J Mater Sci: Mater Med (2008) 19:2009–2013
DOI 10.1007/s10856-007-3286-0

Polymer assisted hydroxyapatite microspheres suitable for biomedical application

A. Sinha · T. Mishra · N. Ravishankar

Received: 27 June 2007 / Accepted: 19 September 2007 / Published online: 19 October 2007

Abstract Hollow Microspheres of hydroxyapatite-polymer composite can be used as carriers in drug delivery and fillers in tissue engineering. Based on the concept of soft chemistry, a battery of technique is available in the literature to synthesize hollow microspheres, however, an economically viable synthesis route, having good control over the microarchitect and easy to be scaled up, is yet to be developed. Polymer matrix mediated synthesis of inorganic nanoparticles is known to synthesize nanoparticles with controlled morphology and dimensions. It is termed as biomimetic synthesis. Integrating the biomimetic synthesis of nano-particles and spray drying techniques, a novel process of producing hydroxyapatite-polymer composite hollow microspheres is briefly discussed here.

1 Introduction

Hydroxyapatite (HAp), the most stable calcium phosphate polymorph under physiological conditions, has been extensively researched for a variety of medicinal applications. In recent years, polymer-HAp nano-composites, having good biocompatability and high osteoconductive activity, have been developed into excellent scaffolds for biomedical applications [1–4]. Among the different forms, polymer-HAp microspheres are ideal vehicles for the delivery of cells, proteins and drugs in the treatment of defective tissues and their regeneration [5]. A battery of techniques is now available to synthesize polymer-HAp microspheres. Such techniques include template-based synthesis, layer by layer self-assembly technique, micro-emulsion technique and spray drying [6–8]. Each of the techniques has its own merits, however, except spray drying rest of the techniques involve multi steps process, produce a large size distribution and may not be found economically attractive [9]. Microstructural features of polymer-HAp nanoparticles, synthesized by spray drying technique, depend on spray drying process parameters as well as on the particle size distribution and degree of agglomeration of HAp particles dispersed in polymer medium. Hence, a good morphological control over the dispersed particles is a necessary condition. In numerous biological structures, Mother Nature has manifested highly controlled synthesis of functional inorganic nanoparticles through biomineralization. A highly controlled and soft chemistry based aqueous process of biomineralization has been adopted as a working model in material science to introduce the concept of biomimetic synthesis. Exploiting the concept of in situ mineralization of polymer and protein matrix (*akin to* biomineralization), we have already established biomimetic process for the synthesis of magnetic, semiconducting and bioceramic nanoparticles (particle size < 30 nm) for various applications [10, 11]. However, a polymer mediated synthesis route coupled with a simple filtration process fail to produce microporous polymer-HAp microspheres as required for biomedical applications [7]. In the present communication, integrating the biomimetic synthesis with spray-drying technique, we describe polymer matrix mediated synthesis of sub-micron sized HAp-polymer nano-composite microporous hollow spheres, intended for biomedical applications. The method,

A. Sinha (✉) · T. Mishra
Materials Science & Technology Division, National Metallurgical Laboratory, Jamshedpur 831007, India
e-mail: arvind@nmlindia.org

N. Ravishankar
Materials Research Centre, Indian Institute of Science, Bangalore, India

inspired by the biomineralization process, controls the nucleation and growth kinetics of HAp nano particles and their assembly into a porous microsphere. The scope of the paper is limited to the synthesis and characterization of HAp micro spheres.

2 Experimental

Freshly prepared 200 ml calcium nitrate tetrahydrate solution of strength 0.4 M was made alkaline using ammonia: water in the ratio 1:2. The pH of the solution was maintained at 10.50. Freshly prepared, 200 ml of 0.5% aqueous solution of PVA was added to the above (PVA obtained from Fluka, India, average molecular weight 1,25,000). The mixture was stirred thoroughly to obtain a homogeneous solution, incubated at a temperature of 30 °C for 48 h. About 0.156 M-diammonium hydrogen phosphate was made alkaline using ammonia: water in the ratio 1:1, pH maintained at 10, and the required volume was added gradually to the above-incubated mixture of calcium salt and PVA. A milky white coloration was observed almost instantaneously, which was allowed to age for a week at a temperature of 30 ± 2 °C. The precipitate slowly settled down, leaving colorless supernatant liquid that was decanted and again the volume was restored by adding distilled water. The process was repeated twice a week for two more weeks as to remove most of the water-soluble salts formed as by product during the reaction. Washed slurry was spray dried by using a tabletop spray dryer fitted with a nozzle of 0.50 mm diameter and two cyclones (supplied by SMST Kolkata, India). Slurry was sprayed in the drying chamber along with the hot air circulating at a rate of 300 l/h and maintained at a temperature 160 °C. The spray-dried powder was collected within the container fitted with spray dryer. Collected powder was stored under moisture free conditions. Spray-dried HAp powder samples were structurally characterized using scanning electron microscopy (SEM, JSM-840 A, JEOL) (powder was gold coated for SEM), transmission electron microscopy (TEM, CM 200, Philips), X-ray diffractometry (XRD PTS 3003, Seifert, targets Co Kα and Cu Kα, respectively) and Fourier transform infrared spectroscopy (FT-IR-410 (JASCO)).

