Preparation and Characterization of Alginate and Hydroxyapatite-Based Biocomposite

Purnendu Parhi,[1,2,3] A. Ramanan,[2] Alok R. Ray[1,3]

[1]*Center for Biomedical Engineering, Indian Institute of Technology, Delhi*
[2]*Department of Chemistry, Indian Institute of Technology, Delhi, India*
[3]*All India Institute of Medical Science, Delhi, India*

Received 18 November 2005; accepted 10 April 2006
DOI 10.1002/app.24706
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: In this article, we report a novel route for the preparation of alginate-hydroxyapatite biocomposite. Hydroxyapatite has been nucleated on alginate chains by precipitation method to obtain a biomimetic artificial bone-like composite. The composite was characterized by powder XRD, FTIR, TGA, DTA, and SEM to ascertain its phase homogeneity and particle size distribution. Hydroxyapatite particles on alginate matrix are around 500–1000 nm in diameter. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 5162–5165, 2006

Key words: alginate; hydroxyapatite; biocomposite

INTRODUCTION

Bone is a complex and highly specialized form of connective tissue, which provides mechanical support to the body as well as serves as a reservoir of minerals, particularly calcium and phosphate. It is a good example of dynamic tissue, since it has unique capability of self-regenerating or remodeling to a certain extent throughout the life without leaving a scar.[1] However, bone degenerates due to polio, osteoporosis, rheumatoid arthritis, Vitamin D deficiency, etc. Hence, there is an increase in the demand for materials that can potentially replace, repair, or regenerate injured or diseased bone. Bone is, in fact, among the most frequently transplanted tissues.[2,3] For the last few decades, the focus of research has moved toward the synthesis of new materials that mimic natural bone tissue.[4–7] Both bioactive ceramics and polymers have been developed for use as tissue engineering scaffolds. Bioactive ceramics have chemical composition resembling that of natural bone but they are inherently brittle and have low biodegradation rates, which limit their clinical uses.[8,9] Biopolymers, on the other hand, have distinct advantages over the ceramic materials. The mechanical properties and biodegradation rate of these polymers can be tailored to a certain extent for specific applications. The low manufacture cost of the biopolymers, related to their large agricultural availability and renewability are an additional advantage. Among the biopolymers, polysaccharides have the widest medical applications due to their nontoxicity, water solubility, or high swelling ability induced by simple modification, stability to pH variation, and a broad variety of chemical structures.[10,11] Alginate, a naturally occurring polysaccharide, is biocompatible, hydrophilic, and biodegradable under normal physiological conditions.[12,13]

Alginates are a family of unbranched binary copolymers consisting of 1–4 glycosidically-linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G) as shown in Figure 1.[14,15] Alginates on binding with monovalent ions are generally water soluble, while bivalent cations like Ca^{2+} form hydrogels, which are partially soluble in water.[16] Alginate can be crosslinked with calcium under very mild conditions, such as at low temperatures and in the absence of any organic solvents. Calcium crosslinked alginate hydrogels have been used in drug delivery and cell transplantations for decades. Moreover, alginate hydrogels have been widely studied for cartilage and bone regeneration applications as scaffolds.[17,18] With the aim of preparing biomimetic composite, the direct nucleation of hydroxyapatite (HAp) on alginate copolymers was attempted, which is an excellent example of self-assembling process. The term self-assembling process refers to the assembly of building blocks of various natures, from organic molecules and polymers to inorganic entities in the form of tubes, sheets, and rods.[19] The driving forces behind assembling of such building blocks are essentially hydrogen-bonding, van der Waals, electrostatic forces and electron-transfer interactions.[20] In bone tissue engineering, deposition of a matrix and subsequent mineralization are required for the development of tissue to form the new bone.[21] In this work, alginate chains were employed as a template

Correspondence to: A. R. Ray (alokray@cbme.iitd.ernet.in).
Contract grant sponsor: ICMR.

