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The stimulation of proliferation and differentiation of periodontal ligament cells by the ionic products from $Ca_7Si_2P_2O_{16}$ bioceramics

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ABSTRACT

The ultimate goal of periodontal tissue engineering is to produce predictable regeneration of alveolar bone, root cementum, and periodontal ligament, which are lost as a result of periodontal diseases. To achieve this goal, it is of great importance to develop novel bioactive materials which could stimulate the proliferation, differentiation and osteogenic/cementogenic gene expression of periodontal ligament cells (PDLCs) for periodontal regeneration. In this study, we synthesized novel $Ca_7Si_2P_2O_{16}$ ceramic powders for the first time by the sol–gel method and investigated the biological performance of PDLCs after exposure to different concentrations of $Ca_7Si_2P_2O_{16}$ extracts. The original extracts were prepared at 200 mg ml^{-1} and further diluted with serum-free cell culture medium to obtain a series of diluted extracts (100, 50, 25, 12.5 and 6.25 mg ml^{-1}). Proliferation, alkaline phosphatase (ALP) activity, Ca deposition, and osteogenesis/cementogenesis-related gene expression (ALP, Col I, Runx2 and CEMP1) were assayed for PDLCs on days 7 and 14. The results showed that the ionic products from $Ca_7Si_2P_2O_{16}$ powders significantly stimulated the proliferation, ALP activity, Ca deposition and osteogenesis/cementogenesis-related gene expression of PDLCs. In addition, it was found that $Ca_7Si_2P_2O_{16}$ powders had excellent apatite-mineralization ability in simulated body fluids. This study demonstrated that $Ca_7Si_2P_2O_{16}$ powders with such a specific composition possess the ability to stimulate the PDLC proliferation and osteoblast/cemenoblast-like cell differentiation, indicating that they are a promising bioactive material for periodontal tissue regeneration application.

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1. Introduction

Periodontitis is an inflammatory disease that causes progressive destruction of the tooth-supporting tissues, including alveolar bone, root cementum, and periodontal ligament [1]. Currently, the reconstitution of lost periodontal structures still remains a significant challenge. Following traditional manual or mechanical disinfection procedures, various treatment options for periodontitis have been developed, such as root surface conditioning [2,3], bone grafts [4,5], guided tissue regeneration (GTR) [6,7], application of enamel matrix derivative [8,9] or growth factors [10,11]. Although some acquired favorable results in animal studies or clinical trials, only a few of these approaches may be regarded as true regenerative techniques, as the outcomes are limited [12], with only a little new connective tissue attachment and cementogenesis being achieved. Recently, bioactive materials have been shown to be an attractive alternative with great potential for periodontal structure regeneration by stimulating responsive cell infiltration, promoting cell differentiation and new bone formation [13–15]. In addition, being the key cell type for periodontal tissue regeneration, periodontal ligament cells (PDLCs) have been demonstrated to be capable of differentiating into osteoblasts and cementoblasts in vitro [16] due to their unique localization and mesenchymal stem-cell-like properties with multilineage differentiation capacity [17]. However, sources of autologous PDLCs are limited, especially for patients with advanced periodontitis, thus impeding the clinical application of the cell-based therapy. Therefore, it is of great interest to develop novel bioactive materials which possess the ability to stimulate the proliferation, differentiation and osteogenic/cementogenic gene expression of PDLCs for better periodontal regeneration.

Conventional calcium-phosphate (CaP)-based hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP) ceramics have been used for periodontal bone regenerations due to their similar

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mineral phase composition with human bone and their excellent osteoconductivity. However, these bioactive materials have some limitations. Sintered stoichiometric HAp ceramics have a limited ability to form interface tissues, and to stimulate the development of new bone tissue [18,19]. Also, the sintered HAp does not degrade significantly but rather remains as a permanent fixture susceptible to long-term failure [20]. Although β-TCP ceramics are degradable, with a quicker degradation than HAp, the in vivo osteogenesis of sintered β-TCP ceramics is far from satisfactory [21–23]. In addition, it has not been found that CaP-based ceramics possess the ability to stimulate the osteogenic/cementogenic differentiation of PDLCs.

Silicon (Si) has been found to be one of the important trace elements in the human body [18]. Si is located at active calcification sites in the bones and is directly involved in the mineralization process of bone growth [24]. Due to the significant function of Si in human bone, Si has been widely incorporated into biomaterials in order to enhance their bioactivity [25–30]. In the past several years, it has been found that the silicate-based bioactive glasses and bioceramics have a great osteostimulatory effect on a series of stem cells, including bone marrow stromal cells, human dental pulp cells, and adipose-derived stem cells [31–40]. Recently, Varanasi et al. reported that 45S5 bioactive glasses have shown the ability to enhance the early mineralization of PDLCs [41]. Therefore, it is speculated that the combination of CaP-based composition (for mimicking the mineral composition of bone) and silicate-based composition (for osteogenic stimulation) will be of great interest in preparing novel bioceramics for better periodontal tissue regeneration. $Ca_7Si_2P_2O_{16}$ is a Ca-, Si- and P-containing mineral with a single-phase crystal structure (unlike Si-doped HAp and TCP, for example). Due to its specific composition and crystal structure, we speculated that $Ca_7Si_2P_2O_{16}$ ceramics have particular effects on the biological response of PDLCs. To our knowledge, there is no report about the preparation of $Ca_7Si_2P_2O_{16}$ materials and the investigation of their effects on the proliferation and osteogenic/cementogenic differentiation of PDLCs. Therefore, the aim of this study is to synthesize $Ca_7Si_2P_2O_{16}$ powders and investigate their in vitro apatite-mineralization ability, and the effect on PDLC proliferation and osteogenic/cementogenic differentiation for potential application of periodontal tissue regeneration.