3 Results and discussion

Spray drying is a simple process, however, optimization of number of process parameters (like inlet temperature, air flow rate, feed rate, slurry composition etc.) is required to produce the hollow micro spheres of required geometry [6]. The present results have been obtained after optimizing the process and establishing the reproducibility. PVA being a water-soluble polymer, capable of undergoing gellation, is known for its different biomedical applications. As a habit modifier during inorganic crystallization, PVA also regulates the growth rates and directions by preferential adsorption on specific crystallographic planes of the growing crystals. In the present study, *akin to* biomineralization, a long range order present in PVA conformation has been used as a supramolecular matrix for in situ nucleation and growth of HAp nanoparticles [12]. Due to limited solubility in water, PVA chains form a micelle like structure with a hydrophilic nano-sized core suitable for in situ mineralization [13]. The lone pair of electrons available with the oxygen atoms of hydroxyl groups attached to PVA carbon chains chelate the calcium ions into available nanosized core. On addition, the phosphate ions slowly diffuse into the polymer network via collisions and react with immobilized Ca ions to nucleate HAp phase in constrained reactors [12, 13]. HAp nanocrystals precipitated in PVA undergoes an ordered assembly as the PVA micelles tend to self assemble in order to minimize their surface energy [14]. SEM studies of as precipitated HAP particles in PVA revealed a thick film like structure, exhibiting dispersion of HAp submicron sized particles (200–500 nm) in polymeric matrix (Fig. 1a). EDX analysis also confirmed the presence of Ca and P in the sample maintaining a near stochiometric ratio of Ca:P $\sim$ 1.62 (Fig. 1b).

SEM studies of the spray-dried powder confirmed the formation of composite microspheres having diameter in the range 2–5 μm (Fig. 2). TEM studies confirmed the formation of hollow spheres having microporous surface suitable for loading and unloading of the drug molecules (Fig. 3a, b). Two microstructures differ with each other in terms of the wall thickness of the microsphere. Selected area diffraction pattern confirmed the polycrystalline nature of the microspheres revealing the diffraction rings corresponding to reflections from (002), (211) and (310) planes of hydroxyapatite (Fig. 3c). Production of spherical ceramic particles by spray drying is a known phenomenon applied in different industrial processes. However, the mechanism of porous and hollow microsphere formation has been proposed on the basis of co-operative phenomenon of sluggish solute diffusion, rapid solvent evaporation and porosity-induced stability of the structure [6]. When the slurry is sprayed into the drying chamber, a concentration gradient of the solute sets in along the radius of the droplet. Concentration of the solute being maximum on the surface and minimum at the centre of the droplet is caused by the sluggish solute diffusion and a rapid solvent evaporation kinetics. This leads to the formation of a thick solid shell. This shell inhibits the instant release of moisture that increases the internal pressure. An increase in the internal pressure ruptures the shell surface and inhibits the formation of hollow micro-spheres [15–17]. If the shell has nano