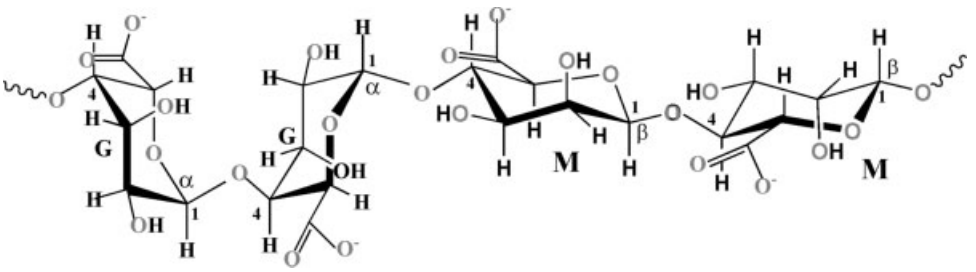

Figure 1 Structure of alginate.

for the growth of the inorganic component, HAp, and Ca ions present in the hydroxyapatite were exploited to partially crosslink the alginate, which can later be used as a scaffold for bone growth. There have been reports of hydroxyapatite/collagen,[22] hydroxyapatite/alginate,[16] and hydroxyapatite/collagen/alginate[23] composites being used as scaffold materials for bone tissue engineering.

EXPERIMENTAL

Materials

All chemicals used were of analytical reagent grade. Sodium alginate, liquor NH_3, $CaCl_2$, and Na_3PO_4 are purchased from CDH, India. Chemicals were used without any further purification.

Preparation of HAp-alginate composite

The direct nucleation of an apatite phase on alginate copolymers was performed by the following precipitation method. Two grams of alginate was taken in 100 mL distilled water and stirred continuously, and 5 mL of liquor NH_3 was added to dissolve the alginate. Once a clear solution was obtained, 1 g of $CaCl_2$ was added. After 30 min of stirring, 1.547 g of Na_3PO_4 was added keeping Ca/P ratio 1.67. The solution was stirred overnight at room temperature (25°C). The resultant solution was aged for a day. The precipitate was filtered and dried at 50°C before characterization. The product was characterized using powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM).

IR spectra

The samples of the HAp-alginate composite and pure alginate were dried in a vacuum oven at 50°C for 24 h, before they were ground to a suitable size for IR analysis with a Nicolet 5DX spectrophotometer.

XRD analysis

The ground samples of pure alginate and HAp-alginate were characterized by X-ray diffraction (XRD). The patterns were recorded with a Bruker D8 Advance diffractometer using Ni-filtered Cu Kα radiation ($\lambda = 1.542$ Å) generated at 40 kV and 30 mA. The samples were scanned from 10° to 60° in 2θ (where θ is the Bragg angle) in a continuous mode.

SEM observation

Morphological investigations of the composite and pure alginate were carried out using Cambridge Stereoscan 360 SEM. Pure alginate and HAp-alginate composite were dried properly and coated with gold before observing in the microscope. SEM picture has been taken at different magnifications.

Thermal analysis

Thermogravimetric investigation of pure alginate and HAp-alginate composite were carried out using Perkin-Elmer TGA7 and DTA7 system on well ground samples in flowing nitrogen atmosphere with a heating rate of 5°C/min.

RESULTS

Powder XRD pattern of HAp synthesized by precipitation method on alginate is shown in Figure 2(a). The low degree of crystallinity shows that *in situ* nucleation leads to the formation of very small nuclei and interac-

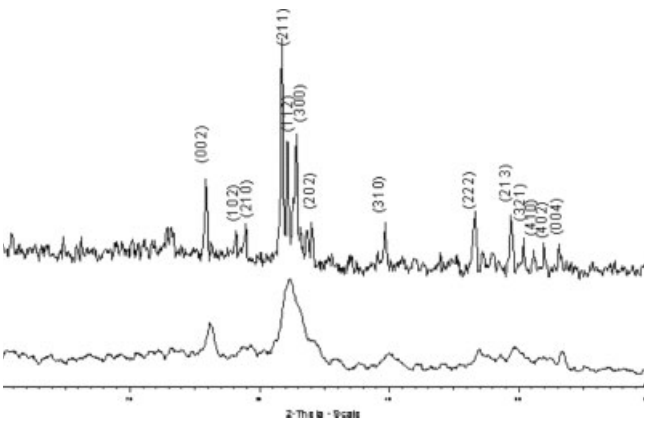

Figure 2 XRD pattern of (a) HAp-alginate composite (b) composite heated to 800°C.

