J Mater Sci (2009) 44:4020–4025
DOI 10.1007/s10853-009-3555-z

Fabrication of superparamagnetic hydroxyapatite with highly ordered three-dimensional pores

Deping Wang · Xiang Duan · Jiying Zhang · Aihua Yao · Liyun Zhou · Wenhai Huang

Received: 24 October 2008 / Accepted: 9 May 2009 / Published online: 29 May 2009

Abstract Hydroxyapatite (HA) with highly ordered three-dimensional pores, whose size is about 300 nm, was prepared by colloidal template method. The effect of the surface modification of silica spheres on the order degree of porous structure was investigated by field emission scanning electron microscopy (FESEM). Then, superparamagnetic Fe_3O_4 nanoparticles were fabricated via redox reaction, followed by coating with silica via a sol–gel process, in which a certain amount of TEOS was used in order to control the thickness of the silica shell. X-ray diffraction (XRD), transmission electron microscopy (TEM), and magnetometry were applied to characterize the properties. Finally, Fe_3O_4 magnetic nanoparticles coated with silica were adsorbed in the mesopores of HA with highly ordered three-dimensional pores by capillarity. The influence of dispersing agent on the adsorption results has been studied. Magnetometry was applied to characterize the magnetic properties of superparamagnetic HA. The quantities of adsorbed SiO_2/Fe_3O_4 nanoparticles with core–shell have been compared by variation of saturation magnetization before and after adsorption.

Introduction

Recently, there has been great interest in fabricating materials with highly ordered three-dimensional pores due to their potential applications in non-linear optics [1], optical and magnetic information processing and storage [2], sensors [3], catalyst [4], or as removable scaffolds for the formation of highly ordered, macroporous materials [5]. Hydroxyapatite (HA) is readily biocompatible with hard bone tissues, which has been broadly used in healing of bone defect. Therefore, HA with highly ordered three-dimensional pores, which possesses excellent properties of both three-dimensional materials and HA, has great application foreground in bone repair areas. It can also be used as drug and functionalized particles delivery, which was efficient in the treatment of osteomyelitis and osteocarcinoma.

Magnetite nanoparticles have remarkable properties such as superparamagnetism and high saturation field, so they have received sustainable attention for their potential application in various fields such as in biomedicine and bioengineering, which can be used as targeted drug delivery, cell separation, magnetic resonance imaging, immunoassay, and separation of biochemical products [6–8]. However, magnetite nanoparticles could lose the special magnetic properties associated with single-domain, because they would tend to aggregate into large clusters caused by anisotropic dipolar attraction [9]. Usually, coating magnetite with silica could help shielding the magnetic dipolar attraction, which prevent the aggregation in liquid and improve their chemical stability [10]. At the same time, abundant silanol groups are existed on the silica surface, which could improve the reaction activity of the magnetite nanoparticles with core–shell structure [11].

Numerous works [12–14] have been done to give some ferromagnetic properties to bioceramics so that it can be

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D. Wang · X. Duan (✉) · J. Zhang · A. Yao · L. Zhou · W. Huang
School of Materials Science and Engineering, Tongji University, Shanghai 200092, China
e-mail: duanxiang30181614@hotmail.com

D. Wang · W. Huang
Key Laboratory of Advanced Civil Engineering Materials, Tongji University, Shanghai 200092, China

used for the treatment of bone cancer by hyperthermia [15] and to reinforce the bone formation. However, up to now, few works have been systematically done to study using HA with highly ordered three-dimensional pores to deliver controllable quantities of ferromagnetic particles. In this paper, we design a novel style of magnetite HA with highly ordered three-dimensional pores which can be used for magnetite-targeting and bone repairing at the same time. Firstly, HA with highly ordered three-dimensional pores is fabricated by colloidal template method. Subsequently, we prepare superparamagnetic Fe_3O_4 nanoparticles which are coated with silica via a sol–gel process. Finally, SiO_2/Fe_3O_4 nanoparticles will be loaded in HA highly ordered three-dimensional pores by adsorption process. In our research, the effect of surface modification by H_2O_2 on the order degree of HA with highly ordered three-dimensional pores has been studied. In addition, the influence of dispersing agent on the adsorption process and the magnetic properties of SiO_2/Fe_3O_4 nanoparticles are also discussed.

