Materials Science and Engineering C 78 (2017) 398–404

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Doped tricalcium phosphate bone tissue engineering scaffolds using sucrose as template and microwave sintering: enhancement of mechanical and biological properties

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ARTICLE INFO

Article history:
Received 15 December 2016
Received in revised form 16 March 2017
Accepted 17 March 2017
Available online 20 March 2017

Keywords:
Tricalcium phosphate scaffolds
MgO and ZnO
Microwave sintering
PCL incorporation
Mechanical property
Osteoinductivity

ABSTRACT

β-tricalcium phosphate (β-TCP) is a widely used biocompatible ceramic in orthopedic and dental applications. However, its osteoinductivity and mechanical properties still require improvements. In this study, porous β-TCP and MgO/ZnO-TCP scaffolds were prepared by the thermal decomposition of sucrose. Crack-free cylindrical scaffolds could only be prepared with the addition of MgO and ZnO due to their stabilization effects. Porous MgO/ZnO-TCP scaffolds with a density of 61.39 ± 0.66%, an estimated pore size of 200 μm and a compressive strength of 24.96 ± 3.07 MPa were prepared by using 25 wt% sucrose after conventional sintering at 1250 °C. Microwave sintering further increased the compressive strength to 37.94 ± 6.70 MPa, but it decreased the open interconnected porosity to 8.74 ± 1.38%. In addition, the incorporation of polycaprolactone (PCL) increased 22.36 ± 3.22% of toughness while maintaining its compressive strength at 25.45 ± 2.21 MPa. Human osteoblast cell line was seeded on scaffolds to evaluate the effects of MgO/ZnO and PCL on the biological property of β-TCP *in vitro*. Both MgO/ZnO and PCL improved osteoinductivity of β-TCP. PCL also decreased osteoblastic apoptosis due to its particular surface chemistry. This novel porous MgO/ZnO-TCP scaffold with PCL shows improved mechanical and biological properties, which has great potential in bone tissue engineering applications.

1. Introduction

Musculoskeletal diseases, including osteoporosis, fracture, bone trauma, *etc.*, have been causing chronic conditions, disability, and death resulting in a huge economic and human capital loss [1]. Direct health care cost and indirect lost wage expenditure caused by musculoskeletal diseases are enormous and estimated to escalate every year due to the increase of aging population, which has drawn a compelling interest in developing potential treatments [2]. Bone tissue engineering scaffolds, as one of the most effective therapies, are intriguing to alleviate this burden. However, several requirements must be satisfied for scaffolds to become ideal for treating musculoskeletal diseases.

The first requirement is excellent biocompatibility and appropriate biodegradability. β-TCP has high compositional similarity to bone mineral resulting in its excellent biocompatibility. In addition, the biodegradation of β-TCP is much better (solubility product, $K_{sp} = 1.25 \times 10^{-29}$) than another popular bioceramic, hydroxyapatite ($K_{sp} = 2.35 \times 10^{-59}$), which is more advantageous in fitting bone remodeling cycle [3,4].

The second requirement is adequate open interconnected porosity which provides space for biological fixation between scaffolds and host tissues. Thermal decomposition is a cost-effective way to create open interconnected porosity by simply burning off the porogen while maintaining pores randomly distributed just like natural bones. Porogens, such as polyethylene glycol (PEG) [5], sucrose [6,7] and naphthalene [8,9], were exploited to fabricate porous structures in previous studies. Sucrose is a biocompatible, nontoxic and cost-effective porogen. It was reported that large porous calcium deficient apatite blocks were prepared using sucrose as the porogen to control their pore size and porosity [7]. However, small porous β-TCP scaffolds using sucrose as the porogen and their biological characterizations were not reported.

