before and after soaking in SBF. Fig. 2(a) is the image of composite material before immersion. As seen from Fig. 2(a), the Hap particles can be homogeneously incorporated with Cs and CMC matrix which possess better density. Besides, the inorganic

phase Hap bonded with organic phase, which led to many small pores in composite material. The Hap particles were composed of almost spherical aggregates with uniformly dispersion, and the particles size was about 20 nm in diameter. Fig. 2 (b), (c), (d) were SEM images of composites materials with different soaking time periods. It was evident that the microstructure had been changed with immersion time increasing, the surface of composite material had sediment of amorphous accumulation consisting of small particles after one week soaking, and crystallized particles of nano-hydroxyapatite on the surface are obviously decreased. For the above phenomenon, it might be explained that Cs and CMC occurred partial degradation. Then the interaction among degradation products, such as calcium, phosphorus and other ionic in SBF solution, resulted in the formation of sediment of calcium salt on the surface (Fig. 2b). With the increase of immersion time, the amorphous deposits gradually disappeared and the fine particles emerged on the surface of composite material. The number of particles shows the increasing trend. Three weeks later, the surface of the composite material is substantially covered with nano-particles of the sediments (Fig. 2(c), (d)). The reason might be that Ca^{2+} 、 PO_4^{3-} and other ions in-SBF were exchanged. The tiny apatite crystals particles were formed when the exchange gradually arrived to balance with immersion periods going.

In order to study the changes of material component, the semiquantitative analysis of major elements content was determined by energy dispersive spectroscopy (EDS). Five measurements of Ca and P content were performed at different places of each sample, and average Ca/P ratios with standard deviations were calculated. Fig. 3 showed the typical spectrum of material before and after immersion, which showed that the composite material mainly contain Ca、P、O、C and a few amount of sodium element before soaking. Apparently, it also showed that there are some new additional elements (Mg, K, Cl, S, etc) in composite material after materials were soaked in SBF solution. Fig. 4 showed the change of Ca/P ratios at different soaking time period. The Ca/P ratio of surface layers had an abrupt decrease during the first three weeks, then increase to about 1.65 at soaking weeks. Subsequently, it showed a continuous slow increase trend with the extension of immersion time. By the end of the fourth week, the Ca/P ratio of material maintained at about 1.75. This phenomenon

could be due to the presence of richer Ca and P content. Because of poorly Hap crystalline with high solubility in SBF solution, the ion-exchange, dissolving, deposition, re-crystallization process

occurred on the surface firstly. With the increase of soaking time, these processes tended to equilibrium, which generated the Ca/P ratio of Hap/Cs-CMC composite maintained unchanged.