Experimental

Materials

Trimethyl phosphate (TMP) and calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) of analytic purity were used as the HA precursors. Tetraethyl orthosilicate (TEOS) was analytical grade and was used as the silica precursor. Ferric chloride ($FeCl_3$) and sodium sulfite (Na_2SO_3) were used to fabricate Fe_3O_4 nanoparticles. NH_4OH (25 mass% aqueous solution), acetone (C_3H_6O), H_2O_2 (30 mass% aqueous solution), ethanol and all the above reagents were all purchased from Sinopharm Chemical Reagent Co. Ltd. All chemicals were used as received.

Preparation of HA with highly ordered three-dimensional pores

HA with highly ordered three-dimensional pores was fabricated by using the method already described in our recent paper [16], which was in accordance with the process as shown in Fig. 1. Firstly, monodisperse silica spheres were synthesized by the hydrolysis of TEOS at alkaline condition (Fig. 1a). Before obtaining a close-packed colloidal template (Fig. 1b), monodisperse silica spheres were surface modified by H_2O_2. One gram monodisperse silica spheres were dispersed in 100 mL H_2O_2 and then stirred for 48 h. HA precursor was prepared by mixing 5 g $Ca(NO_3)_2 \cdot 4H_2O$, 1.9 mL TMP and 7.6 mL ethanol together, stirring for 24 h in a oil bath at 80 °C. The colloidal silica template was infiltrated with HA precursor (Fig. 1c), followed by calcimine at 600 °C for 2 h (Fig. 1d). Finally, the colloidal silica template was removed by immersing in 5 M NaOH solution for 4 days (Fig. 1e).

Preparation of SiO_2/Fe_3O_4 nanoparticles

Magnetite nanoparticles were prepared using the method already described based on the redox reaction between $SO_3^{2-}$and Fe^{3+} in which pH value was about 11 adjusted by a concentrated NH_4OH aqueous solution (25 mass%) under argon protection [17]. Magnetic nanoparticles modified by citric acid and coated with silica as described in our previous work [18]; in our research, 3 mL magnetite solution was ultrasonically dispersed in a mixture system of 80 mL ethanol and 18 mL deionized water, then under continuous stirring, different amounts of TEOS and 2.5 mL NH_4OH (25 mass%) were consecutively added. The reaction was allowed to proceed for 8 h, and the resulting product was washed three times with ethanol.

Assembly of HA with highly ordered three-dimensional pores and SiO_2/Fe_3O_4 nanoparticles

Different amounts of SiO_2/Fe_3O_4 nanoparticles were dispersed in 10 mL ethanol and 10 mL deionized water separately, following by adding 0.02 g HA with highly ordered three-dimensional pores under continuous stirring, which was allowed to proceed for 24 h. After this reaction, the product was collected by filtration and washed with

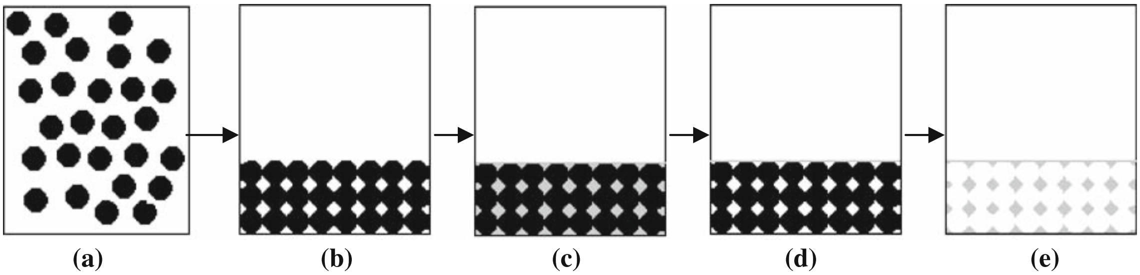


Fig. 1 Preparation flow chart of HA with highly ordered three-dimensional pores. **a** Preparation of SiO_2 spheres; **b** Preparation of ordered SiO_2 template; **c** Infiltration of HA precursor; **d** Calcimine; **e** Removal of template

water and ethanol for several times. Finally, it was desiccated for 8 h at 50 °C in vacuum drying oven.

