Applied Surface Science 255 (2008) 304–307

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Osteoblastic cell response on magnesium-incorporated apatite coatings

Guochao Qi [a,b], Sam Zhang [a,*], Khiam Aik Khor [a], Sun Woh Lye [a], Xianting Zeng [c], Wenjian Weng [d], Chunming Liu [b], Subbu S. Venkatraman [e], Lwin Lwin Ma [e]

[a] School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore
[b] School of Materials and Metallurgy, Northeastern University, Shenyang 110004, PR China
[c] Singapore Institute of Manufacturing Technology, 71 Nanyang Drive, Singapore 638075, Singapore
[d] Department of Materials Science and Engineering, Zhejiang University, Hangzhou, Zhejiang 310027, PR China
[e] School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

ARTICLE INFO

Article history:
Available online 26 June 2008

PACS:
81.05.−t
87.17.Ee

Keywords:
Magnesium apatite
X-ray photoelectron spectroscopy (XPS)
Cell culture
MG63 cell
Hydroxyapatite

ABSTRACT

Magnesium is one of the most important bivalent ions associated with biological apatite. A series of magnesium-substituted calcium apatite coatings $(Ca_{10-x}Mg_x)(PO_4)_6(OH)_2$, where $x = 0$, 0.50, 1.00, 1.50 and 2.00, are synthesized onto Ti6Al4V substrate by sol–gel dip-coating method to determine how magnesium influences the synthesis and the resulting structural and biological properties. X-ray diffraction (XRD) analysis shows that the incorporation of magnesium helps formation of Mg-containing β-TCP (β-TCMP) phase. X-ray photoelectron spectroscopy (XPS) is used to study the chemical composition and the results show that the apatite structure can only host magnesium less than ~2.4 wt.% beyond which magnesium aggregates on the surfaces. The incorporation of magnesium slows down the dissolution of Ca^{2+} from the coating. The *in vitro* behavior of the coatings is evaluated with human osteosarcoma MG63 cells for cell morphology and proliferation. Similar cell morphologies are observed on all coatings. The cell proliferation results show that the incorporation of magnesium up to $x = 2$ has no adverse effect on cell growth.

1. Introduction

Hydroxyapatite (HA) is the major inorganic component of natural bone and has been used as orthopedic and dental material [1]. As a material for osseous implants, HA shows excellent properties because of its biocompatibility and ability to regenerate calcified tissues [2]. When used to coat an orthopedic or dental implant, synthetic HA provides a surface for the anchorage-dependent osteoblasts to deposit calcium-containing mineral. This promotes osseointegration and stabilization of an implant and prevents motion-induced damage [3].

Despite these bio-advantages, HA is limited in use due to high *in vivo* solubility and poor mechanical properties. Synthesizing materials that mimic natural bone thus becomes objective of many technological researches [3,4]. Mg is one of the main substitutes for calcium in biological apatite. Enamel, dentin and bone contain 0.44, 1.23 and 0.72 wt.% of Mg, respectively [5–7]. Magnesium indirectly influences mineral metabolism and directly influences or even controls the crystallization processes of mineral substance as well as the pattern of mineral formation [8]. It is reported that magnesium inhibited the crystallization of hydroxyapatite but increased the thermal conversion into β-TCMP (Mg-containing β-TCP) [9]. Apatite doped with carbonate and/or Mg ions can improve the behaviors of MSC and MG-63 cells in term of adhesion, proliferation and metabolic activation compared to stoichiometric HA [4]. HA Doped with 2 mol% of Mg^{2+} significantly enhances osteoblast adhesion as compared to pure HA [10]. The adverse effects of magnesium are also reported in the literature. Serre et al. [8] studied the influence of magnesium substitution on a collagen-apatite biomaterial and demonstrated that the apatite containing 4.5 mol% magnesium adversely affected the osteoinductive properties since too much magnesium intoxicated the apatite thus inhibited osteoblastic phenotype.

