Journal of the Ceramic Society of Japan 117 [4] 521-524 2009 **Paper**

Preparation and characterization of periodic porous frame of hydroxyapatite

Liyun ZHOU, Deping WANG,† Wenhai HUANG, Aihua YAO, Masanobu KAMITAKAHARA* and Koji IOKU*

The Institute of Bioengineering and Information Technology Materials, School of Material Science and Engineering, Tongji University, Shanghai 200092, People's Republic of China
***Graduate School of Environmental Studies, Tohoku University, 6−6−20, Aoba, Aramaki, Aoba-ku, Sendai 980−8579**

Three-dimensional, periodic macroporous frame of hydroxyapatite(HA) has been fabricated via a template-assisted colloidal processing technique. In the present method, colloidal template was firstly prepared with SiO_2 spheres by gravitational sedimentation, which was then infiltrated with HA precursor prepared by sol–gel process. The resulting HA therefore replicated the three-dimensionally ordered macroporous structure of SiO_2 template. After removal of the template by immersing in NaOH solution, the periodic macroporous frame made from HA was obtained. The arrangement of the pore structure was hexagonal close-packed and pores were connected with each other. The resulting highly ordered macroporous frame of HA could have potential applications in bio-medical field.

Key-words : Periodic macroporous materials, Colloidal template, Hydroxyapatite, Monodisperse SiO_2 spheres

[Received December 8, 2008; Accepted February 19, 2009]

1. Introduction

Recently, ordered porous materials have been regarded as a new star in the whole family of porous materials. Because of their special structures, ordered porous materials exhibit such many unique properties as similar pore size, large surface areas and high pore volume,[1),2)] which have been widely used in many fields, such as optical grating, photonic crystal, catalyst carrier and biomedicine fields.[3),4)] Undoubtedly, the preparation techniques for these materials are also very attractive.

So far, various kinds of fabrication methods have been applied to prepare various ordered porous materials, of which, the colloidal templating approach is the most commonly used method.[5)–8)] The general concept of colloid templating is simple and this approach includes the three steps: (1) to pack the spherical colloid into close-packed arrays, (2) to fill the interstitial spaces of the spherical colloid with a fluid precursor capable of solidification, and (3) after precursor solidifying, to remove the template to obtain a porous inverse replica.[9)] For this procedure, the precursors do not undergo a special pre-treatment process, and the pore sizes of the resulting materials could be easily controlled by using different size of colloidal spheres. With the merit, the method has been widely used to prepare various kinds of periodic porous materials.

As a template in the method, colloidal spheres must meet the following requirements: similar diameters with narrow distribution, good monodispersity, stable physical and chemical properties without reacting with the stuffing, easily removal, etc. Both polymer spheres (such as PS and PMMA) and silica spheres could be easily prepared and their diameters could also be controlled. Besides, both of them could be dispersed in some impregnant very well and easily removed, leading to wide application in the preparation of ordered porous materials. However, polymer spheres could be easily removed by sintering, which sometimes would release some gas to destroy the pore structures. Correspondingly, silica spheres could be quickly removed by immersing in HF acid[3)] or basic solution whose pH value is higher than 13, which is more suitable in some occasions.

Hydroxyapatite (HA) is one of the major constituents of the inorganic component in human hard tissues(bones and teeth), and it is one of the most common biomaterials being exclusively studied because of its great biomedical properties. With good biocompatibility and bioactivity, HA would be preferably used as adsorption and separation materials in biomedical fields.[10)] With apatite particles as the precursor, ordered macroporous apatite has been prepared by electrophoretic deposition method.[5)] Even so, the array of pore structure needs improving. With HA sol as precursor, the macroporous HA prepared by colloidal template method would possess more ordered pore structures. And the resulting material could be good carrier for drug or protein because of its excellent adsorption.

The goal of this work, therefore, is to explore a method to prepare HA with ordered porous frame by colloidal template techniques. Monodispersed silica spheres (MSS) and HA sol would be used as a template and ceramic precursor, respectively. In addition, NaOH solution would be used to remove the template. The experiment results from this method would provide with technical parameters for the development of mass production of periodic HA, which might have unprecedented applications to transporting of active substances in biological media.