Characterization

XRD data were collected using a Rigaku D/MAX2550 diffractometer with CuK$_\alpha$ radiation ($\lambda = 0.15406$ nm). The morphology of the product was characterized by transmission electron microscopy (TEM) (HITACHI, H-800). The morphology of colloidal template and the periodic porous HA frames were characterized by field emission scanning electron microscopy (FESEM, Quanta 200 FEG, Japan). Electrophoretic mobilities of particles were measured on a Malvern Zetasizer Nano Z. A vibrating sample magnetometer (VSM) (Nanjing University Instrument Plant, LH-3) was used to measure the magnetic moment.

Results and discussion

In the present study, SiO_2 colloidal template with high ordered degree is prepared with SiO_2 spheres by gravitational sedimentation. For gravitational sedimentation method, the order degree of the colloidal silica template is determined by the size and surface charge condition of the silica spheres. The SiO_2 spheres, whose size is about 350 nm as shown in Fig. 2a, are prepared by sol–gel method. The SiO_2 spheres with the same size are chosen by controlling the speed of sedimentation. It is reported [19] that the surface charge of silica spheres prepared via sol–gel process is imbalance; there are certain quantities of dissociative hydroxyls on the surface of SiO_2 spheres. In order to avoid these problems, the silica spheres are modified by H_2O_2 before sedimentation. Figure 3 shows the zeta potential of silica spheres before and after H_2O_2 surface modification. It can be seen in the picture that zeta potential declines and isoelectric point declines from 2.00 to 1.50 after surface modification, which indicates that H_2O_2 surface modification can repair the dissociative hydroxyls, and thus the uniformity of the surface charge distribution is improved, just as shown in Fig. 2b. Therefore the surface modification by H_2O_2 can improve the order degree of the colloidal silica template, which is beneficial to the formation of HA with highly ordered three-dimensional pores.

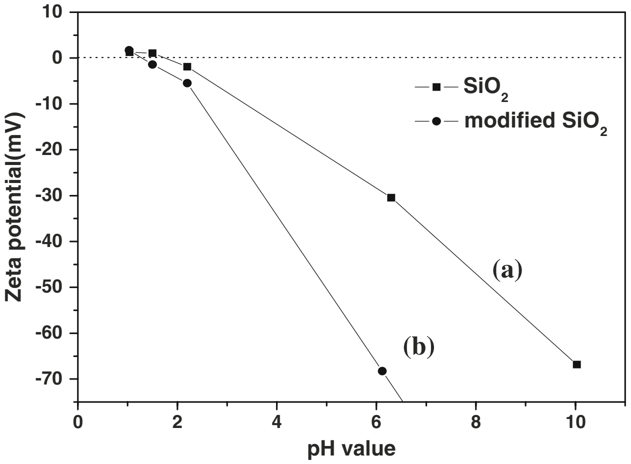


Fig. 3 Zeta potential of SiO_2 (a) and SiO_2 after H_2O_2 surface modification (b)

Figure 4 shows the FESEM image of HA with highly ordered three-dimensional pores, whose pore size is approximately 300 nm. As compared with the original diameter of SiO_2 spheres, the pores of HA shrank slightly. Furthermore, it is found that there are a great number of mesopores whose apertures are about 30–40 nm on the pore walls of HA, which make it possible for the magnetic nanoparticles to adsorb on the walls of the pores.