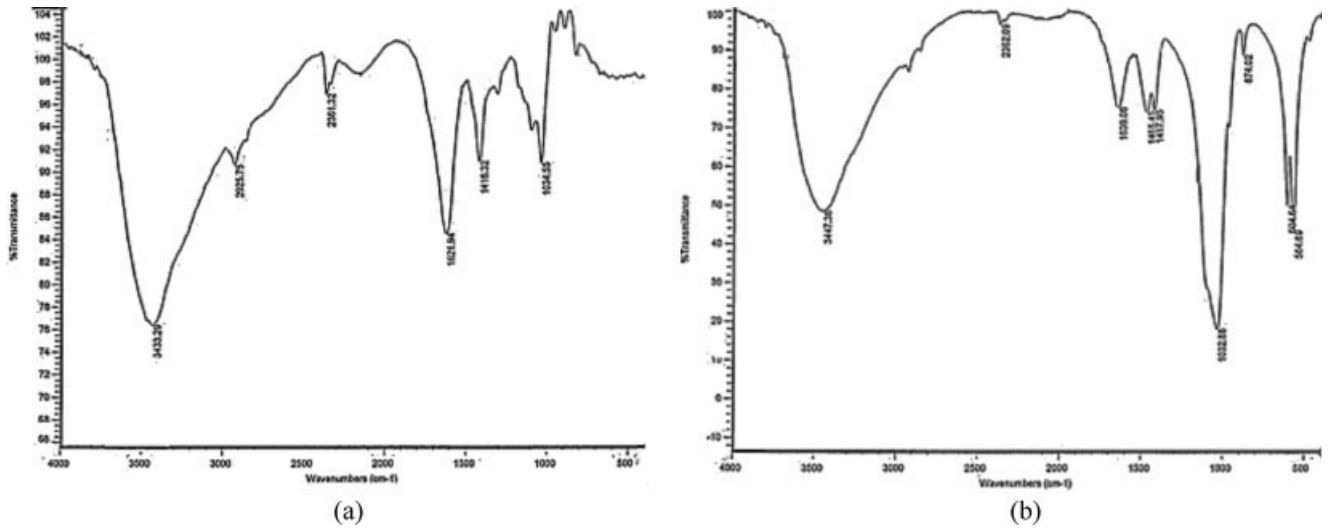

Figure 3 FTIR Spectra of (a) sodium alginate (b) HAp-alginate composite.

tion with the polymer prevents any further crystallization of hydroxyapatite. Crystalline phase of hydroxyapatite appears when the composite was heated at 800°C as shown in Figure 2(b). The FTIR absorption spectra, recorded for sodium alginate, and HAp-alginate composite are shown in the Figures 3(a) and 3(b), respectively. The FTIR spectrum of sodium alginate shows a broad peak at 3433 cm^{-1} representing the hydroxyl groups. The peaks at 1621 and 1416 cm^{-1} have been assigned to the asymmetric and symmetric stretching of carboxyl groups. In Figure 3(b), the band at 874 and 1639 cm^{-1} corresponding to the bending of OH group and stretching of C=O groups of carboxylic appear to be shifted. This is probably due to the interaction with the Ca atoms of the HAp with the oxygen sites of alginate. In Figure 3(b), the band at 1032 cm^{-1} corresponds to phosphate stretching and the bands at 564 and 604 cm^{-1} correspond to the phosphate-bending vibrations. TGA of sodium alginate and HAp-alginate composite are shown in Figures 4(a) and 4(b), respectively. TGA of sodium alginate shows loss of water below 100°C. The two weight loss at 240°C (around 35%) and 640°C (around 13%) indicates the rupture of chains, fragments, and monomers resulting in 25% residual. The TG curve of composite shows broad transition, but the relative weight loss is the same as that of pure alginate. The weight loss at 250°C (around 10%) and 700°C (around 3%) shows the rupture of alginate chains. SEM of sodium alginate and the HAp-alginate composite are shown in Figures 5(a) and 5(b), respectively. The hydroxyapatite particles that grow on the alginate surface are agglomerated as shown in Figure 5(b). The size of the hydroxyapatite particles are in the range of 500–1000 nm (1 μ).