The third requirement is excellent osteoinductivity, which is generally defined as the ability to induce the osteoblastic differentiation towards bone lining cells or more simply, the ability to induce osteogenesis [10]. Substantial studies adopted additives to address the lack of osteoinductivity in calcium phosphates. Magnesium oxide (MgO) was reported to have effects on the skeletal metabolism, leading to a prominent increase in new bone formation and induced osteoblastic activities [3,11,12]. Zinc oxide (ZnO) was also reported to boost osteoinductivity of calcium phosphates by inducing not only osteoblastogenesis but also osteoblastic differentiation and mineralization [13,14]. Besides adding additives to TCP, an *in vivo* study indicated that the combination of polycaprolactone (PCL) and β-TCP improved the osteoinductivity and new bone formation [15].

Great mechanical properties, which determine if scaffolds can endure the stress during surgery and recovery, are the last requirement.

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http://dx.doi.org/10.1016/j.msec.2017.03.167
0928-4931/

Polycaprolactone (PCL) is one of the well-known polymers, which has been reported to improve mechanical properties of porous ceramic scaffolds [16–19]. We previously reported that PCL was able to enhance the compressive strength of 70 vol.% porous β-TCP scaffolds nearly three times, however, the compressive strength was 2.41 MPa after PCL coating, which was only above the threshold of the compressive strength of cancellous bones [19]. We also found that ZnO increased the densification of β-TCP scaffolds and the addition of MgO and ZnO significantly increased the compressive strength of dense TCP scaffolds [12,20].

According to the requirements for bone tissue engineering scaffolds and related literature review, the combination of porous TCP structure, PCL, and MgO/ZnO should be promising to have excellent mechanical and biological properties, while related studies have not been conducted. The objective of this work is to prepare porous TCP scaffolds with excellent mechanical and biological properties and study the effects of MgO/ZnO and PCL on mechanical and biological properties of porous TCP scaffolds. Our hypothesis is that both PCL and MgO/ZnO should improve mechanical and biological properties of porous TCP scaffolds. To testify the hypothesis, porous TCP and MgO/ZnO-TCP scaffolds were prepared by the thermal decomposition of sucrose. The incorporation of PCL was conducted by using a 2 wt% PCL ($M_w = 100{,}000$) solution. Physical properties of scaffolds were characterized by density, surface microstructure, and phase formation. Mechanical properties of scaffolds were characterized by compressive strength and toughness. Besides physical and mechanical characterizations, human osteoblast cells were seeded on scaffolds for 3, 7, and 11 days to evaluate their biological responses *in vitro*. MTT and ALP assays were used to evaluate the effects of MgO/ZnO and PCL on osteoblastic proliferation and differentiation.

2. Materials and methods

2.1. Powder preparation

β-TCP was synthesized *via* a solid state method using dicalcium phosphate ($CaHPO_4$) and calcium carbonate ($CaCO_3$) from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). Briefly, 2 mol of $CaHPO_4$ were mixed with 1 mol of $CaCO_3$ at 70 rpm for 2 h in a 5:1 milling media to powder weight ratio. Then the mixture was heated up to 1050 °C for 24 h for β-TCP synthesis.

Magnesium oxide (MgO) and zinc oxide (ZnO) were purchased from Sigma-Aldrich Co. LLC. MgO/ZnO-TCP powder was prepared by mixing 1 wt% MgO, 0.25 wt% ZnO, and β-TCP in a bottle with a 5:1 milling media to powder weight ratio and anhydrous ethanol at 70 rpm for 6 h. Pure β-TCP powder was prepared by mixing only β-TCP under the same condition as MgO/ZnO-TCP powder. Mixtures were dried at 70 °C overnight followed by further mixing with 2 wt% polyvinyl alcohol in water for 2 h. Then they were dried at 70 °C for another 72 h prior to use.

2.2. Scaffold preparation

Sucrose crystals (Avantor, PA, USA) were ball milled with zirconia balls until they could be filtered by two sieves (180 and 212 μm in diameter). 25 wt% filtered sucrose powder was further mixed with TCP and MgO/ZnO-TCP, respectively. Cylindrical scaffolds (around 7 mm in diameter and 12 mm in length) were prepared by uniaxial pressing for density and compressive strength measurements. Disc scaffolds (around 12.5 mm in diameter and 3 mm in thickness) were also prepared by uniaxial pressing for *in vitro* characterizations. Finally, scaffolds were sintered in a conventional or microwave furnace at different temperatures for the optimization of sintering process.