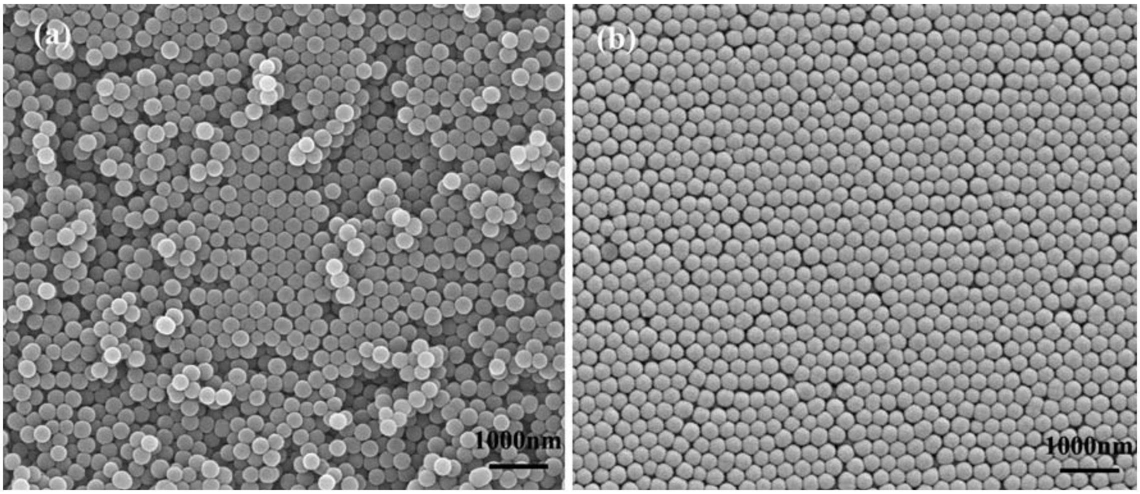


Fig. 2 FESEM image of SiO_2 collodial template before H_2O_2 modified (**a**) and after H_2O_2 modified (**b**)

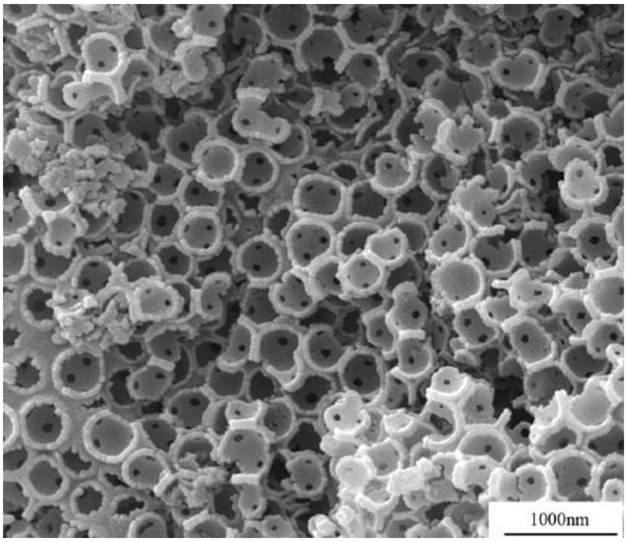
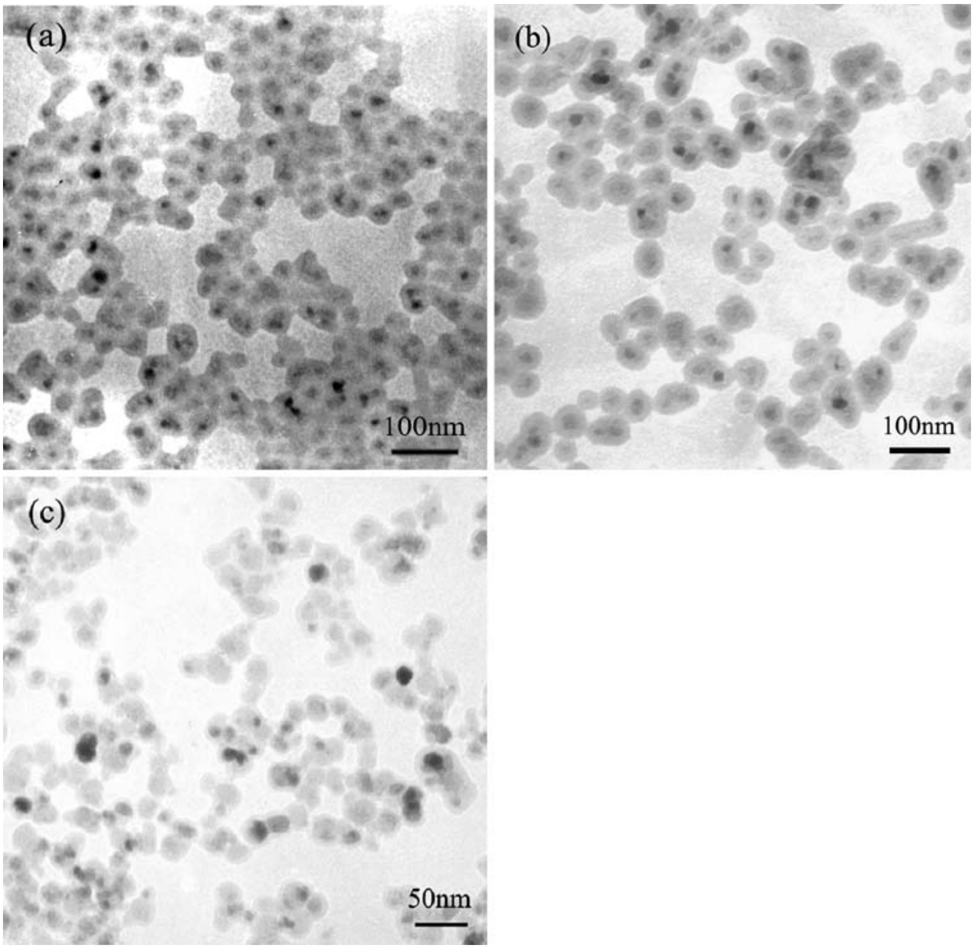


