Journal of Colloid and Interface Science 482 (2016) 112–120

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

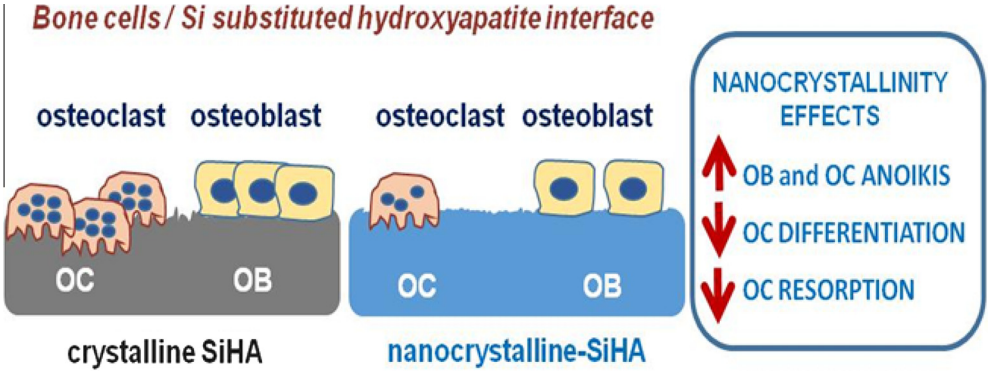
Regular Article

Nanocrystallinity effects on osteoblast and osteoclast response to silicon substituted hydroxyapatite

Laura Casarrubios [a,b], María Concepción Matesanz [a], Sandra Sánchez-Salcedo [c,d], Daniel Arcos [c,d], María Vallet-Regí [c,d], María Teresa Portolés [a,b,*]

[a] Department of Biochemistry and Molecular Biology I, Faculty of Chemistry, Universidad Complutense de Madrid, Spain
[b] Instituto de Investigación Sanitaria San Carlos IdISSC, Spain
[c] Department of Inorganic and Bioinorganic Chemistry, Faculty of Pharmacy, Universidad Complutense de Madrid, Instituto de Investigación Hospital 12 de Octubre i+12, Spain
[d] Networking Research Center on Bioengineering/Biomaterials and Nanomedicine, CIBER-BBN, Spain

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 June 2016
Revised 27 July 2016
Accepted 28 July 2016
Available online 29 July 2016

Keywords:
Nanocrystallinity
Hydroxyapatite
Silicon
Osteoclast
Osteoblast
Anoikis
Osteoporosis
Cell adhesion
Apoptosis
Cell cycle

ABSTRACT

Hypothesis: Silicon substituted hydroxyapatites (SiHA) are highly crystalline bioceramics treated at high temperatures (about 1200 °C) which have been approved for clinical use with spinal, orthopedic, periodontal, oral and craniomaxillofacial applications. The preparation of SiHA with lower temperature methods (about 700 °C) provides nanocrystalline SiHA (nano-SiHA) with enhanced bioreactivity due to higher surface area and smaller crystal size. The aim of this study has been to know the nanocrystallinity effects on the response of both osteoblasts and osteoclasts (the two main cell types involved in bone remodelling) to silicon substituted hydroxyapatite.
Experiments: Saos-2 osteoblasts and osteoclast-like cells (differentiated from RAW-264.7 macrophages) have been cultured on the surface of nano-SiHA and SiHA disks and different cell parameters have been evaluated: cell adhesion, proliferation, viability, intracellular content of reactive oxygen species, cell cycle phases, apoptosis, cell morphology, osteoclast-like cell differentiation and resorptive activity.
Findings: This comparative *in vitro* study evidences that nanocrystallinity of SiHA affects the cell/biomaterial interface inducing bone cell apoptosis by loss of cell anchorage (anoikis), delaying osteoclast-like cell differentiation and decreasing the resorptive activity of this cell type. These results suggest the potential use of nano-SiHA biomaterial for preventing bone resorption in treatment of osteoporotic bone.