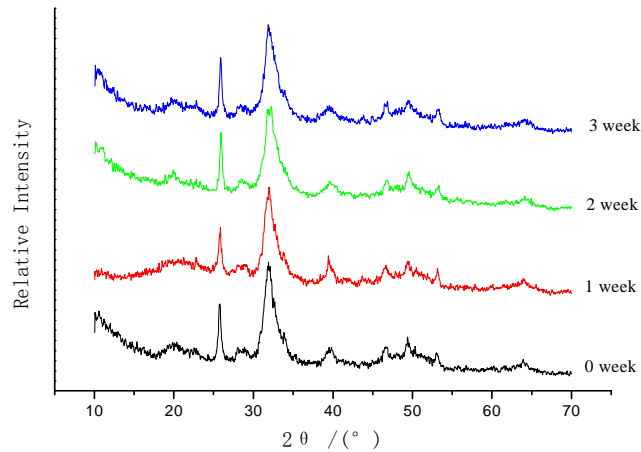


Fig. 1. XRD patterns of composite dipped in SBF for different times

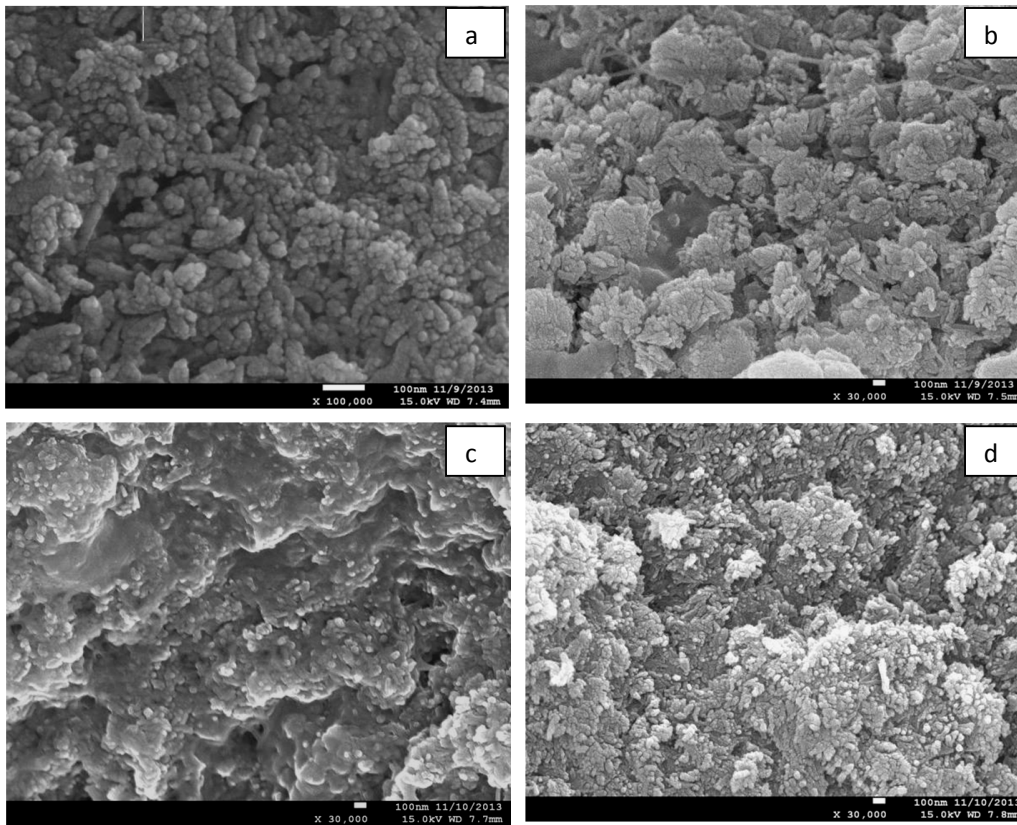


Fig. 2. Surface SEM micrographs of n-HA/Cs-CMC (50/50) composite dipped in SBF for different times (a) 0 week; (b) 1 week; (c) 2 week; (d) 3 week