Fig. 4 FESEM image of HA with highly ordered three-dimensional pores

To obtain monodisperse SiO_2/Fe_3O_4 nanoparticles with uniform core–shell structure, the nanoparticles were dispersed in different dispersing agents. Figure 5a shows the TEM images of SiO_2/Fe_3O_4 nanoparticles dispersed in water, in which the used amount of TEOS is 0.12 mL. The thickness of the particles shell is about 20 nm. It is obvious that the particles are not uniform, and form three-dimensional chain structure rather than individual core–shell structure. This is probably because the abundant silanol groups existed on the silica surface lead to the particle adhesion. Figure 5b shows the TEM image of SiO_2/Fe_3O_4 nanoparticles dispersed in ethanol, in which the same amount of TEOS is used. The core–shell structure is obvious, and the particles are well dispersed. Therefore, the SiO_2/Fe_3O_4 nanoparticles dispersed in ethanol were used for the following adsorption process.

It was found in our experiments that the particles sizes of SiO_2/Fe_3O_4 had a marked influence on the adsorption process of the nanoparticles on the pore walls of HA. The used amount of TEOS, which controlled the thickness of silica shells, is therefore a crucial factor to determine the adsorption process [20, 21]. When the used amount of TEOS is 0.12 mL, the particle size of the SiO_2/Fe_3O_4 is about 35 nm, which is large relative to the average size of the mesopores on the pore wall of HA. So it is difficult for the SiO_2/Fe_3O_4 nanoparticles to be adsorbed on the pore

Fig. 5 TEM images of SiO_2/Fe_3O_4 nanocomposite particles dispersed in water (**a**), in ethanol (**b**), in which the amount of TEOS is 0.12 mL; SiO_2/Fe_3O_4 nanocomposite particles dispersed in ethanol, in which the amount of TEOS is 0.02 mL (**c**)



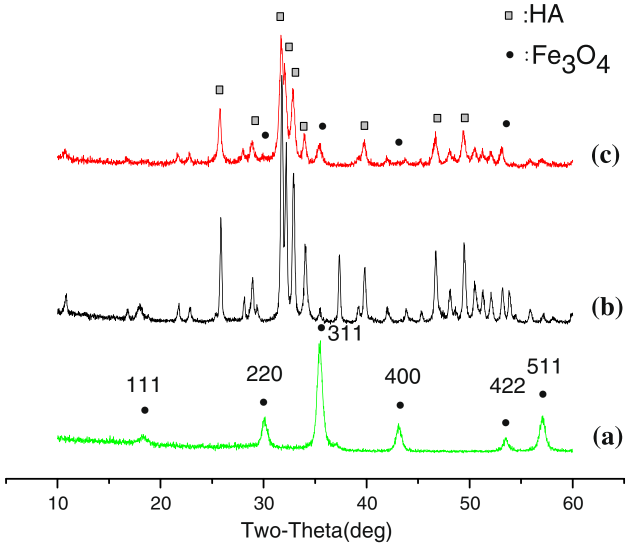


Fig. 6 XRD pattern of Fe_3O_4 nanoparticles (a); the HA with highly ordered three-dimensional pores (b); the HA with highly ordered three-dimensional pores adsorbing SiO_2/Fe_3O_4 nanoparticles (c)

walls of HA [22]. Comparatively, when the used amount of TEOS is 0.02 mL, as shown in Fig. 5c, the SiO_2 shell is relatively thin, and the average particle size is only 15 nm, which is a suitable particle size for the adsorption process.