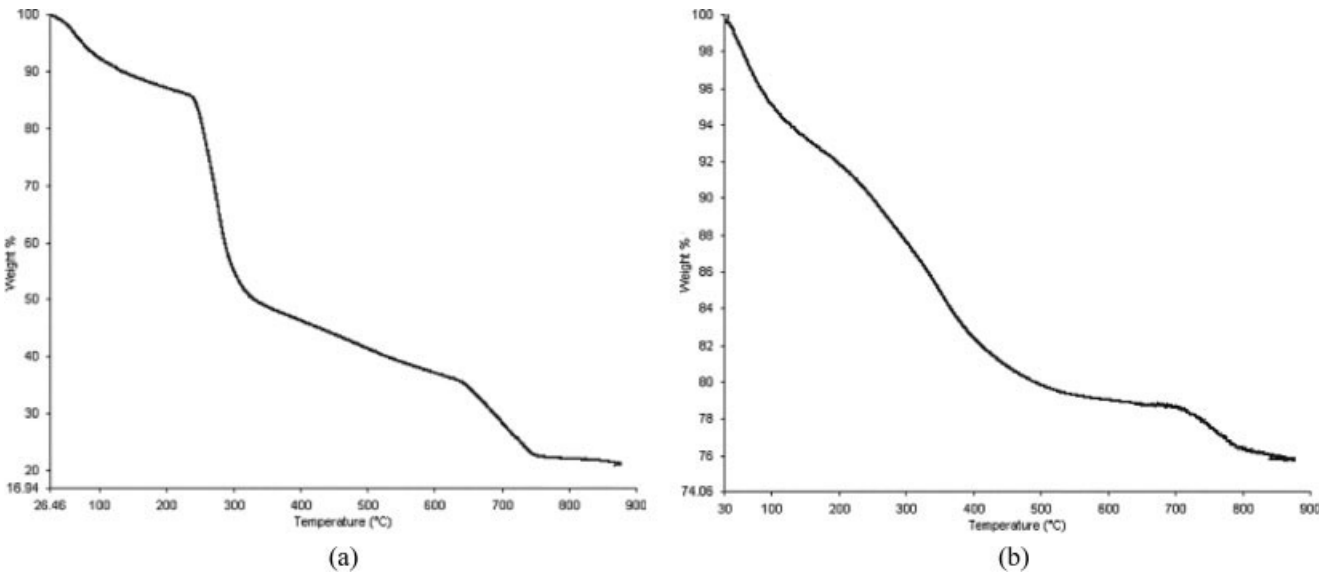

Figure 4 TGA of (a) sodium alginate (b) HAp-alginate composite.

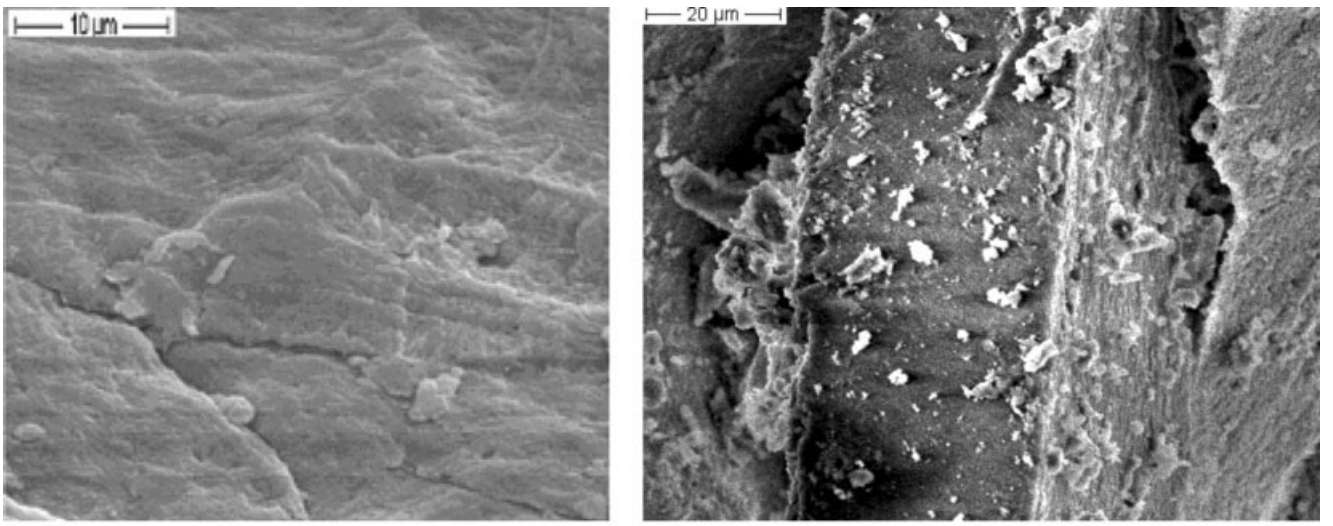

Figure 5 Scanning electron micrographs of (a) alginate and (b) hydroxyapatite grown on alginate.

