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Biological response of human bone cells to zinc-modified Ca–Si-based ceramics

Yogambha Ramaswamy [a], Chengtie Wu [a], Hong Zhou [b], Hala Zreiqat [a,*]

[a] Biomaterials and Tissue Engineering Research Unit, Biomedical Engineering, School of AMME, The University of Sydney, Sydney, NSW, Australia
[b] Bone Biology Program, ANZAC Research Centre, Sydney, NSW, Australia

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Abstract

Calcium silicate ($CaSiO_3$) ceramics have received considerable attention in recent years due to their excellent bioactivity and degradability. However, their poor chemical stability limits their biological applications. Hardystonite ($Ca_2ZnSi_2O_7$) ceramics are Ca–Si-based materials developed by incorporating zinc into the Ca–Si system to improve their chemical stability. However, the biological responses of $Ca_2ZnSi_2O_7$ to bone cells are unknown. The objective of this study is to investigate and compare the in vitro responses of human osteoblast-like cells (HOBs) and osteoclasts when cultured on $Ca_2ZnSi_2O_7$ and $CaSiO_3$ ceramic disks. The ability of $Ca_2ZnSi_2O_7$ ceramics to support HOB attachment, cytoskeleton organization, proliferation and differentiation was assessed by scanning electron microscopy, confocal microscopy, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, alkaline phosphatase activity and quantitative real-time polymerase chain reaction. Our results show that $Ca_2ZnSi_2O_7$ supported HOB attachment with a well-organized cytoskeleton structure, and significantly increased cellular proliferation and differentiation compared to $CaSiO_3$. In addition, $Ca_2ZnSi_2O_7$ showed increased expression levels of osteoblast-related mRNAs (alkaline phosphatase, collagen type I, osteocalcin, receptor activator of $NF_\kappa B$ ligand and osteoprotegerin) compared to $CaSiO_3$. $Ca_2ZnSi_2O_7$ ceramic supported the formation of mature and functional osteoclasts and formed resorption imprints. On $CaSiO_3$ ceramics, the cells failed to differentiate from the monocytes into osteoclasts. Taken together, these results indicate that Hardystonite ceramics are conducive to both types of bone cells, osteoblast-like cells and osteoclasts, suggesting their potential use for skeletal tissue regeneration and as coatings onto currently available orthopedic and dental implants.

Keywords: Hardystonite; Human osteoclasts; Human osteoblasts; Gene expression; Resorption

1. Introduction

A range of bioactive ceramics, such as hydroxyapatite (HAp), tricalcium phosphate and bioglass®, have been employed to treat bone defects because of their excellent bone bonding ability; however, their poor mechanical properties limit their biological application. In recent years, calcium silicate-based ceramics have shown promise as bone implant materials, opening new avenues in the biomaterials field. $CaSiO_3$ is a typical group of Ca–Si-based bioceramics, regarded as potential bioactive material for bone tissue regeneration and implant coating due to their excellent bioactivity and degradability [1–4]. However, a major drawback of the $CaSiO_3$ ceramics is their high dissolution rate, leading to a high pH value in the surrounding environment, which is detrimental to cells [5–7]. Indeed, the quick dissolution rate of the $CaSiO_3$ coatings onto titanium substrate degrades fast, which limits further biological applications [8].

Recently, zinc was incorporated into the Ca–Si system, forming a material referred to as Hardystonite ($Ca_2ZnSi_2O_7$), which possesses improved mechanical properties (increased bending strength and fracture toughness) compared to HAp [9].

* Corresponding author. Tel.: +61 2 93512392; fax: +61 2 93517060.
E-mail address: hzreiqat@usyd.edu.au (H. Zreiqat).

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We hypothesize that Zn-modified calcium silicate ($Ca_2ZnSi_2O_7$) ceramics are chemically stable (low dissolution) and can improve the biological response of bone cells. Ideally, implant surfaces should be conducive to cell interactions that balance osteoblastic and osteoclastic activity. The coordinated activity of osteoblasts and osteoclasts (OCs) is critical for normal bone remodelling. Osteoblasts are responsible for the deposition of bone extracellular matrix and regulate the differentiation and activity of bone resorbing OCs, thereby maintaining the skeletal architecture [10,11]. It is therefore important to determine how different biomaterials and their modification may mediate the expression of bone-related genes and their proteins, together with some of the factors that regulate bone lysis.

The aim of the present study is to determine the biological activity of primary human osteoblast-like cells (HOBs) and human OCs when cultured on $Ca_2ZnSi_2O_7$ and $CaSiO_3$ ceramics.

