Key Engineering Materials Vols. 330-332 (2007) pp. 525-528
online at http://www.scientific.net

Characterization of Crystalline Hydroxyapatite Thin Coatings for Biomedical Applications

Z. Hong[1,a], A. Mello[2-3,b], L. Luan[1], M. Farina[4], L.R.Andrade[4], Ferreira C.L.[3], S. Paik[1], B. Deng[1], J. Eon[5], J. Terra[2], A. M. Rossi[2], D.E. Ellis[1], and J. B. Ketterson[1,c]

[1]Department of Physics and Astronomy, Northwestern University, Evanston, IL 60208, USA

[2]CBPF, Rua Dr. Xavier Sigaud, 150, Rio de Janeiro, 22290-180, RJ, Brazil

[3]Instituto Militar de Engenharia,IME, Rio de Janeiro, RJ, Brasil

[4]Departamento de Histologia e Embriologia, ICB, CCS, UFRJ, Ilha do Fundão, Rio de Janeiro, RJ, CEP 21941-590, Brazil

[5]Inst. Química, PUC/RJ, Rio de Janeiro, 21941-590, RJ, Brazil

[a]hong@northwestern.edu, [b]mello@cbpf.br, [c]j-ketterson@northwestern.edu

Keywords: hydroxyapatite, thin coatings, magnetron sputtering, crystallinity, heat treatment, cell culture

Abstract. Crystalline hydroxyapatite thin coatings have been prepared using a novel opposing RF magnetron sputtering approach at room temperature. X-ray diffraction (XRD) analysis shows that all the principal peaks are attributable to HA, and the as-deposited HA coatings are made up of crystallites in the size range of 50-100nm. Fourier transform infrared spectroscopy (FTIR) studies reveal the existence of phosphate, carbonate and hydroxyl groups, suggesting that HA coatings are carbonated. Finally, in vitro cell culture experiments have demonstrated that murine osteoblast cells attach and grow well on the as-sputtered coatings. These results encourage further studies of hydroxyapatite thin coatings prepared by the opposing RF magnetron sputtering approach as a promising candidate for next-generation bioimplant materials.

Introduction

Hydroxyapatite (HA) thin coatings on biocompatible metals have been extensively studied in connection with bioimplants[1]. Conventional sputtering techniques have shown some advantages over the commercially utilized plasma spray method; however, the as-sputtered coatings are usually amorphous which can cause serious adhesion problems when post-deposition heat treatment is necessitated[2,3,4,5]. In this work we have utilized an alternative magnetron sputtering system based on a right-angle geometry to prepare thin and adherent HA coatings on single crystal silicon substrates. It is found out that under chosen experimental conditions the as-sputtered HA coatings are phase-pure and crystalline.

Materials and Methods

Stoichiometric HA powder (Ca/P = 1.67) were synthesized from the dropwise addition of calcium nitrate and ammonium phosphate solutions. The precipitate was separated by filtration, vacuum drying and sieving into fine powder. The sputtering targets with a diameter of 25 mm were prepared by uniaxial pressing HA under 30MPa, followed by sintering at 1100ºC. The substrates we utilized were single crystal Si(111). All substrates were ultrasonically cleaned with 10% hydrofluoric acid and acetone before deposition to remove the surface oxidization layer. After deposition some of the coatings were photolithographically patterned, using hydrochloric acid as an etchant, to generate a discrete circular dot pattern for cell culture experiments. X-ray diffraction (XRD) was used to identify the phase composition and to estimate the mean crystallite size of the as-sputtered coatings. Fourier transform infrared spectroscopy (FTIR) was used to characterize the various functional

groups, most importantly the phosphate and hydroxyl groups. Cell morphology on the coated surfaces at different stages of cell growth was observed by scanning electron microscopy (SEM). Biocompatibility tests were performed on HA coatings by seeding murine osteoblasts over the whole silicon substrate surface. These cells were obtained from femurs, where cortical bones free of bone marrow were macerated in small fragments (5 mm), rinsed with 0.1M phosphate buffered saline (PBS) and transferred to 6-well plates containing DMEM culture medium for cell proliferation. The osteoblasts originating from the bone fragments were counted and used for cell adhesion experiments. The HA coatings were sterilized at 100°C for 4 hours, followed by soaking in 70% ethanol for 2 hours. Approximately 2 x 10^5 cells were seeded on HA coatings in 24-well plates with DMEM, maintained at 37°C for 4 hours and 3 days in a humid atmosphere containing 5 % CO_2. After these periods, the cells were prepared for SEM observations. Cells that adhered on the HA coatings were fixed with 2.5 % glutaraldehyde in 0.1M sodium cacodilate buffer (pH 7.2) for 1 hour, rinsed in the same buffer and dehydrated in graded ethanol series till absolute. The samples were dried by liquid CO_2 critical-point method (Baltec CPD 020), deposited on conductive carbon tape covering aluminum stubs, and gold-sputtered. The samples were observed in a JEOL 5310 SEM operated at 15 kV.