2.3. PCL incorporation

PCL ($M_w = 100{,}000$) was first dissolved in chloroform to achieve 2 wt% PCL solutions. Then the incorporation was completed by a food saver machine (V2244, Walmart, WA) to apply PCL on both inside and outside surfaces of porous scaffolds. Each scaffold went through five vacuum cycles in order to get a homogeneous PCL incorporation.

2.4. Microstructure, density, and compressive strength

The cross-sectional microstructure of scaffolds was observed under a field-emission scanning electron microscope (FESEM) (FEI Inc., Hillsboro, OR, USA). Open interconnected porosities of sintered scaffolds were calculated by Archimedes' principle based on ASTM C830-00 [21]. Bulk densities of sintered scaffolds were determined by scaffolds mass divided by scaffolds volume. Compressive strength was tested by a screw-driven mechanical test machine (AG-IS, Shimadzu, Japan) with a constant crosshead speed of 0.33 mm/min and a load cell of 50 kN. Compressive stress/strain curve was plotted before and after PCL incorporation to analyze their toughness. All data above were achieved based on five scaffolds ($n = 5$).

2.5. Cell-scaffold interactions

Scaffolds were sterilized in an autoclave at the temperature of 121 °C for 20 min. After autoclaving, several porous pure TCP and MgO/ZnO-TCP scaffolds were incorporated with PCL. Scaffolds with PCL were then immersed in ethanol for 2 h followed by exposing under UV light for 1 h for further sterilization. Human preosteoblast cell line, hFOB 1.19 (ATCC, Manassas, VA), was cultured for *in vitro* cell-scaffold interactions. After cells reached their confluency, they were transferred onto each scaffold at a density of 3×10^5 cells/scaffold. The cell medium for this cell line was prepared by mixing Ham's F12 and Dulbecco's Modified Eagle's Medium (Sigma, St. Louis, MO) with 10% fetal bovine serum (PBS), 0.3 mg/ml G418 (Sigma, St. Louis, MO), and 2 ml/l of Penicillin-Streptomycin-Amphotericin B solution (ATCC, Manassas, VA). Cells were incubated at 34 °C and 5 vol.% CO_2 environment according to the manufacturer recommended protocol for this particular cell line. Cell medium was changed every alternate day for the following 11 days.

2.6. Cellular morphology

Scaffolds for cell morphology observation were transferred to new well plates after 3, 7, and 11 days of incubation. Then they were fixed in a 2% paraformaldehyde/2% glutaraldehyde in 0.1 M phosphate buffer solution overnight at 4 °C followed by a post-fixation in 2% osmium tetroxide (OsO_4) at 4 °C overnight. After fixation, each scaffold was dehydrated in an ethanol series from 30% to 100% followed by hexamethyldisilane (HMDS) drying before gold coating. Gold coating was conducted using a Technics Hummer V sputter coater at 10 mA for 6 min. After gold coating, cell morphology was observed under FESEM.

2.7. Cellular proliferation

Osteoblastic proliferation was evaluated by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma, St. Louis, MO). MTT assay solution was made by dissolving 50 mg MTT powder in 10 ml filter sterilized PBS. 100 μl of as prepared MTT solution and 900 μl of cell media were mixed and added to each scaffold followed by incubation at 34 °C for 2 h. After aspirating MTT solution, 1 ml of solubilization solution (10% Triton X-100, 0.1 N HCl and isopropanol) was added on scaffolds. Resulting supernatant was transferred into a 96-well plate and read by an ultraviolet–visible spectroscopy (UVS, Synergy 2 microplate reader, Biotek, Winooski, VT) at 570 nm. Three biological and technical replicates were used in MTT measurements to confirm their reproducibility.

2.8. Cellular differentiation

Osteoblastic differentiation was evaluated by an alkaline phosphatase (ALP) kit purchased from Abcam (ab83369, Cambridge, MA). The