Journal of the Mechanical Behavior of Biomedical Materials 86 (2018) 215–223

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

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

Tailoring the mechanical property and cell-biological response of β-tricalcium phosphate composite bioceramics by $SrO-P_2O_5-Na_2O$ based additive

Fupo He[a,*,1], Teliang Lu[b,1], Ye Tian[a], Xue Li[c], Fei Zuo[a], Xuetao Shi[b], Jiandong Ye[b,*]

[a] School of Electromechanical Engineering, Guangdong University of Technology, Guangzhou 510006, People's Republic of China
[b] School of Materials Science and Engineering, South China University of Technology, Guangzhou 510641, People's Republic of China
[c] 8th Department of Orthopaedics, Foshan Hospital of Traditional Chinese Medicine, Guangzhou University of Chinese Medicine, Foshan 528000, People's Republic of China

ARTICLE INFO

Keywords:
Calcium phosphate
Compressive strength
Osteoclastic activity
Osteogenic differentiation
Bone repair

ABSTRACT

β-tricalcium phosphate (β-TCP) bioceramic, which is a prevalent bone graft, is deficient in mechanical strength and mediating the biological functions. In the present study, β-tricalcium phosphate composite bioceramics (TCP/SPNs) were prepared by introducing $SrO-P_2O_5-Na_2O$ based (SPN) sintering additive. With increasing mole ratio of SrO to P_2O_5, the SPN tended to crystallize. In the liquid-phase sintering process, β-TCP reacted with SPN, producing new compounds. The difference in characteristic of SPN additive affected the compressive strength and cell-biological response of the fabricated TCP/SPNs. By selecting SPN with appropriate formulation, the TCP/SPNs not only could more than double their compressive strength, but also improved the cell viability, promoted osteogenic differentiation and inhibited osteoclastic activities. Taken together, this work establishes a beneficial strategy to improve the overall performance of calcium phosphate bioceramic for application in bone regeneration.

1. Introduction

β-tricalcium phosphate (β-TCP) bioceramics are well-documented bone repair materials, owing to their biodegradability, excellent biocompatibility and osteoconductivity, and chemical similarity to bone mineral (Dorozhkin, 2010). However, the β-TCP is short of the capacity of mediating biological responses (stimulating osteogenesis, inhibiting osteoclastic activities, and promoting vascularization, etc.), which are crucial for accelerating regeneration of bone defect with large size or in the osteoporosis pathological condition (Wang et al., 2012, 2018; Kim et al., 2017). Moreover, β-TCP bioceramics are encountered with the drawback of poor mechanical strength (Ryu et al., 2002; Champion, 2013).

It has been well established that the addition of trace elements (Zn, Si, Sr, Mg, etc.) can influence the biological response of β-TCP (Roy and Bose, 2012; Parra et al., 2017; Yamada et al., 2008). Of the trace elements, Sr (strontium) draws a great deal of attention recently, because it is able to promote bone formation by osteoblasts and inhibit bone resorption by osteoclasts (Saidak and Marie, 2012). Osteoporosis, characterized by loss of bone density, is caused by imbalance between bone formation by osteoblastic activity and bone resorption by osteoclastic activity (Teitelbaum, 2000). Studies have proved that the biomaterials containing Sr promoted osteoblastic differentiation, inhibited osteoclastic activity, and boosted bone regeneration of bone defect in the normal and osteoporotic animals (Luo et al., 2015; Kuang et al., 2015; Lin et al., 2013; Schumacher and Gelinsky, 2015; Zhang et al., 2016).

The poor mechanical strength of β-TCP bioceramics is mainly caused by the poor sinterability of β-TCP (Ryu et al., 2002; Champion, 2013). β-TCP transforms to α-TCP at the temperatures ranging from 1120 to 1170 °C (Itatani et al., 1994). The density of α-TCP (2.86 g/cm^3) is lower than that of β-TCP (3.07 g/cm^3). The β-TCP samples sintered above the transformation temperature will be subjected to volume expansion, which prevents the TCP ceramics from densification (Ryu et al., 2002; Itatani et al., 1994). The temperatures of β → α-TCP transformation can be elevated by introducing calcium pyrophosphate and substituting some metal ions (K^+, Na^+, Mg^{2+}, Zn^{2+} and Sr^{2+}, etc.) for Ca^{2+} in the β-TCP (Banerjee et al., 2010; Bandyopadhyay et al., 2012; Torres et al., 2016; Yoshida et al., 2006). This enables β-TCP to be sintered at higher temperatures without phase transformation, thus