DISCUSSION

Sodium alginate is soluble in basic medium. Addition of liquor NH_3 increases the pH to 9–10. Addition of $CaCl_2$ to the above solution precipitates calcium alginate. Calcium ions being divalent replace the monovalent sodium ions of sodium alginate. Na_3PO_4 added to the calcium alginate precipitates hydroxyapatite on the alginate surface. A close examination of the PO_4 peaks in the 500–600 cm^{-1} region of the FTIR and the broad nature of the XRD pattern suggest that the apatitic phase has an amorphous nature. The SEM of the composite indicates that in HAp-alginate composite the hydroxyapatite particles are in the nanometer range.

CONCLUSIONS

We are able to develop a polymeric composite made up of nanohydroxyapatite and alginate, which can be used as scaffold for bone-tissue growth.[16,23] HAp-alginate composite was formed as a result of *in situ* nucleation of hydroxyapatite on alginate polymeric chain.

PP acknowledges CSIR for a research fellowship. AR acknowledges DST-IRHPA for a powder diffractometer to the Department of Chemistry, Indian Institute of Technology, New Delhi, India.

References

1. Murugan, R.; Ramakrishna, S. Compos Sci Technol 2005, 15, 2385.
2. Hanker, J. S.; Giammara, B. L. Science 1988, 242, 885.
3. Addadi, L.; Weiner, S. Proc Natl Acad Sci USA 1996, 82, 4110.
4. Sato, K.; Kumagai, Y.; Tanaka, J. J Biomed Mater Res 2000, 50, 16.
5. Silva, C. D.; Pinheiro, A. G.; Figueiro, S. D.; Goes, J. C.; Sasaki, J. M.; Miranda, M. R. J Mater Sci 2002, 37, 2061.
6. Chang, M. C.; Ikoma, T.; Kikuchi, M.; Tanaka, J. J Mater Sci Lett 2001, 20, 1199.
7. Rhee, S. H.; Do, L. J.; Tanaka, J. J Am Ceram Soc 2000, 83, 2890.
8. Jarcho, M. Clin Orthop 1981, 259.
9. Hench, L. L.; Wilson, J. Science 1984, 226, 630.
10. Miyamoto, T.; Takahashi, S.; Ito, H.; Inagaki, H.; Noishiki, Y. J Biomed Mater Res 1989, 23, 125.
11. Hayashi, T. Prog Polym Sci 1994, 19, 663.
12. Gutowska, A.; Jeong, B.; Jasionowski, M. Anat Rec 2001, 263, 342.
13. Becker, T. A.; Kipke, D. R.; Brandon, T. J Biomed Mater Res 2001, 54, 76.
14. Gombotz, W. R.; Wee, S. F. Adv Drug Delivery Rev 1998, 31, 267.
15. Smidsrod, O.; Draget, K. I. Carbohydr Eur 1996, 14, 6.
16. Tampieri, A.; Sandri, M.; Landi, E.; Celotti, G.; Roveri, N.; Mattioli-Belmonte, M.; Virgili, L.; Gabbanelli, F.; Biagini, G. Acta Biomater 2005, 1, 343.
17. Eiselt, P.; Yeh, J.; Latvala, R. K.; Shea, L. D.; Mooney, D. J Biomater 2002, 21, 1921.
18. Alsberg, V.; Anderson, K.; Albeiruti, A.; Franceshi, R. T.; Mooney, D. J. J Dent Res 2001, 80, 2025.
19. Casal, B.; Ruiz-Hitzky, E.; VanVaeck, L.; Adams, F. C. Mol Cryst Liq Cryst 1988, 161, 433.
20. Ruiz-Hitzky, E. Chem Rec 2003, 3, 88.
21. Lee, K. Y.; Alsberg, S.; Mooney, D. J. J Biomed Mater Res 2001, 56, 228.
22. Wang, R. Z.; Cui, F. Z.; Lu, H. B.; Wen, H. B.; Ma, C. L.; Li, H. D. J Mater Sci Lett 1995, 14, 490.
23. Zhang, S. M.; Cui, F. Z.; Liao, S. S.; Zhu, Y.; Han, L. J Mater Sci: Mater Med 2003, 14, 641.