2. Experimental procedure

2.1 Preparation of monodispersed silica spheres(MSS)

According to Stöber method,[13)] the MSS were prepared on the basis of the hydrolysis and condensation of Tetraethyl orthosilicate (TEOS, Shanghai Chemical Supply Station) in a mixture of water, ammonia and alcohol. In a typical process, 22 cm^3 of

† Corresponding author: D. Wang; E-mail: wdpshk@tongji.edu.cn

TEOS were mixed with 24 cm^3 of ethanol (solution A), meanwhile, 30 cm^3 of ethanol, 18 cm^3 of distilled water and 6 cm^3 of concentrated ammonia (25–28 vol%) were mixed together for at least 15 min (solution B). Then, solution A was dripped into solution B and the mixture was stirred for 2 h. The resulting silica spheres were centrifugally separated from the suspension and ultrasonically washed with distilled water, and this wash process was repeated for three times. The separated spheres with uniform size were then oven-dried at 50°C for 12 h.

2.2 Preparation of colloidal template

In a typical process, 1 g of MSS were dispersed in 50 cm^3 of ethanol, and then ultrasonic agitated for 15 min, followed by stirring for 1 h to ensure complete dispersal. The resulting suspension underwent static gravitational sedimentation for about 48 h in order to obtain a close-packed colloidal template. After ethanol volatilization, the templates consist of nothing but dried and close-packed MSS.

2.3 Preparation of HA precursor

In a typical process, 10 g of $Ca(NO_3)_2{\cdot}4H_2O$ (Chemical Agent Co. Ltd., China) were dissolved in 20 cm^3 of distilled water (solution C), meanwhile, 5 cm^3 of trimethyl phosphate (TMP, Chemical Agent Co. Ltd., China) and 10 ml of ethanol were mixed together for 30 min(solution D). Then, solution D was dripped into solution C and the mixture was stirred for 12 h in a water bath at 80°C, resulting in the solution used as the ceramic precursor (The following abbreviates "solution which used as the ceramic precursor" to "HA sol").

2.4 Preparation of HA with periodic porous frame

The colloidal templates were immersed in HA precursor sol for 3 min, and the coated templates were dried at 100°C for 12 h to transform HA sol into HA gel. Then, the mixture of HA precursor and template was calcined at 600°C for 2 h at a heating rate of 1°C·min^{-1} to obtain the lumps made of silica spheres and crystalline HA.[11),12)] And the silica template was removed by immersing the lumps in 5 mol·dm^{-3} NaOH(room temperature, static) for 4 d, followed by washed by distilled water and oven-drying at 50°C for 12 h. The resulting lumps were the HA with periodic porous frame.

2.5 Characterization

The pH values of washing water were measured by pH meter (ORPPHB–9905, China)followed by these steps: (1) taking porous HA frames out of NaOH solution and putting them into a beaker filled with distilled water (samples /distilled water needed = 1 g/500 cm^3); (2) stirring water with glass rod for 5 min two times in an interval of 30 min;(3) measuring and recording the pH value of the water at the bottom of beaker; (4) changing the water and repeating the steps(1)–(3) for several times up to 10 times.

The size and morphology of MSS were examined by transmission electron microscopy (TEM, Hitachi Ltd., H–800, Japan). The morphology of colloidal template and the periodic porous HA frames were characterized by scanning electron microscopy (SEM, Quanta 200 FEG, Japan) and the phase identification of the samples were achieved using X-ray diffraction (XRD, Rigaku Co., D/Max = 2250, Japan).

3. Results

3.1 Characterization of MSS and colloidal template

Figure 1 shows TEM photograph of SiO_2 spheres (a) and SEM photograph of colloidal template (b). The diameter of colloidal silica droplets was 250 ± 20 nm in narrow size distribution. It's obvious that the as-prepared silica droplets were monodispersed, spherical and homogeneous. After gravitational sedimentation, the silica spheres were ordered into hexagonal close-packed domains forming colloidal template with less point defects, line defects or large disordered regions.

3.2 Characterization of HA with periodic porous frame

Silica spheres are widely used as templates in colloidal templating method and could be quickly removed by immersing in HF acid.[3)] In this experiment, however, MSS had to be removed by immersing in NaOH solution instead of HF acid, because HA is stable in basic solution and dissolved in acid. **Figure 2** shows the SEM photographs of two samples consisting of HA and silica spheres after immersing in NaOH solution for 12 h(a) and 96 h(b). Obviously, the silica spheres had not been removed completely when it was immersed in 5 mol·dm^{-3} NaOH solution for 12 h. Moreover, the silica spheres were still well arrayed after the penetration of HA precursor, which proved the effectiveness of gravitational sedimentation method. There were gaps between the silica spheres and the interstitial HA skeletons (shell-like in morphology) shown in Fig. 2(a), whereas, all silica spheres were removed after immersing for 96 h, as shown in Fig. 2(b), since all gaps were interconnected.