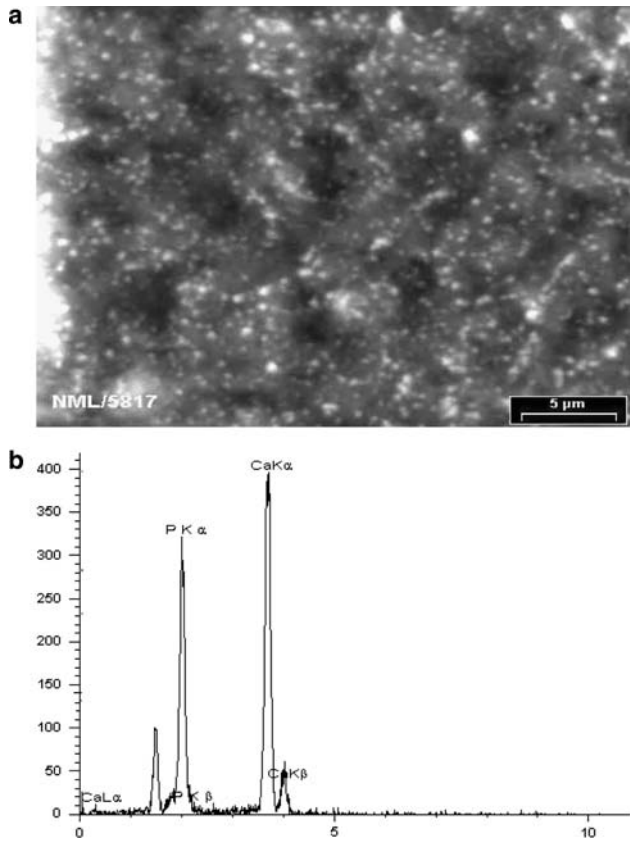

Fig. 1 (**a**) SEM image of as precipitated PVA-HAp composite. (**b**) EDX of as precipitated PVA-HAp composite

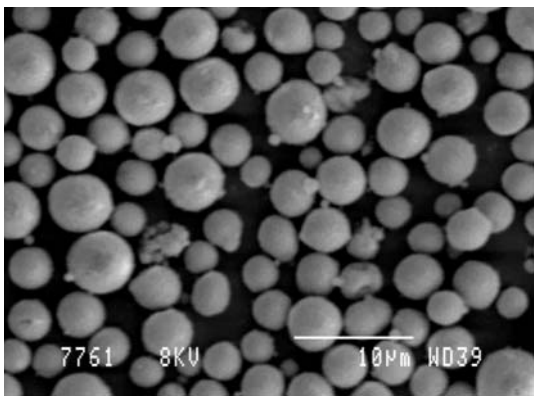

Fig. 2 SEM image of the spray dried PVA-HAp microsphers

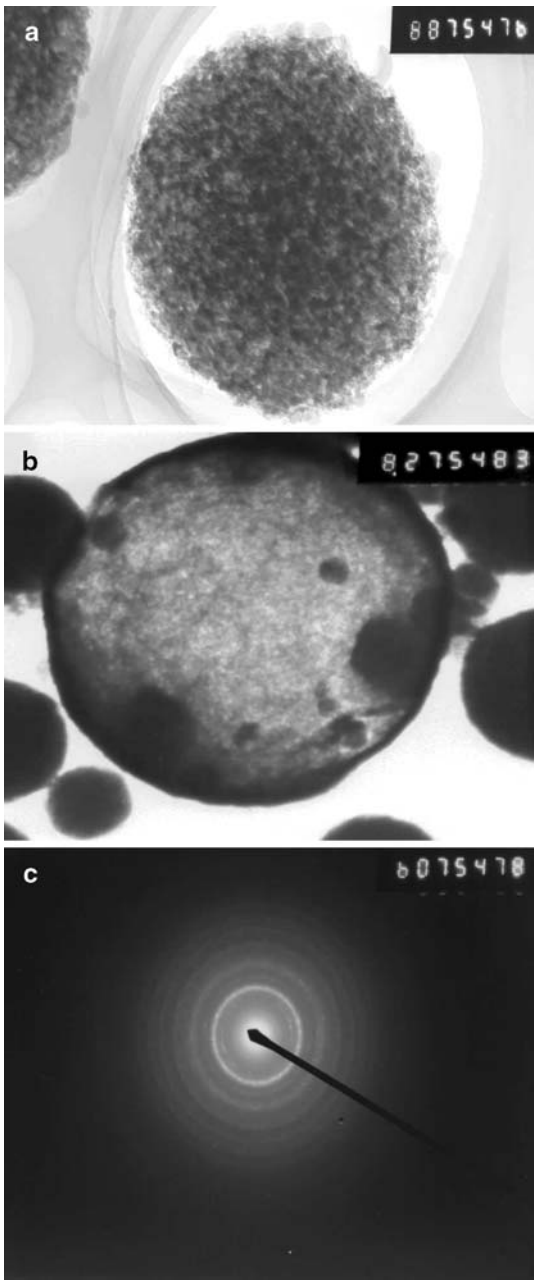

Fig. 3 (**a**) Bright field image of HAp-PVA microsphere exhibiting thicker shell surface. (**b**) Bright field image of HAp-PVA microsphere exhibiting thinner shell Surface. (**c**) Selected area electron diffraction pattern of HAp microspheres

or micro-porosity on its surface, the internal pressure is released slowly and the hollow structure of the microsphere is retained [18–20]. PVA solution having a microgel like structure does facilitate the formation of porous surface under the combined effect of surface tension and solvent evaporation during spray drying.