Results and Discussion

The XRD patterns of the as-sputtered coatings show sharp peaks like those of sintered HA powder, indicating that as-sputtered HA coatings have high crystallinity. All the principal peaks are attributable to HA. Although the XRD patterns are typical of a polycrystalline material, preferred

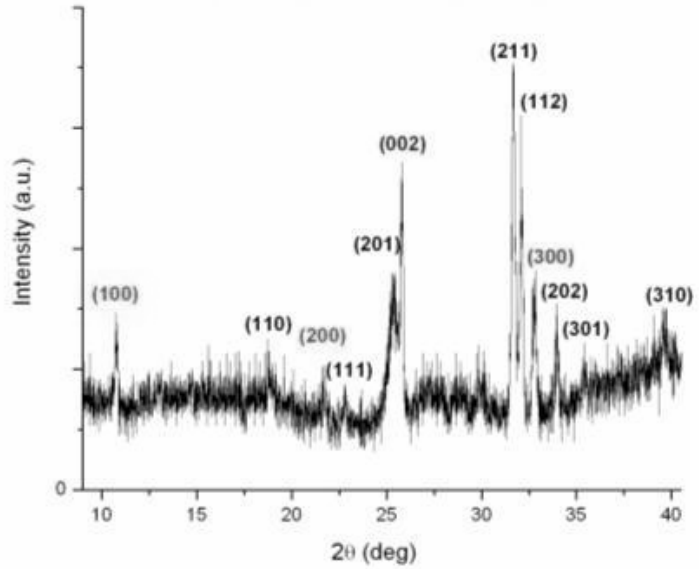

Fig. 1. X-ray diffraction pattern of the as-sputtered HA thin coatings on Si(111) substrates.

orientations of growth along (h00) and (00k) are observed. The crystallite size broadening of a diffraction peak can be related to the mean crystallite size via the Scherer equation, $t = 0.9\lambda / (B\cos\theta_B)$. The (002) and (300) reflections were chosen for analysis of the broadening of the Bragg line, the Gaussian symmetrical profile function was fitted to the two Bragg peaks for extraction of FWHM. The mean crystallite size was then estimated to be within the range of 50 to 100nm. It was also found that the intensity ratio between the (002) and (300) diffraction peaks of HA coatings (1.64) is markedly larger than that of standard HA powder (0.67). This result shows that crystallization along (002) planes is more preferred in HA coatings than in HA powder precipitated in aqueous solution.

The FTIR spectrum of the HA coating on Si(111) (Fig. 2) is composed of sharp phosphate and hydroxyl absorption bands, indicating that PO_4^{3-} and OH^- sites are well ordered in coating structure.