Figure 3 shows the XRD patterns of three different samples,

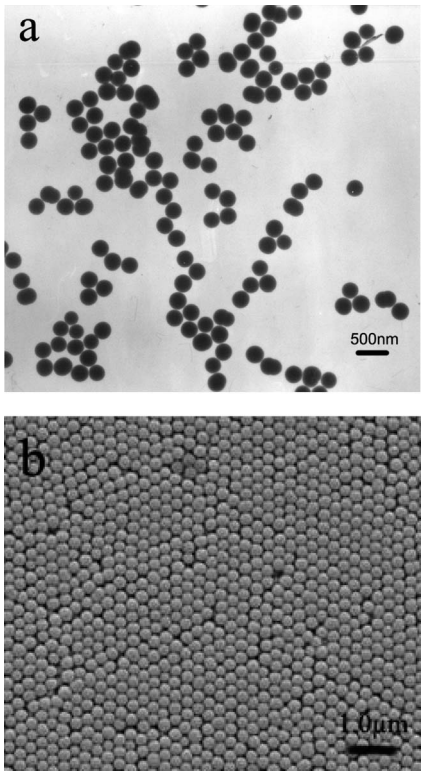

Fig. 1. (a) TEM photograph of SiO_2 spheres. (b) SEM photograph of colloidal templates.

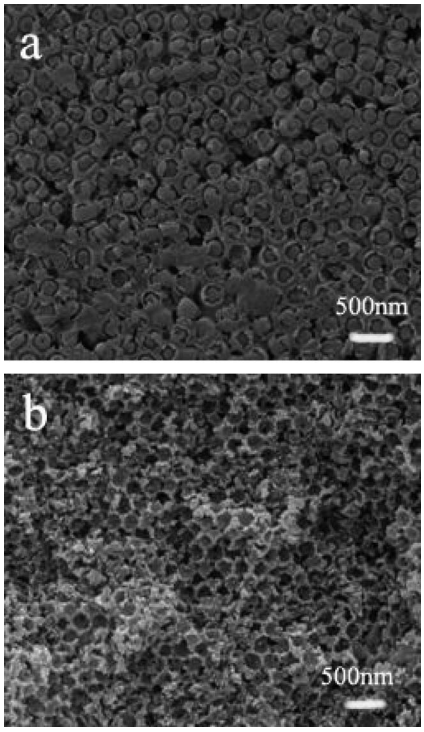

Fig. 2. SEM photographs of two samples consisted of HA and SiO_2 spheres immersed in NaOH solution for 12 h (a) and for 96 h (b).

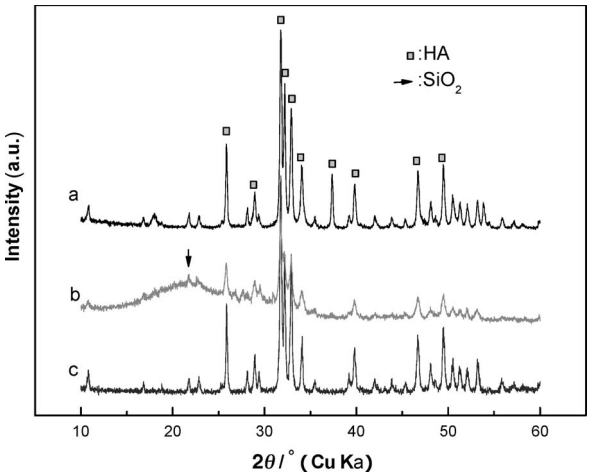

Fig. 3. XRD patterns of three different samples,(a) sol–gel HA after calcinations at 600°C, (b)the composite consisting of sol–gel HA and SiO_2 spheres before immersing in NaOH, (c) the composite consisting of sol–gel HA and SiO_2 spheres after immersing in 5 mol·dm^{-3} NaOH for 96 h.

that is (a) sol–gel HA after calcinations at 600°C, (b)the composite consisting of sol–gel HA and SiO_2 before immersing in NaOH, and (c) the composite consisting of sol–gel HA and SiO_2 after immersing in 5 mol·dm^{-3} NaOH for 96 h. The phase of sample (a) was identified as being HA. There were some characteristic peaks of HA and a peak envelope of amorphous SiO_2 shown in pattern(b), which meaned that HA and silica co-existed in the composite before immersing in NaOH. The weak characteristic peaks of HA resulted from the minority HA in the composite. The phase of sample (c) was identified as being HA.