Magnesium-containing apatite coatings on metal substrate are expected for the future medical applications. However, the bioresponse of these coatings are not reported in literature. The present study employs the sol–gel dip-coating method to fabricate magnesium-containing apatite coatings to study the dissolution and biocompatibility behaviors of the coatings in physiological saline solution and the response of human osteosarcoma MG63 cells.

* Corresponding author. Tel.: +65 67904400; fax: +65 67911859.
E-mail address: msyzhang@ntu.edu.sg (S. Zhang).

0169-4332/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsusc.2008.06.106

2. Experimental

2.1. Deposition of the magnesium-doped apatite coatings

The processing of the dipping sols and deposition of the magnesium apatite coatings are detailed in our previous publications [11,12]. In short, Titanium alloy (Ti-6Al-4V) plates were used as substrates. Calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, Sigma–Aldrich, AR), phosphorus pentoxide (P_2O_5, Merck, GR) and magnesium nitrate hexahydrate ($Mg(NO_3)_2{\cdot}6H_2O$, Sigma–Aldrich, AR) were selected to prepare Ca-precursor, P-precursor and Mg-precursor, respectively. The degree of substitution of Ca^{2+} by Mg^{2+} in the mixture was indicated by the x value in the general formula of $(Ca_{10-x}Mg_x)(PO_4)_6(OH)_2$, where $x = 0$, 1/2, 2/2, 3/2 and 4/2. The subsequent coatings were labeled as HA, MA1, MA2, MA3, MA4, respectively. The phase characterization of the coatings was conducted by X-ray diffraction (XRD, PW1830). The magnesium concentration in the coating was determined by X-ray photoelectron spectroscopy (XPS, Kratos-Axis Ultra System) using monochromatic Al Kα X-ray source (1486.7 eV).

2.2. Dissolution test

The dissolution behavior of the coatings was investigated by soaking in a Tris-buffered physiological saline solution (0.9% NaCl, pH 7.4) at a constant temperature of 37 °C for fixed periods of time. At the end of each period, the samples were taken out and the concentration of Ca^{2+} and mg^{2+} in the solution were analyzed with Atomic Absorption spectrometer (AAnalyst 100, PerkinElmer). An average of five measurements was taken for each sample.

2.3. Cell culture

Human osteosarcoma MG63 cells (ATCC, Rockville, MD) were used to assay the osteoblastic cell response on the coating surface. In the standard incubation condition (5%CO_2, 37 °C), the cells were incubated in a standard culture medium containing Eagle's Minimum Essential Medium (EMEM, ATCC) supplemented with 10% fetal bovine serum (FBS, ATCC) and 1% penicillin/streptomycin (ATCC). Cells were seeded on the coating surface at a density of 4.4×10^4 cells/cm^2. In characterization of cell attachment, the cultured cells were detached from the coatings with trypsin/EDTA solution, and then the cell numbers were counted by hemocytometer (Becton Dickinson, Germany). Statistical analysis was carried out on cellular tests using one-way analysis of variance (ANOVA) at an average of 5 replicates. Differences were considered statistically significant at $p < 0.05$. For cell morphology observation, the osteoblast-like cells attached on the coatings were fixed with 2.5% glutaraldehyde for 1 h at room temperature followed by dehydration with a series of graded ethanol/water solutions (50∼100%). Then 0.5 ml hexamethyldisilazane was added to each well to preserve the original morphology of the cells. The samples were coated with gold (for conduction) before observation under a Scanning Electron Microscope (SEM, Leica S360).