2. Materials and methods

2.1. Synthesis and characterization of $Ca_7Si_2P_2O_{16}$ powders

$Ca_7Si_2P_2O_{16}$ powders were synthesized by the sol–gel process using tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS), triethyl phosphate ($(C_2H_5O)_3PO$, TEP) and calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) (Sigma-Aldrich, Castle Hill, Australia). Briefly, the TEOS was mixed with water and 2 M HNO_3 and ethanol and hydrolyzed for 30 min under stirring. Then, TEP and $Ca(NO_3)_2{\cdot}4H_2O$ were added to the mixture (mol ratio: TEOS/TEOS/$Ca(NO_3)_2{\cdot}4H_2O$ = 2:2:7), and reactants were stirred for 5 h at room temperature. After the reaction, the solution was maintained at 60 °C for 1 day and dried at 120 °C for 2 days to obtain the dry gel. The dry gel was ground and sieved to 250-mesh, transferred into a corundum crucible and calcined at 1450 °C for 3 h. The calcined powders were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS).

2.2. Apatite mineralization of $Ca_7Si_2P_2O_{16}$ powders

To investigate apatite mineralization of $Ca_7Si_2P_2O_{16}$ powders, simulated body fluids (SBFs) were prepared according to Kokubo [42]. $Ca_7Si_2P_2O_{16}$ powders were soaked in SBF at 37 °C for 1, 3 and 7 days, and the ratio of powder mass to SBF volume was 1.5 mg ml^{-1}. After soaking, the apatite mineralization on the surface of $Ca_7Si_2P_2O_{16}$ powders was determined by SEM, XRD, EDS and Fourier transform infrared spectroscopy (FTIR).

2.3. Preparation of the dissolution extracts of $Ca_7Si_2P_2O_{16}$ powders

The dissolution extracts of $Ca_7Si_2P_2O_{16}$ were prepared by soaking $Ca_7Si_2P_2O_{16}$ powders to low glucose Dulbecco's Modified Eagle Medium (DMEM; Invitrogen Pty Ltd., Australia) at the concentration of 200 mg ml^{-1} according to International Standard Organization (ISO/EN) 10993–5 [43]. 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

Table 2
Primer sequences for the gene observed in this study.

Gene name	Forward sequences	Reverse sequences
ALP	5′TCAGAAGCTCAACACCAACG	5′TTGTACGTCTTGGAGAGGGC
COL1	5′CTTTGGAGCCAGCTGGA	5′GTGGGCTTCCTGGTGA
RUNX2	5′GCCTTCAAGGTGGTAGCCC	5′CGTTACCCGCCATGACAGTA
CEMP1	5′GGGCACATCAAGCACTGACAG	5′CCCTTAGGAAGTGGCTGTCCAG
18s	5′TTCGGAACTGAGGCCATGAT	5′CGAACCTCCGACTTCGTTC

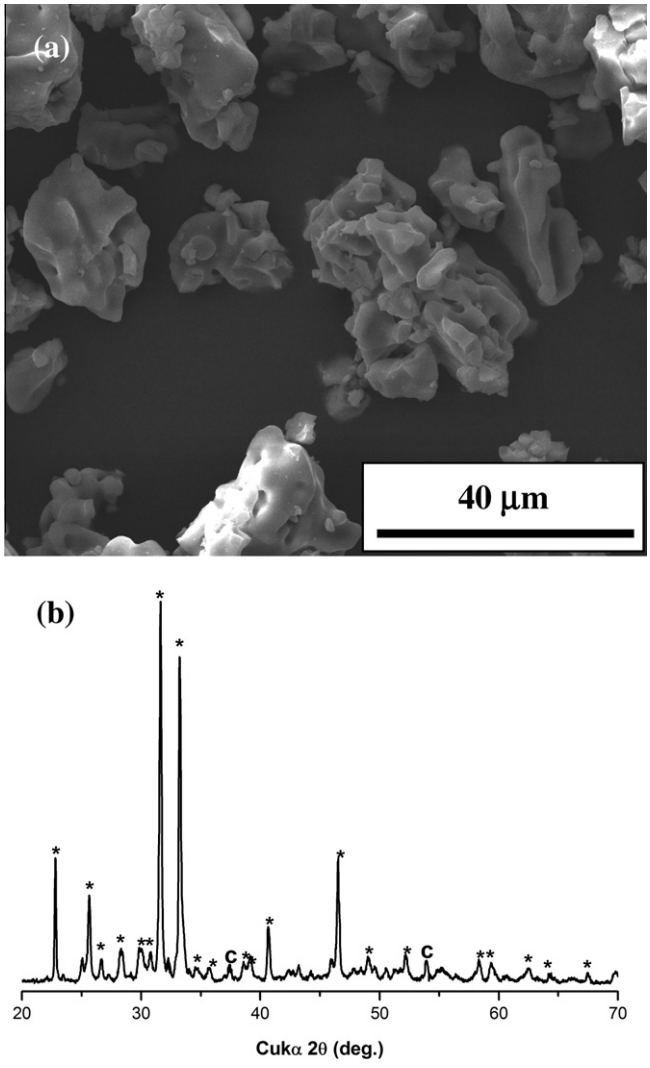

Fig. 1. SEM (a) and XRD (b) analysis for the synthesized $Ca_7Si_2P_2O_{16}$ powders. (*: $Ca_7Si_2P_2O_{16}$ phase, c: CaO).

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