XRD pattern of the as-synthesized Fe_3O_4 nanoparticles is shown in Fig. 6a. The diffraction peaks corresponding to (220), (311), (400), (422), and (511) are the characteristic peaks of the Fe_3O_4 crystal with a cubic spinel structure (PDF No#89-691). No peaks could be assigned to impurities such as γ-Fe_2O_3 and α-Fe_2O_3. In addition, the mean diameter of the magnetic particles derived from XRD peaks based on Scherrer's equation is 12.4 nm and Fe_3O_4 nanoparticles should possess superparamagnetism, due to the diameter is less than 15 nm [23]. In Fig. 6b, the XRD pattern of HA with highly ordered three-dimensional pores fabricated via a sol-gel process is matched well to the standard HA PDF No#72-1243. The XRD pattern of HA with highly ordered three-dimensional pores after adsorbing SiO_2/Fe_3O_4 nanoparticles is seen in Fig. 6c. The pattern consists of a series of diffraction peaks of Fe_3O_4 nanoparticles and HA, but the peak intensity of Fe_3O_4 nanoparticles is weak due to low adsorption amount.

The hysteresis loops of SiO_2/Fe_3O_4 nanoparticles with different thickness of silica shell and magnetic HA with highly ordered three-dimensional pores were measured at room temperature using a vibrating sample magnetometer. As shown in Fig. 7, all of the curves present extremely low coercive forces, meaning the products exhibit superparamagnetic behaviors and silica shell has almost no effect on the coercive force. The saturation magnetization (Ms) of SiO_2/Fe_3O_4 nanoparticles is 34.14 and 23.16 emu/g, when the amount of TEOS is 0.02 and 0.12 mL, respectively.

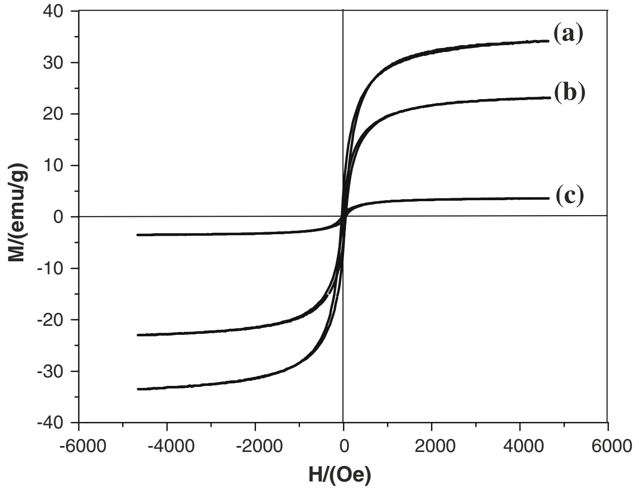


Fig. 7 The magnetic hysteresis loops of SiO_2/Fe_3O_4 nanoparticles. (a) 0.02 mL TEOS; (b) 0.12 mL TEOS and the HA adsorbing SiO_2/Fe_3O_4 nanoparticles ((c) 0.02 ml TEOS)

The Ms of SiO_2/Fe_3O_4 nanoparticles decreases correspondingly upon the increase of the thickness of silica shell and the decrease of mass proportion of magnetite. Figure 7c shows the hysteresis loop of HA after adsorbing the SiO_2/Fe_3O_4 nanoparticles (0.02 mL TEOS). The Ms of HA is 3.57 emu/g after ultrasonic washing for several times. It was found that while the SiO_2/Fe_3O_4 nanoparticles prepared by using 0.12 mL of TEOS was adsorbed, the Ms of HA approached 0 emu/g after the same ultrasonic washing was performed. Therefore, it indicates that when the SiO_2/Fe_3O_4 nanoparticles prepared by using 0.02 mL of TEOS, the SiO_2/Fe_3O_4 nanoparticles have been successfully assembled into the pores of HA, so that the SiO_2/Fe_3O_4 nanoparticles will not easily break away from HA during the ultrasonic washing process.