3. Results and discussion

3.1. Magnesium Incorporation

The surface chemical compositions of the coatings are analyzed with XPS. Fig. 1 presents the XPS survey scan spectra showing that the coating surfaces comprise Ca, P, O, C and Mg. The Mg 2p and Mg 2s peaks become prominent when $x > 1.00$, and the intensity increases with increasing Mg. The Auger peaks of Mg KLL (at about 301 eV [13]) show clearly the increase of magnesium in the coating. The more magnesium in the sols, the stronger the intensities of Mg KLL peaks are observed. The Mg^{2+} concentrations indicated by the x value in the general formula of $(Ca_{10-x}Mg_x)(-)(PO_4)_6(OH)_2$ in the coatings are shown in Fig. 2. The dash line indicates the ideal situation where all Mg^{2+} in the coatings are completely incorporated in the apatite structure to replace Ca^{2+}. The result shows, however, this happens only when $x < 1.00$ (∼2.4 wt.%), the Mg concentrations in the coatings almost match that designed in the sol. As $x > 1.00$, the difference between that measured in the coating and that designed in the sol aggravates: Mg in the coating becomes more than that designed in the sols. The higher Mg concentrations detected on the coating surfaces when $x > 1.00$ shows that the apatite crystal structure can host Mg only up to $x = 1.00$(∼2.4 wt.%).

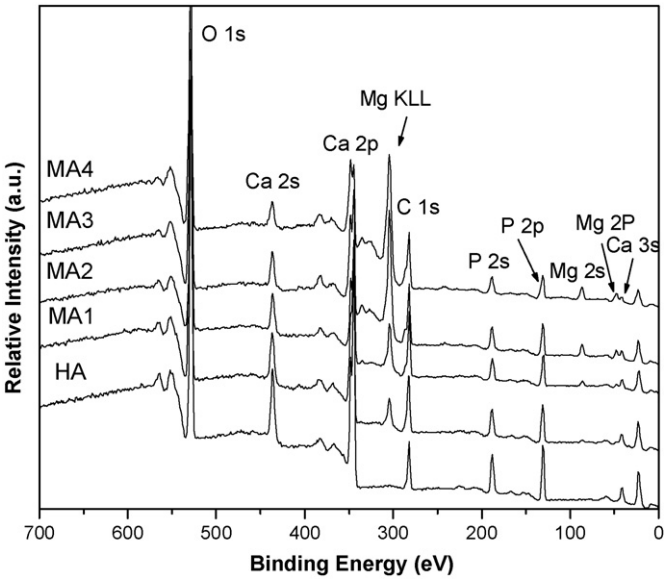

Fig. 1. XPS survey scan spectra of magnesium apatite coatings.

The differences of magnesium in the designed sols and the measured coatings are from the limitation of the magnesium

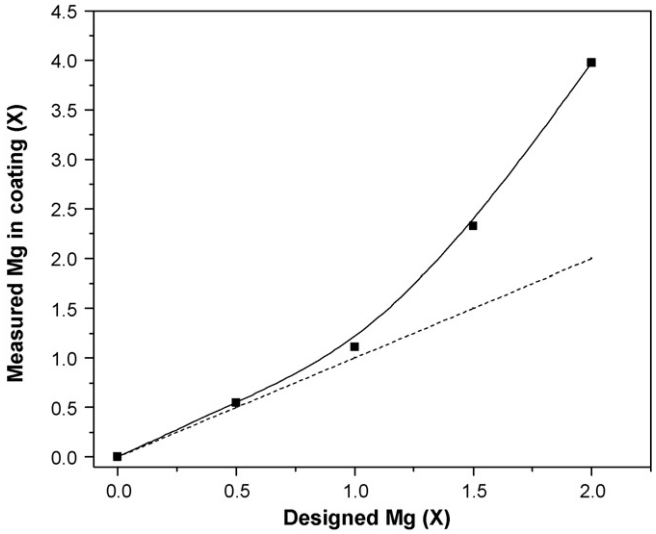

Fig. 2. Mg concentrations indicated by the x value in the general formula of $(Ca_{10-x}Mg_x)(PO_4)_6(OH)_2$ in the coatings: broken line: designed; solid line: measured Mg concentration in the coatings: broken line: designed; solid line: measured.