XRD confirmed the formation of single phase HAp, revealing the diffraction peaks from (002), (210), (211), (202), (310), (311), (113), (222) and (213) respectively (Fig. 4a). Appearance of broad diffraction peaks confirms the presence of HAp nanocrystals in the composite microspheres. Diffuseness associated with almost all the diffracted peaks can be attributed to the presence of PVA in the HAp structure. The peak analysis carried out using Scherer's equation: Average crystallite size = $K\lambda/\beta \cdot \cos\theta$, where K is the constant related with crystallite shape and can be approximated to unity, λ is the wave length of the

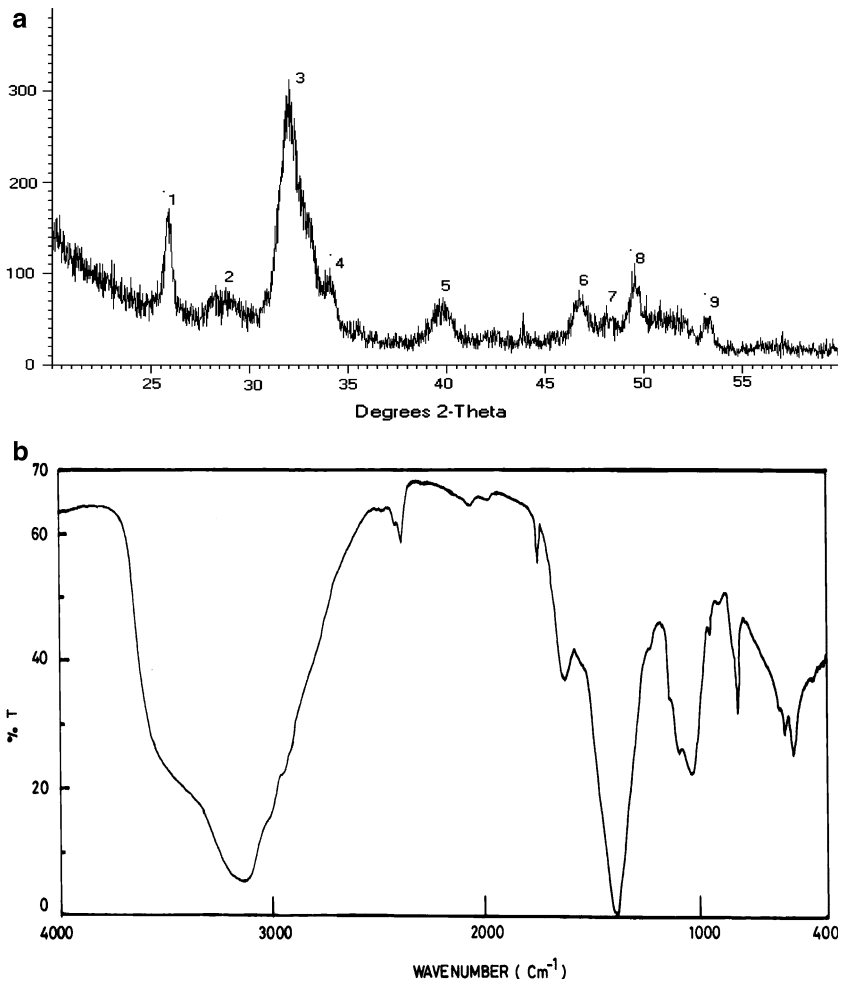

Fig. 4 (**a**) XRD of spray dried PVA-HAp microspheres. (**b**) FTIR spectrum of PVA-HAp microspheres

radiation, β is the peak width in radians at half of the intensity maximum. To get the approximate size of the crystallites, contributions from lattice strain and instrumental broadening were ignored. Calculations yielded an average crystalline size of 15 nm, when calculated from (002), (211) and (310) peaks. It indicates that the microspheres as observed under SEM, in fact, may be an assembly of HAp nanocrystals. An empirical relation, $((\beta)^3 \times X_c = K_A)$, correlating the peak width (β) with the degree of crystallinity (X_c) has been used to evaluate the X_c where K_A is a constant and set at 0.24 [21, 22]. Analysis revealed $X_c = 0.81$, signifying a high degree of lattice perfection in the precipitated crystallites. Effect of different reaction conditions on the average particle size and Xc is the subject of further investigation.