Conclusions

The formation of magnetic HA with highly ordered three-dimensional pores via an adsorption approach was systematic studied in the present work. HA with highly ordered three-dimensional pores was firstly prepared by using SiO_2 spheres as a template. During the process, surface modification of the colloidal silica template by H_2O_2 had a marked effect on the order degree of the template and the structure uniformity of the resultant HA. Subsequently, magnetic SiO_2/Fe_3O_4 nanoparticles with core–shell structure were prepared by adjusting the used amount of TEOS. When the amount of TEOS is 0.02 mL, the size of the SiO_2/Fe_3O_4 nanoparticles was about 15 nm, which was suitable for the following adsorption process. After adsorption process, the SiO_2/Fe_3O_4 nanoparticles were successfully assembled into the pores of HA with highly ordered three-dimensional pores. The resulting magnetic HA showed favorable superparamagnetic behavior. However, further work is needed to improve the saturation magnetization of the magnetic HA by increasing the adsorption amount of magnetic nanoparticles.

Acknowledgements This work was supported by the National Natural Science Foundation of China (NSFC) (No. 50572072) and Special Fond from Science and Technology Commission of Shanghai Municipality (SCTSM) (No. 0452nm059).

References

1. Lebeau B, Fowler CE, Mann S, Farcet C, Charleux B, Sanchez C (2000) J Mater Chem 10:2105
2. Jeong U, Wang YL, Ibisate M, Xia YN (2005) Adv Funct Mater 15:1907
3. Qian WP, Gu ZZ, Fujishima A, Sato O (2002) Langmuir 18:4526
4. Wu Q, Liao J, Yin Q, Li Y (2008) Mater Res Bull 43:1209
5. Wijnhoven J, Bechger L, Vos WL (2001) Chem Mater 13:4486
6. Kim J, Lee JE, Lee J, Yu JH, Kim BC, An K, Hwang Y, Shin CH, Park JG, Kim J, Hyeon T (2006) J Am Chem Soc 128:688
7. Arruebo M, Galan M, Navascues N, Tellez C, Marquina C, Ibarra MR, Santamaria J (2006) Chem Mater 18:1911
8. Zhang HR, Meyerhoff ME (2006) Anal Chem 78:609
9. Bee A, Massart R, Neveu S (1995) J Magn Magn Mater 149:6
10. Grüttner C, Rudershauscn S, Teller J (2001) J Magn Magn Mater 225:17
11. Deng Y, Qi D, Deng C, Al E (2008) J Am Chem Soc 130:28
12. Wang W, Shi DL, Lian J, Guo Y, Liu GK, Wang LM, Ewing RC (2006) Appl Phys Lett 89:183106
13. Li GD, Zhou DL, Xue M, Yang WZ, Long Q, Cao B, Feng DG (2008) Appl Surf Sci 255:559
14. del Real RP, Arcos D, Vallet-Regi M (2002) Chem Mater 14:64
15. Hilger I, Frühauf K, Andrä W, Al E (2002) Acad Radiol 9:198
16. Zhou LY, Wang DP, Huang WH, Yao AH, Xia CH, Duan X (2009) Mater Res Bull 44:259
17. Lou MY, Wang DP, Huang WH, Chen D, Liu B (2006) J Magn Magn Mater 305:83
18. Liu B, Wang D, Huang W, Yao A, Kamitakahara M, Ioku K (2007) J Ceram Soc Jpn 115:877
19. Huang ZB, Gao JN, Wang J, Tang FQ, Yuan CW (2004) Acta Phys-Chim Sin 20:651
20. Lu Z, Dai J, Song X, Al E (2008) Colloids Surf A Physicochem 317:450
21. Lu Y, Yin YD, Mayers BT, Xia YN (2002) Nano Lett 2:183
22. Chen DH, Li Z, Yu CZ, Shi YF, Zhang ZD, Tu B, Zhao DY (2005) Chem Mater 17:3228
23. Lee J, Isobe T, Senna M (1996) J Colloid Interf Sci 177:490