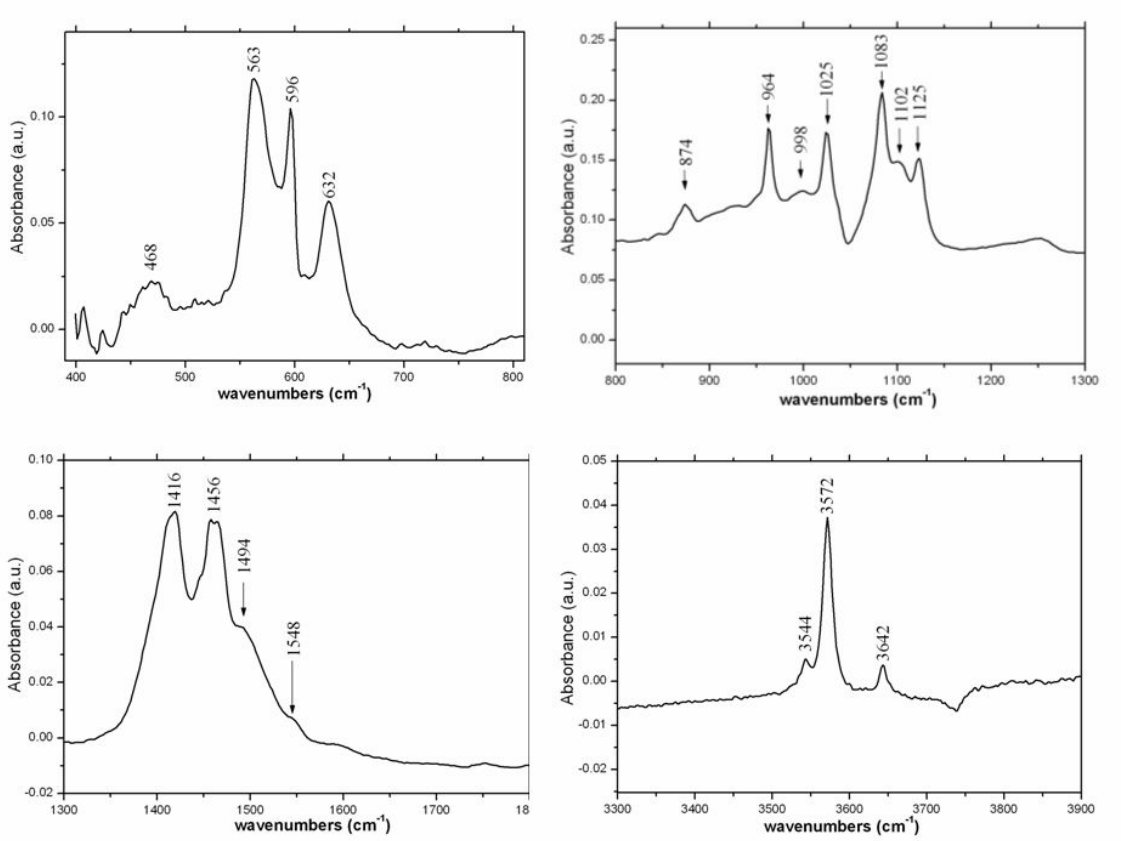

Fig. 2. FTIR spectra of the as-sputtered HA thin coatings on Si(111) substrates.

The pronounced hydroxyl bands at 632 and 3572 cm^{-1} are typical of hydroxyapatite with a high degree of crystallinity. The hydroxyapatite phosphate absorption bands ν_1 ,ν_2 and ν_4 were found at 468, 563, 596, and 964 cm^{-1} which are very close to those of a stoichiometric HA (ν_1 at 962 cm^{-1}; ν_2 at 462 and 474cm^{-1}; ν_4 at 571 and 601 cm^{-1}). In the region 1000 - 1150 cm^{-1}, the ν_3 bands of stoichiometric HA at 998 and 1083 cm^{-1} were identified but in the position of ν_3 components at 1046 cm^{-1} and 1032 cm^{-1} a broad band centered at 1025 cm^{-1} was seen. This band has been identified in calcium deficient HA.[24] All coatings showed carbonate bands at 1416 cm^{-1}, 1456 cm^{-1}, 1494 cm^{-1}, and 1548 cm^{-1}. These bands are typical of carbonate occupying OH^{-1} and PO_4^{3-} sites in HA hexagonal structure and suggests the formation of carbonated HA coatings. The carbonate contamination may come from adsorption and diffusion of CO_2 molecules in the air during the sputtering process and after.

SEM images show that the surface of the HA coatings was smooth with regularly distributed circular platforms, generated by wet etching with hydrochloric acid using photolithography. These platforms are around 0.6μm high and are 50μm in diameter (Fig. 3(a)). After exposure for 4 hours numerous osteoblasts adhered to the wafer surface. It was also observed that some cells surrounded (embraced) while others ascended the platforms, indicating that the cells could interact and move across the HA surface (Fig. 3(b)). After 3 days the osteoblasts proliferated and spread on the wafer, forming a monolayer of cells (Fig. 3(c)). The osteoblasts could also adhere, spread and proliferate under the HA coatings, showing that membrane receptors interact with HA deposited on silicon wafers. Fig. 3(d) shows a cross-sectional view of a platform edge that was broken up by a cell, suggesting the interaction between the cell and coating is very strong.