The presence of the polymer in the spray-dried powder was established by FTIR analysis (Fig. 4b). Absorbance bands at 3435.56 and at 603.61 cm^{-1} correspond to the presence of hydroxyl groups into the system, bands at 1639 and 1384.64 cm^{-1} reveals the presence of C=O and CH_2 asymmetric bending and bands at 1039.44 and 567.93 cm^{-1} reveal the presence of phosphate group in the composite system [14, 23].

4 Conclusions

In summary, the present communication reports a novel method of producing HAp-PVA microspheres suitable for biomedical application. Spray drying is a well established industrial process to produce fine sized ceramic powders, integrating it with a method *akin to* biomineralization provides a direct route to produce HAp microspheres with highly controlled morphological features. Controls being exerted right from the nucleation and growth of HAp nanocrystals and it lead to their ordered assembly during spray drying forming microporous microspheres. Being a soft chemical process, it can be easily scaled up to meet industrial requirements.

Acknowledgements Author (AS) acknowledges the financial support received from Department of Biotechnology (DBT) New Delhi for Biomimetics project.

References

1. H. W. KIM, H. E. KIM and V. SALIH, *Biomaterial* **26** (2005) 5221
2. H. W. KIM, J. C. KNOWELS and H. E. KIM, *J. Biomed. Mater. Res.* **72A** (2005) 136
3. T. KATSUMURA, T. KOSHINO and T. SAITO, *Biomaterial* **19** (1998) 1839
4. M. SIVAKUMAR and T. P. RAO, *Biomaterial* **23**, 3175 (2002)
5. N. K. VARDE and D. W. PACK, *Expert Opin. Biol. Th.* **4** (2004) 35
6. A. J. WANG, Y. P. LU and R. X. SUN, *Mater. Sci. Engg. A.* **460–461** (2007) 1
7. F. NAGATA, T. MIYAJIMA and Y. YOKOGAWA, *J. Eur. Cer. Soc.* **26** (2006) 533
8. Q. Q. QIU, P. DUCHEYNE and P. S. AYYASWAMY, *Ann. NY Acad. Sci.* **974** (2002) 556
9. J. G. ZHANG, S. Q. XU and E. KUMACHEVA, *J. Am. Chem. Soc.* **126** (2004) 7908
10. A. SINHA, S. NAYAR, A. AGRAWAL, V. RAO and P. RAMACHANDRA RAO, Indian patent on "A process for preparatin of nanosized hydroxyapatite by a biomimetic route" Patent Number: 192392 Dated 28 Feb. 2005
11. A. SINHA, S. K. DAS, V. RAO and P. RAMACHANDRARAO, *Scripta Met. Mater.* **44** (2001) 1933
12. A. SINHA, S. KUMAR DAS, V. RAO and P. RAMACHANDRARAO, *J. Mater. Res.* **46** (2001) 1846
13. A. SINHA, S. NAYAR, A. AGRAWAL, D. BHATTACHARYA and P. RAMACHANDRARAO, *J. Am. Cer. Soc.* **86** (2003) 357
14. S. NAYAR and A. SINHA, *Coll. Surf. B.* **35** (2004) 29
15. B. JURGEN, B. JAN and K. ROLF, *Chem. Eng. Technol.* **27** (2004) 829
16. P. LUO and T. G. NIEH, *Mater. Sci. Eng.* **C3** (1995) 75
17. R. X. SUN, M. S. LI and Y. P. LU, *Mater. Rev.* **19** (2005) 10
18. A. M. GOULA and K. G. ADAMOPOULOS, *J. Food Eng.* **66** (2005) 35
19. M. G. ATHANASIA and G. A. KOUSTANTINOS, *J. Food Eng.* **66** (2005) 25
20. I. FERRY, G. LEON and O. KIKUO, *J. Colloid Interf. Sci.* **265** (2003) 296
21. V. M. RUSU, N. CHUEN-HOW, M. WILKE, T. BRIGTTE, F. PETER and M. G. PETER, *Biomaterial* **26**, 5414 (2005)
22. E. LANDI, A. TAMPIERI, G. CELOTTI and S. SPRIO, *J. Eur. Ceram. Soc.* **20** (2000) 2377
23. N. DEGIRMENBASI, D. M. KALYON and E. BIRINCI, *Coll. Surf. B* **48** (2006) 42