2. Materials and methods

2.1. Material preparation

$Ca_2ZnSi_2O_7$ ceramics were prepared according to the method described previously [9]. Briefly, $Ca_2ZnSi_2O_7$ powders were synthesized by the sol–gel method using zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$; Sigma–Aldrich, USA), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$; Sigma–Aldrich) and tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS; Sigma–Aldrich) as raw materials. For the preparation of ceramic disks, the calcined $Ca_2ZnSi_2O_7$ powders were sieved to 230 meshes and then were mixed with 6 wt.% polyvinyl alcohol (PVA; Sigma–Aldrich) water solution binders (weight ratio: PVA solution/powders = 1:9). The mixture was uniaxially pressed at 200 MPa to get $Ca_2ZnSi_2O_7$ green disks with a dimension of Ø15 × 2 mm. Subsequently, the green disks were sintered at 1280 °C for 3 h, with a heating rate of 2 °C min^{-1}, to obtain ceramic disks with a weight of 0.45 g. $CaSiO_3$ ceramics were prepared using the same method and were used as controls for cell cultures.

2.2. Isolation and culture of primary HOBs

HOBs were grown from outgrowths of normal human trabecular bone as previously described [12]. Briefly, bone was broken up into 1 mm^3 pieces, washed several times in phosphate-buffered saline (PBS) and digested for 90 min at 37 °C with 0.02% (w/v) trypsin (Sigma–Aldrich) in PBS. Digested cells were cultured in osteogenic medium containing α-minimal essential medium (α-MEM; Gibco Laboratories, USA), supplemented with 10 vol.% fetal calf serum (Gibco Laboratories), 2 mM L-glutamine (Gibco Laboratories), 25 mM HEPES buffer (Gibco Laboratories), 2 mM sodium pyruvate, 30 mg ml^{-1} penicillin, 100 mg ml^{-1} streptomycin (Gibco Laboratories) and 0.1 M L-ascorbic acid phosphate magnesium salt (Wako Pure Chemicals, Osaka, Japan). The confluent cells were trypsinized and used for the experiments of the attachment, viability, differentiation and gene regulation of HOB-specific markers, as described below. Permission to use discarded human tissue was granted by the Human Ethics Committee of the University of Sydney.

2.3. Inductively coupled plasma atomic emission spectroscopy (ICPAES) test

The ionic concentrations of Ca, Si, and Zn in the culture medium were measured using ICPAES analysis at 3 and 7 days of culturing HOBs on the test materials and tissue culture plastic without any ceramics.

2.4. Cytotoxicity test

$Ca_2ZnSi_2O_7$ powder extract was mixed in full culture medium according to International Standard Organization (ISO/EN 10993-5). The dissolution extracts of ceramics were prepared by adding $Ca_2ZnSi_2O_7$ powders to serum-free α-MEM culture medium at a ratio of 200 mg ml^{-1} (powder to medium). After incubation at 37 °C for 24 h the mixture was centrifuged and the supernatant was collected. Serial dilutions of extracts (100, 50, 25, 12.5, and 6.25 mg ml^{-1}) were prepared using serum-free α-MEM medium (without L-glutamine or ascorbic acid). Subsequently, the diluted extracts were sterilized by filtration (0.22 μm) and used in cell culture experiments. HOBs were seeded at a density of 3×10^3 cells cm^{-2} into 96-well plate and incubated for 24 h. The culture medium was then removed and replaced by 50 μl of α-MEM medium supplemented with 20% FCS and 50 μl of appropriate concentration of extracts. The culture medium supplemented with 10% FCS without addition of diluted extracts was used as a blank control (Blank), and a solution of 50 μl of 0.2% Triton X-100 and 50 μl of α-MEM medium supplemented with 20% FCS was used as a negative control (Ctr-). Cells were then incubated at 37 °C in 5% CO_2 for 1, 3, and 7 days. Viable cell numbers were evaluated at appropriate time intervals (1, 3, and 7 days) using the MTS assay (Promega, Madison, WI) according to the manufacturer's instructions. The MTS (tetrazolium) compound is a soluble version of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. At the predetermined time point, 100 μl of 0.5 mg ml^{-1} MTS solution was added to each well and was incubated for 4 h at 37 °C to take up the dye. The absorbance of the formazan was read at 490 nm using an ELISA plate reader and software Accent\MTS.

2.5. Attachment of HOBs

HOBs were cultured on $Ca_2ZnSi_2O_7$ and $CaSiO_3$ disks placed in individual wells of a 24-well culture plate at an initial concentration of 1.5×10^4 cells cm^{-2} and incubated for 24 h in α-MEM culture medium supplemented with

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