Figure 4 shows the low magnification(a) and high magnification(b) SEM photographs of periodic porous HA after washed

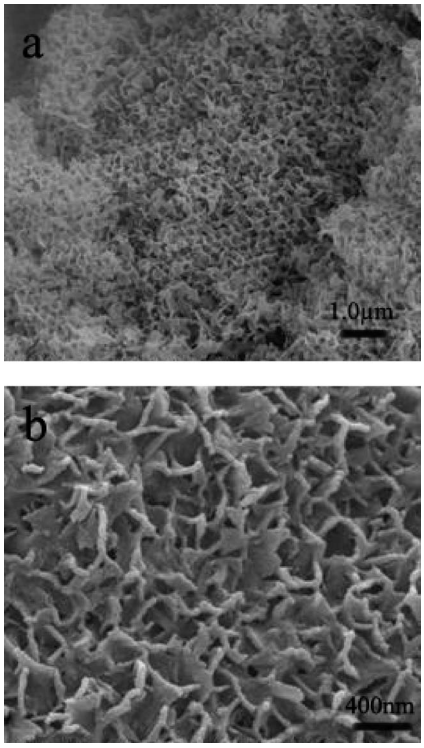

Fig. 4. (a) Low magnification and (b) high magnification SEM photographs of periodic porous HA after washed for 5 times.

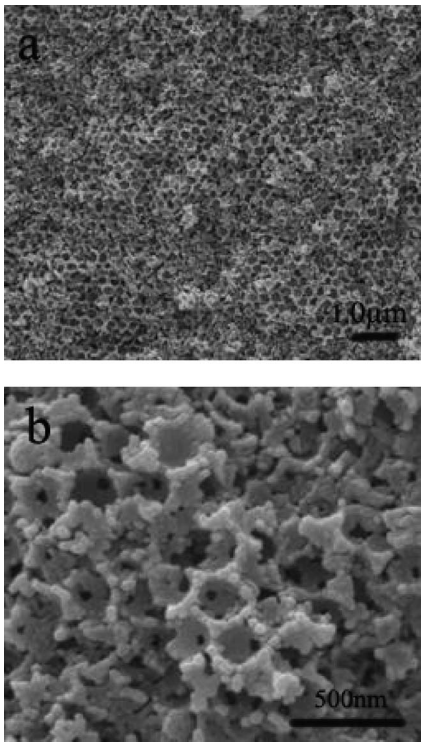

Fig. 5. (a) Low magnification and (b) high magnification SEM photographs of periodic porous HA after washed for 10 times.

for 5 times. Although the pores in the sample were ordered, it's seen that HA crystal grew from interstitial spaces to outside.

Figure 5 shows low magnification(a) and high magnification(b) SEM photographs of periodic porous HA after washed

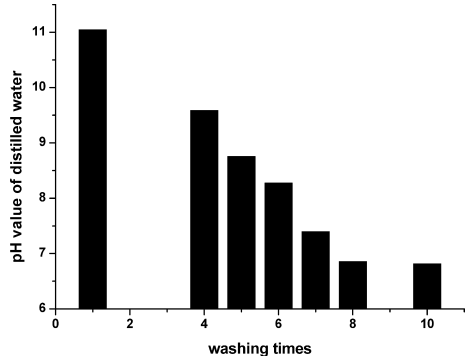

Fig. 6. Change in pH value of distilled water with the washing times increasing.

for 10 times. In this experiment, periodic porous frame of HA is three-dimensional. The size of ordered pore in the as-prepared sample was about 210 ± 20 nm, which was 16% smaller than that of the initial silica spheres due to the shrinkage and growth of HA crystal (Figs. 5(a) and (b)). It also can be seen that the thickness of pore wall was about 50 nm. Moreover, a large amount of mesopores existed in the pore walls.

Figure 6 shows the change in pH value of distilled water with washing times increasing. It could be learnt that the pH value of water was about 8.7 after the samples were washed for 5 times, which left a weak basic environment in the pores of the sample. However, after the samples were washed for 10 times, the pH value of water was below 7, which was near to that of distilled water (about 6.88).

4. Discussion

For colloidal template method, the microstructure of porous materials strongly depends on the structure of colloid templates. The close-packed colloidal spheres generally lead to well-ordered porous materials. Moreover, spheres with same diameter tended to be closest packing according to crystal theory. The silica spheres prepared in this experiment were monodispersed, spherical and homogeneous. Therefore, they are very suitable to form well-ordered template in order to prepare HA materials with periodic porous structure. In addition, according to Stöber method,[13] the sizes of silica spheres could be controlled by adjusting the process parameters, such as the concentration of TEOS, water content and catalyst content, etc. Therefore, the pore size of the resulting periodic porous HA can be controlled in the present methods by using different size of silica.