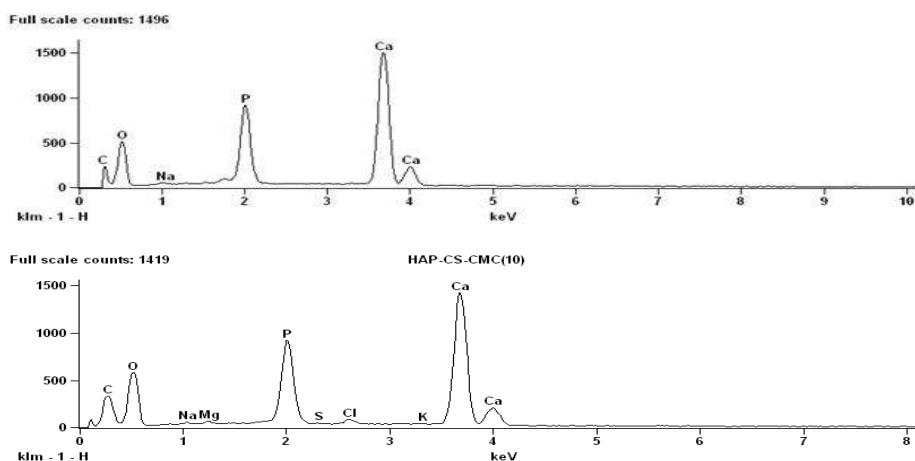


Fig. 3. EDS spectra of n-Hap/Cs-CMC

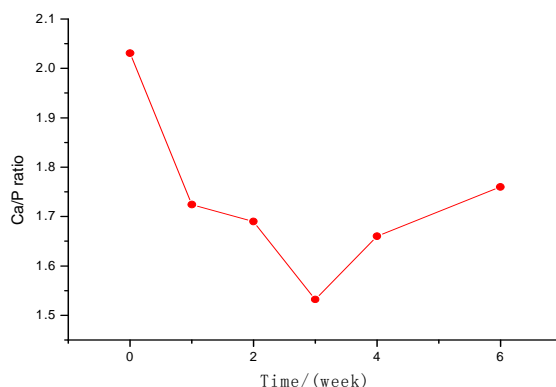


Fig. 4. The change of Ca/P ratios at different soaking time period

3.3 pH Change of SBF Solution

In order to investigate the degradation and ion-exchange process, the pH values of SBF solution was determined at different soaking times (Fig. 5). It can be seen that the pH value of SBF solution increases with the extension of immersion time. By the third week, the pH value increased from initially 7.4 ± 0.03 to 7.54 ± 0.04 , then it decreased to 7.36 ± 0.02 at the four week. The observed results indicated that the Hap/Cs-CMC composite material did not exhibit much variation in pH value. The changes, such as a gradual decrease and an abrupt decrease in the pH value of SBF solution, is usually due to reabsorption of Ca from the SBF solution by the material as observed in the increase of Ca/P to 1.73. In our opinion, it might be also caused by the alkaline effect of the processes of degradation, ion-exchange, dissolution, deposition, recrystallization etc.

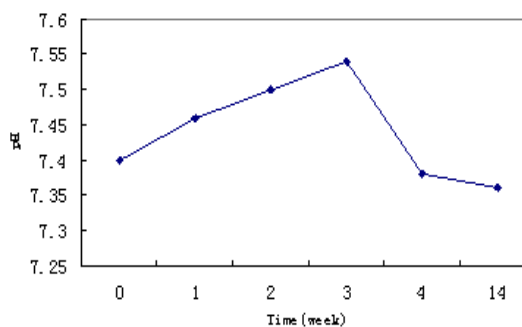


Fig. 5. The change of pH

4. CONCLUSION

The microstructure, phase and component of surface of the composite material were investigated *via* soaking in SBF at different times. It was proven that the phase structure of

composite material was relatively stable in the SBF solution and the microstructure of surface had been changed during the immersion time. These changes showed that the process of ion-exchange, dissolving, deposition, re-crystallization had been occurred between the composite materials and the SBF solution, resulting in a stable Ca/P ratio of Hap/Cs-CMC composite materials.