* Corresponding authors.
E-mail addresses: fphe@gdut.edu.cn (F. He), jdye@scut.edu.cn (J. Ye).
[1] The first two authors contribute equally to this paper.

https://doi.org/10.1016/j.jmbbm.2018.06.036
Received 22 May 2018; Received in revised form 25 June 2018; Accepted 25 June 2018
Available online 28 June 2018
1751-6161/

promoting densification. Nevertheless, the improvement in mechanical strength by stabilizing β-TCP has not been satisfactory (Champion, 2013; Banerjee et al., 2010; Bandyopadhyay et al., 2012).

Phosphate-based glass (PG) based on MeO-P_2O_5-Na_2O system (Me: Mg, Ca, Zn and/or Sr, etc.) is known for hydrolytic degradability and low melting temperature (Ahmed et al., 2004). The low melting temperature of PG makes it an effective sintering agent. PG as a sintering additive can help to achieve liquid-phase sintering for bioorganic materials (calcium phosphate, calcium carbonate, etc.), thereby promoting densification and increasing mechanical strength of the bioceramics (He et al., 2015, 2017; Bellucci et al., 2016; Tian et al., 2018; Yang et al., 2016). However, with rapid degradation rate, the PG and bioceramic with PG additive may release excessive ions, leading to toxicity (Tian et al., 2018; Abou Neel et al., 2009). Abou Neel et al. (2009) found that 5SrO-30CaO-15Na_2O-50P_2O_5 based PG was cytotoxic, owing to the fast release of massive ions. Tian et al. (2018) prepared the β-TCP composite bioceramics using 20 wt% 32SrO-45P_2O_5–23Na_2O based PG as sintering additive; they found that the bioceramics had noticeably compromised cell activity, also due to abrupt release of ions from the bioceramics. What is more, the abnormal grain growth and the presence of residual glass phase may restrict the improvement of mechanical strength for bioceramics (Tian et al., 2018). To some extent, the characteristics (structure, dissolution rate, crystallization, etc.) of MeO-P_2O_5-Na_2O based material can be modulated by adjusting the content of P_2O_5 and metal oxides (Ahmed et al., 2004, 2010).

Therefore, the aim of this study was to tailor the mechanical strength and cell-biological response of β-TCP composite bioceramics (TCP/SPNs) by introducing various SrO-P_2O_5-Na_2O based (SPN) sintering additives. The phase composition, mechanical strength, microstructure and cell behaviors (cell viability, osteogenic differentiation and osteoclastic activities) of the TCP/SPNs were comprehensively investigated.

2. Materials and methods

2.1. Fabrication of TCP/SPNs

β-TCP powders (d_{50} = 3.0 μm) were synthesized by a solid-phase reaction between $CaHPO_4$ and $CaCO_3$ (He et al., 2017). SPN powders were prepared by a melt-quenching method using $SrCO_3$, P_2O_5 and Na_2CO_3 as staring materials. The starting materials with determined formulations were placed into an alumina crucible, and molten after being heated in a furnace (XS3-4-1400, Shenzhen Zhongdaqiang Electric Furnace Factory, China) at 1200 °C for 2 h. The resulting liquid was poured into the deionized water, and the resulting pellets were collected. The pellets were grinded with a planetary ball mill (QM-2SP20, Nanjing University Instrument Factory, China) for 10 h, and then air-dried at 60 °C for 24 h. The SPN powders with median diameter around 5.4 μm were obtained. Three different SPN (SPN1, SPN2 and SPN3) powders were synthesized, and their formulations are listed in Table 1.