* Corresponding author.
E-mail addresses: arualanilom@hotmail.com (L. Casarrubios), conchitamatesanz@hotmail.com (M.C. Matesanz), sansanch@ucm.es (S. Sánchez-Salcedo), arcosd@ucm.es (D. Arcos), vallet@ucm.es (M. Vallet-Regí), portoles@quim.ucm.es (M.T. Portolés).

http://dx.doi.org/10.1016/j.jcis.2016.07.075
0021-9797/

1. Introduction

Bone is a metabolically active and very dynamic tissue in continuous resorption and formation by osteoclasts and osteoblasts respectively, working together via paracrine cell signaling in basic multicellular units [1]. Osteoblasts are mononucleated cells which differentiate from mesenchymal stem cells of the bone marrow stroma and are responsible for deposition of bone matrix and regulation of osteoclasts [2,3]. Osteoclasts are multinucleated giant cells which differentiate from hematopoietic stem cells (that give rise to monocytes and macrophages) and they perform the bone resorption [4]. Osteoclasts attach to the bone surface and initiate resorption by the secretion of hydrogen ions and lysosomal enzymes which degrade all the components of bone matrix producing irregular cavities on the bone surface [5,6]. The balance between bone resorption by osteoclasts and bone formation by osteoblasts is influenced by mechanical, genetic, vascular, nutritional, hormonal and local factors. This bone remodelling is necessary to maintain the structural skeleton integrity and mineral homeostasis. Alterations of this process are involved in the pathogenesis of various skeletal diseases, including osteoporosis [7,8].

Silicon (Si) is an essential element for bone and cartilage formation [9–11] and is present in the areas of greatest osteoblastic activity during bone growth [12]. This element is essential to the normal development of the glycosaminoglycan network in the extracellular matrix [9], increasing bone collagen content [13]. Si also appears to inhibit macrophage and osteoclast activity [14]. Bioactive silicate materials upregulate the expression of vascular endothelial growth factor (VEGF) [15], which is involved in both blood vessel and bone formation [16]. Small levels of ionic substitution by Si in hydroxyapatite (HA) have been shown to have significant effects on thermal stability, solubility, osteoclastic and osteoblastic response both *in vitro* and *in vivo* [17]. Thus, silicon substituted hydroxyapatite (SiHA) presents enhanced bioactivity *in vivo* than HA, showing beneficial effects in the early stages of bone formation [18]. The favourable effects of Si substitution in HA have been explained by considering passive and active mechanisms as material solubility increase, topographical changes, grain size reduction, surface charge modifications and ionic release of Si and Ca, which directly act on bone cells [19–23]. All these facts make SiHA very attractive for use as bone substitute material [24–27] and SiHA has recently been incorporated to the biomaterials market as Actifuse ABXTM (Apatech Ltd, UK) for spinal, orthopedic, periodontal, oral and craniomaxillofacial applications. SiHA approved for clinical use are highly crystalline bioceramics treated at high temperatures (about 1200 °C). However, their preparation with lower temperature methods has been suggested to enhance the bioreactivity of these bioceramics [28–30]. Avoiding the high temperature sintering process, nanocrystalline pieces and grains can be prepared with higher surface area and smaller crystal size. These characteristics could provide very interesting bioresponses in SiHA since the osteogenic effect of silicon is mainly explained by its location at the crystal boundaries [24,25].

The novelty of the present study is the comparison of the action of nanocrystalline and crystalline silicon substituted hydroxyapatites (nano-SiHA and SiHA respectively) on both osteoblasts and osteoclasts, the two main cell types involved in bone remodelling. In this comparative *in vitro* study, Saos-2 osteoblasts and osteoclast-like cells (differentiated from RAW-264.7 macrophages) have been cultured on the surface of nano-SiHA and SiHA disks and different cell parameters have been evaluated: cell adhesion, proliferation, viability, intracellular content of reactive oxygen species (ROS), cell cycle phases, apoptosis, cell morphology, osteoclast-like cell differentiation and resorptive activity.