ACKNOWLEDGEMENTS

This work was financially supported by the Science and Technology Research and Development Projects of the Hebei Province in China (10276732) and Hebei University Dr. Fund.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Supova M. Substituted hydroxyapatites for biomedical applications: A review. *Ceramics International*. 2015;41(8): 9203-9231.
- Sasidharanpillai S, Syamchand, George Sony. Multifunctional hydroxyapatite nanoparticles for drug delivery and multimodal molecular imaging. *Microchimica Acta*. 2015;182(9-10): 1567-1589.
- Xiaofeng Pang, Hongjuan Zeng, Jialie Liu. The properties of nanohydroxyapatite materials and its biological effects. *Materials Sciences and Applications*. 2010; 1(2):81-90.
- Vozzi G, Corallo C, Carta S, et al. Collagen-gelatin-genipin - hydroxyapatite composite scaffolds colonized by human primary osteoblasts are suitable for bone tissue engineering applications: *In vitro* evidences. *J Biomed Mater Res Part A*. 2014;102(5):1415-1421.
- Mututuvari TM, Harkins AL, Tran CD. Facile synthesis, characterization, and antimicrobial activity of cellulose-chitosan—hydroxyapatite composite material: A potential material for bone tissue engineering. *J Biomed Mater Res Part A*. 2013;101(11):3266-3277.
- Rabea EI, Badawy MET, Stevens CV, et al. Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules*, 2003;4(6):1457–1465.
- Pallela R, Venkatesan J, Janapala VR, et al. Biophysicochemical evaluation of chitosan-hydroxyapatite-marine sponge collagen composite for bone tissue engineering. *J Biomed Mater Res Part A*. 2012;100A(2):486-495.
- Chuenjitkuntaworn B, Inrung W, Damrongsri D. Polycaprolactone/ hydroxylapatite composite scaffolds: Preparation, characterization, and *in vitro* and *in vivo* biological responses of human primary bone cells. *J Biomed Mater Res A*. 2010; 94A(1):241–251.
- Phisalaphong M, Jatupaiboon N, Kingkaew J. Chitin, Chitosan, Oligosaccharides and their Derivatives: Biological activities and applications. New York CRC Press. 2011; 53-65.
- Altioek D, Altioek E, Tihminlioglu F. Physical, antibacterial and antioxidant properties of chitosan films incorporated with thyme oil for potential wound healing applications. *J Mater Sci Mater Med*. 2010;21(7): 2227–2236.
- Hong Li, Chang-Ren Zhou, Min-Ying Zhu, et al. Preparation and characterization of homogeneous hydroxyapatite/chitosan composite scaffolds via in-situ hydration. *Journal of Biomaterials and Nanobiotechnology*. 2010;1(1):42-49.
- Chen J, Nan K, Yin S, et al. Characterization and biocompatibility of nanohybrid scaffold prepared via in situ crystallization of hydroxyapatite in chitosan matrix. *Colloids Surf B*. 2010;81(2): 640-647.
- Sergei N Danilchenko, Oksana V Kalinkevich, Maksim V Pogorelov, et al. Characterization and *in vivo* evaluation of chitosan-hydroxyapatite bone scaffolds made by one step coprecipitation method. *Journal of Biomedical Materials Research A*. 2011;96A(4):639-647.
- Bala' zsi C, Bishop A, Yang JHC, et al. Biopolymer-hydroxyapatite scaffolds for advanced prosthetics. *Compos Interfaces*. 2009;16(2-3):191–200.
- Chen JP, Chen SH, Lai GJ. Preparation and characterization of biomimetic silk fibroin/chitosan composite nanofibers by electrospinning for osteoblasts culture. *Nanoscale Res Lett*. 2012;7(1):1-11.
- Shao Zhi Fu, Xiu Hong Wang, Gang Guo, et al. Preparation and properties of nano-hydroxyapatite / PCL-PEG-PCL composite membranes for tissue engineering applications. *Journal of Biomedical*

- Materials Research B: Applied Biomaterials. 2011;97B(1):74-83.
17. Cheng X, Li Y, Zuo Y, et al. Properties and *in vitro* biological evaluation of nano-hydroxyapatite/chitosan membranes for bone guided regeneration. Mater Sci Eng C. 2009;29(1):29-35.
 18. Chesnutt BM, Viano AM, Yuan Y, et al. Design and characterization of a novel chitosan/nanocrystalline calcium phosphate composite scaffold for bone regeneration. J Biomed Mater Res A. 2009; 88(2):491-502.
 19. Daniel L Alge¹, Goebel WS, Chu TM. Effects of DCPD cement chemistry on degradation properties and cytocompatibility: Comparison of MCPM/ β -TCP and MCPM/HA formulations. Biomedical Materials. 2013;8(2): 25010-25017.
 20. Alge D L, Goebel W S, Chu T-M G. *In vitro* degradation and cytocompatibility of dicalcium phosphate dihydrate cements prepared using the monocalcium phosphate monohydrate / hydroxyapatite system reveals rapid conversion to HA as a key mechanism. J Biomed. Mater Res. B Appl. Biomater. 2012;100(3):595-602.
 21. Qiu M, Dai A, Yang P, et al. Preparation, microanalysis and performance of Hap / Cs-Cmc composite materials. American Journal of Materials Engineering and Technology. 2015;3(2):46-50.

© 2015 Qiu et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://sciencedomain.org/review-history/11353>