In this experiment, periodic porous HA could be obtained after removal silica spheres by immersing in NaOH solution for 96 h. The peak envelope of amorphous silica shown in pattern Fig. 3(b) disappeared in pattern Fig. 3(c), and the pattern Fig. 3(a) and Fig. 3(c) were identical, which proved that the existence of silica had no influence on the formation and growth of HA crystal.

When silica templates were removed completely, a porous inverse replica was easily obtained. Obviously, the size of macropores in the samples mainly depended on that of initial silica spheres. Therefore, the morphology of periodical porous HA could be controlled by initial silica spheres. Besides many macropores, there were lots of mesopores existing in the samples. On the one hand, as non-rigid balls, colloidal silica spheres, with a little elasticity, contacted with each other at some area instead of points when they packed closely, and after being removed, therefore, macropores and mesopores left from these spheres and the contacted area. On the other hand, the macropore walls were made of spherical HA crystalline in this experiment, and there must be lots of slots between spherical HA crystalline. Anyway, these mesopores coming from these slots are very helpful to improve the connectivity and to increase surface area of the samples, which are greatly beneficial to biomaterials used in biomedical field.

The morphology of final periodic porous HA frame also depends on the wash process. The samples were immersed in NaOH to remove silica spheres and then washed by distilled water for several times to wipe off the basic solution inside the samples. Growth of HA crystal was found in the samples after washed for 5 times as shown in Fig. 4, but the growth stopped in the samples after washed for 10 times as shown in Fig. 5. The different results from the fact that weak basic environment was contributed to the growth of HA crystal. To get shaped-well periodic porous HA frame, it is necessary to sustain the HA crystal over-growth after silica template removal and to wipe up the basic solution completely.

5. Summary

In summary, three dimensional, periodic porous frame of hydroxyapatite has been successfully fabricated by colloidal template method. The macropores are highly ordered and interconnected with each other. The morphology of periodic porous HA could be controlled by several factors, like initial silica sphere size and washing process in the end. This technique is very useful to fabricate the novel porous HA for biomedical application.

Acknowledgements The authors are grateful to the National Natural Science Foundation of China (50572072) and major projects of the Shanghai Science and Technology Commission (05DJ14006) for financial support.

References

1) H. Wang, D. F. Zhao, X. D. Li, D. Z. Jin and C. M. Zheng, *J. Chi. Ceram. Soc.*, 34, 107 (2006).
2) X. Li, F. F. Tao, Y. Jiang and X. Zheng, *J. Colloid. Interface. Sci.*, 308, 460 (2007).
3) A. A. Zakhidov, R. H. Baughman, Z. Iqbal, C. X. Cui, I. Khayrullin, S. O. Dantas, J. Marti and V. G. Ralchenko, *Science*, 282, 897 (1998).
4) C. W. Kuo, J. Y. Shiu, K. H. Wei and P. L. Chen, *J. Chromatogr.*, A1162, 175 (2007).
5) J. Hamagami, Y. Ato and K. Kanamura, *Solid State Ionics*, 172, 331 (2004).
6) Z. F. Liu, Z. G. Jin, W. Li and J. J. Qiu, *Mater. Res. Bull.*, 41, 119 (2006).
7) F. Q. Tang, T. Uchikoshi and Y. Sakka, *Mater. Res. Bull.*, 41, 268 (2006).
8) Y. Sakka, F. Q. Tang, H. Fudouzi and T. Uchikoshi, *Sci. Tech. Ad. Mater.*, 6, 915 (2005).
9) A. Stein, *Microporous Mesoporous Mater.*, 44, 227 (2001).
10) W. Wang, D. L. Shi, J. Lian, G. Yan, G. K. Liu, L. M. Wang and R. C. Ewing, *Appl. Phys. Lett.*, 89, 1 (2006).
11) Z. L. Huang, Y. Lin, D. W. Wang, S. G. Zhang and L. J. Chen, *J. Inorganic. Mater.*, 16, 661 (2001).
12) W. Y. Hu, *J. Wuhan Polytechnic. Uni.*, 25, 21 (2006).
13) W. Stöber, A. Fink and E. Bohn, *J. Colloid. Interface Sci.*, 26, 62 (1968).