2. Materials and methods

2.1. Synthesis of materials

Silicon-substituted hydroxyapatite (Si-HA) with nominal formula $Ca_{10}(PO_4)_{5.75}(SiO_4)_{0.25}(OH)_{1.75}\square_{0.25}$, where □ means vacancies at the hydroxyl position, was prepared by aqueous precipitation reaction of $Ca(NO_3)_2{\cdot}4H_2O$, $(NH_4)_2HPO_4$ and $Si(CH_3CH_2O)_4$ solutions. Briefly, a 1 M solution of $Ca(NO_3)_2{\cdot}4H_2O$ was added to a second 0.575 M of $(NH_4)_2HPO_4$ and 0.025 M of Si $(CH_3CH_2O)_4$ solution to obtain the composition described above. The mixture was stirred for 12 h at 80 °C. The pH was kept at 9.5 by NH_3 solution addition to ensure constant conditions during the synthesis. The precipitated Si-HA powder was dried, milled and sieved and the powder fraction below 40 μm was selected. Fractions of 300 mg of powder were pressed into disk-shape (11 mm diameter, 2 mm height) by means of 3 tons of uniaxial pressing. Subsequently the disks were treated during 3 h at 700 °C or 1150 °C resulting in nano-SiHA or SiHA, respectively.

2.2. Characterization of materials

The structural characterization was performed by Powder X-ray diffraction (XRD) in a Philips X'Pert diffractometer equipped with a Cu Kα radiation (wavelength 1.5406 Å), with a step size of 0.02° 2θ and 8 s of counting time. In order to determine the crystalline and microstructural characteristics of both samples, Rietveld refinements were carried out over the XRD patterns collected. The refinements were performed using the atomic position set and the space group of the HA structure *P*63/*m*, No. 176 by means of the FullProf 2000 computer program. The instrumental resolution function (IRF) of the diffractometer was obtained from a very-well-crystallized LaB_6 sample and taken into account in a separate input file. The pseudo-Voigt profile function of Thompson, Cox, and Hastings was used with an asymmetry correction at a low angle.

The contact angles were measured to estimate the wettability of the samples. The experiments were performed by the sessile drop method at 25 °C on a CAM 200 KSV contact angle goniometer. Pictures of the drops were taken every 1 s. The software delivered by the instrument manufacturer calculated the contact angles on the basis of a numerical solution of the full Young–Laplace equation.

Textural properties (surface and porosity) were determined by nitrogen adsorption porosimetry in a Micromeritics ASAP 2012. To perform the N_2 adsorption measurements, the samples were previously degassed under vacuum for 24 h at 80 °C. Finally, zeta potential was measured by means of a Zetasizer Nano ZS (Malvern Instruments).

2.3. Culture of osteoblasts in contact with nano-SiHA and SiHA

Human Saos-2 osteoblasts (10^5 cells/ml) were seeded on the surface of nano-SiHA and SiHA disks, previously introduced into 24 well culture (CULTEK S.L.U., Madrid, Spain), in Dulbecco's Modified Eaglés Medium (DMEM, Sigma Chemical Company, St. Louis, MO, USA) supplemented with 10% (vol/vol) fetal bovine serum (FBS, Gibco, BRL), 1 mM L-glutamine (BioWhittaker Europe, Belgium), penicillin (200 μg/ml, BioWhittaker Europe, Belgium), and streptomycin (200 μg/ml, BioWhittaker Europe, Belgium), under a 5% CO_2 atmosphere and at 37 °C for 24 h. Then, the medium was aspirated, cells were washed with PBS and harvested using 0.25% trypsin-EDTA solution. For the analysis of cell proliferation, the cell number was calculated with a Neubauer hemocytometer using 10 μl of each cell suspension. Then, cell suspensions were centrifuged at 310*g* for 10 min and resuspended