7.5% (w/w) paraffin solution was prepared by dissolving paraffin in the heptane. The mixtures of β-TCP and SPN powders were mixed with paraffin solution, then air-dried at 60 °C for 24 h to remove the heptane. The paraffin was used as binder, and its mass fraction in the dried mixtures of β-TCP, SPN and paraffin was about 2%. The mass fraction of SPN in the mixtures of β-TCP and SPN was fixed at 20%. The dried mixtures were placed into a mold, then uniaxially pre-pressed employing a powder pressing machine (FLS, Taizhou Rongmei, China) under a pressure of 10 MPa. Afterwards, the obtained pellets (Φ50 mm × 8 mm) were isostatically pressed with a cold isostatic pressing machine (LDJ100/320-300, Western Sichuan Machinery Co., Ltd, China) under a pressure of 200 MPa for 2 min. The green bodies were heated in air with a heating speed of 1 °C min^{-1}, and then dwelled at 420 °C for 1 h to remove the paraffin. Subsequently, the samples were sintered at 1100 °C for 2 h, then cooled down to room temperature in the closed furnace. The TCP/SPNs were obtained. The β-TCP bioceramic without SPN additive was designated as TCP, and the TCP/SPNs with the addition of SPN1, SPN2, and SPN3 were named as TCP/SPN1, TCP/SPN2, and TCP/SPN3, respectively.

Table 1
Formulations of SPN (SPN1, SPN2 and SPN3) used in this study.

Sample name	SrO (mol%)	P_2O_5 (mol%)	Na_2O (mol%)
SPN1	32	45	23
SPN2	37	40	23
SPN3	42	35	23

2.2. Materials characterization

2.2.1. Phase analysis and microstructure observation

The phase of samples was examined with an X-ray diffractometer (X'Pert PRO, PANalytical Co., Netherlands) employing Cu-Kα X-ray source (40 mA, 40 kV). Data were collected from 10° to 60° for 2θ with a step size of 0.0166°. Microstructure of the bioceramic samples was observed using an environment scanning electron microscope (SEM; Q25, FEI, USA) with an accelerating voltage of 10 kV. The polished bioceramic samples were thermally etched at 950 °C for 30 min. The bioceramic samples were sputter-coated with gold before SEM observation.

2.2.2. Measurement of porosity and compressive strength

Porosity of the bioceramic samples was measured by the Archimedes method using absolute ethyl alcohol as displacement liquid. The bioceramic samples were processed into bars (5 mm × 5 mm × 10 mm), and their compressive strength was tested employing a universal material testing machine (Instron 5567, Instron, USA) with a crosshead speed of 0.5 mm min^{-1}.

2.3. In vitro cell-biological study

2.3.1. Cell culture

Mouse bone mesenchymal stem cells (mBMSCs; ATCC, USA) at passage 5 and RAW264.7 murine monocyte cell line (ATCC, USA) were used to assess in vitro osteogenic behaviors and osteoclastic activities of bioceramic samples, respectively. The bioceramic samples were sterilized by autoclaving. mBMSCs and RAW264.7 cells were cultured with Dulbecco's modified eagle's medium (DMEM; Gibco, USA) containing 10 vol% fetal bovine serum (FBS; Gibco, USA). Cell suspension of either mBMSCs or RAW264.7 was added onto the samples, and cultured in an incubator with 5% CO_2 and 95% humidity atmosphere at 37 °C. After culturing RAW264.7 cells for 1 day, the medium was supplemented with 50 ng mL^{-1} of receptor activator of nuclear factor kappa-B ligand (RANKL; R&D, USA). The medium was refreshed every two days.

2.3.2. mBMSCs viability, F-actin cytoskeleton and proliferation

For evaluation of cell viability, F-actin cytoskeleton and proliferation, 500 μL of mBMSCs suspension (4 × 10^4 cells/mL) was seeded on the samples (Φ10 mm × 2 mm). The cell viability on the bioceramic samples was evaluated using a Live/Dead kit (calcein-AM, Biotium, USA) following the manufacturer's guidelines. In an attempt to observe the cytoskeleton of mBMSCs attaching on the bioceramic samples, the nuclei and F-actin were stained with 40,6-diamidino-2-phenylindole (DAPI, Beyotime, China) and PhalloidiniFluor™488Conjugate (AAT Bioquest, USA), respectively, in accordance to the manufacturer's instructions. The fluorescent photographs were taken with a confocal laser scanning microscope (SP5, Leica, Germany). mBMSCs proliferation on the bioceramic samples was determined employing a CCK-8 kit (Dojindo Laboratories, Japan) according to the manufacturer's protocols.