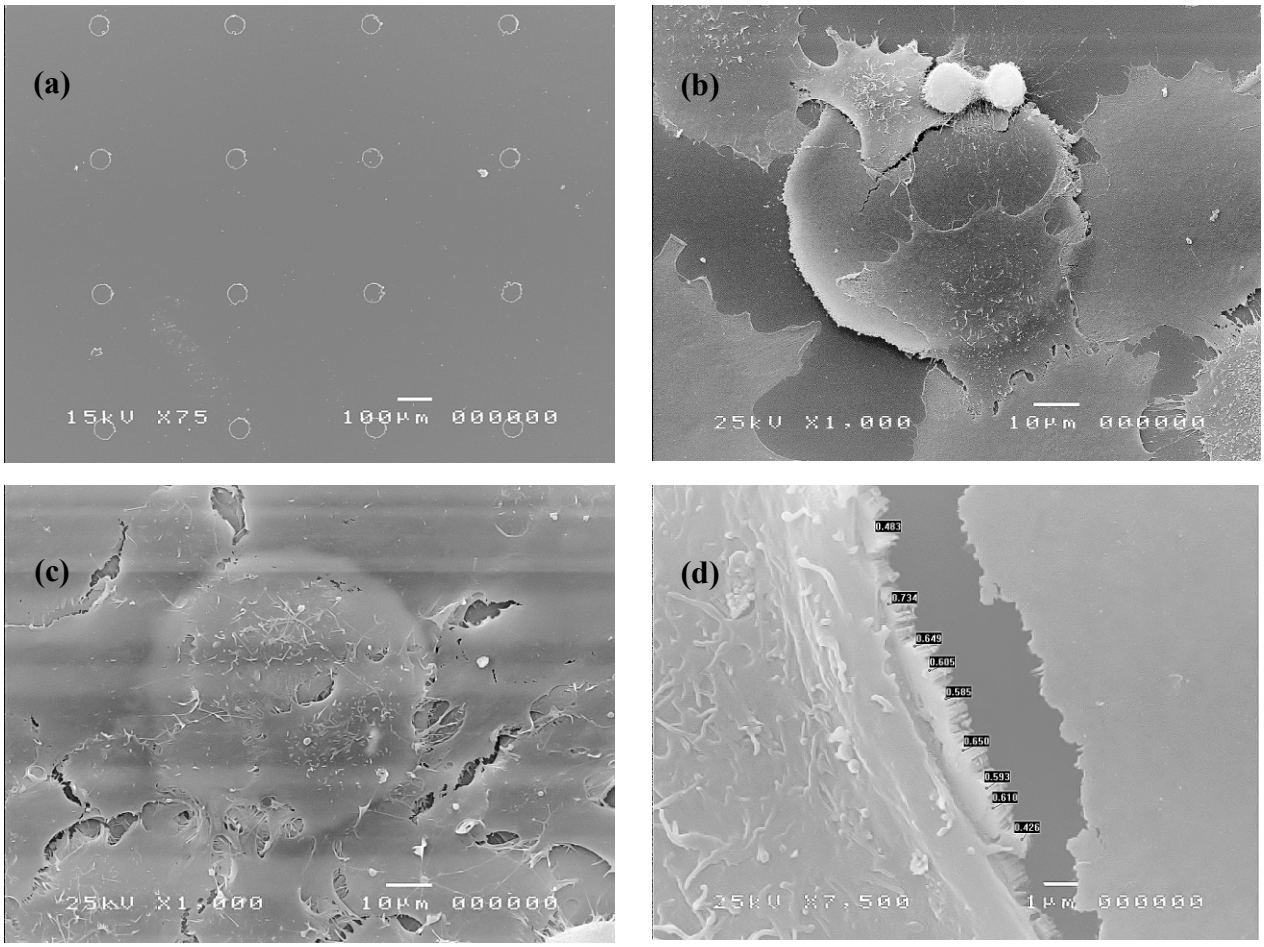

Fig. 3. Cell morphology of as-sputtered HA coating on Si(111) at different stages of cell growth. (a) t=0; (b) t=4hrs; (c)&(d) t=72hrs.

Conclusions

A novel opposing magnetron sputtering system with a right angle geometry has been shown to produce crystalline HA thin coatings without the need for *in-situ* or *ex-situ* annealing. In vitro cell culture studies have demonstrated that the as-sputtered HA coatings promote the growth of murine osteoblast cells. Cells attached and effectively grew on all coated surfaces, and experienced a continuous change in surface morphology as they proliferated with time. In summary, this result suggests that magnetron sputtering with a right angle geometry may be a promising alternative to the plasma spray technique for depositing HA coatings on metallic implants.

Acknowledgements

This work is supported by the U.S. National Science Foundation under grant DMR-0303491 and through CNPq in Brazil.

References

[1] J.E.Lemons: Clin.Orthp. Relat. Res. Vol. 235(1988), p. 220

[2] K.van Dijk, H.G.Schaeken, J.G.C.Wolke, J.A.Jansen: Biomaterials Vol. 17(1996), p. 405

[3] Y.Yang, K.Kim, C.M.Agrawal, J.L.Ong: Biomaterials Vol. 24(2003), p. 5131

[4] A.R.Boyd, B.J.Meenan, N.S.Leyland: Surface & Coatings Technology Vol. 200(2006), p.6002

[5] Y.Yang, K.H Kim, J.L.Ong: Biomaterials Vol. 26(2005), p. 327