Fabrication and Characterization of Porous β-Tricalcium Phosphate Scaffold for Bone Regeneration

M. Khodaei[1,*], A. Valanezhad[2], I. Watanabe[2]

[1] *Dental Research Center, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran.*
[2] *Department of Dental and Biomedical Materials Science, Nagasaki University, Nagasaki, Japan.*
Received: 10 December 2017 - Accepted: 01 June 2018

Abstract

Resorb-able bioceramic scaffolds are often used for bone regeneration purposes. The most important rule of this scaffolds is to act as a bioactive template for cell attachment, proliferation, and differentiation and then bone ingrowth and regeneration. So this scaffolds should have an interconnected porous structure. Calcium phosphate ceramics are the most important groups of bioceramics which includes the mentioned properties. β-tricalcium phosphate (β-TCP) is one of the mostly used bioceramic for bone defect filling during surgery. In this research β-tricalcium phosphate scaffold is fabricated using powder metallurgy-space holder method successfully. And its microstructural and mechanical properties are investigated using scanning electron microscope (SEM) and cold compression test. Results of SEM study indicate that fabricate β-TCP includes some micro pores with size of about 2 micrometer in the struts of scaffold. Results indicate that in the case of fabricated scaffold with macro pore size of about 340 micrometer, by increasing sintering temperature from 1000 to 1100 °C, the compression strength of scaffold increased by 15% without any phase transformation.

Keywords: Porous Scaffold, Mechanical Properties, β-TCP

1. Introduction

In the past decade, the growing number of bone defects caused by disease, trauma, congenital defects, and the accidents has created an urgent need for bone substitutes in the bone tissue regeneration [1,2]. Biodegradable ceramic scaffolds are mainly used for bone regeneration purposes. The most important role of these scaffolds is to act as a bioactive template for cell attachment, proliferation, and differentiation [1]. So, these scaffolds should have an interconnected porous structure with a pore size of at least 150 µm to facilitate nutrition, waste removal and vascularization [3,4].Also, these scaffolds should have enough strength to provide mechanical stability and porosity for the bone ingrowth; however, a modulation is required between the maximum possible porosity and strength of bioceramic scaffolds for hard tissue implantation [5]. While the amount of porosity can affect the mechanical properties of scaffolds negatively [6], the mechanical properties of them decreased with increasing porosity [7,8]. Porous scaffolds, which induce new tissue ingrowth before degradation, are eventually replaced by new tissues. Porous scaffolds have a superior performance than rigid implants; this depends on their porous structure, pore shape, pore size, porosity percentage and pore interconnection pathway [8].

[*] *Corresponding author*
Email address: m.khodaei@ma.iut.ac.ir

So, an exact controlling measure should be applied to porous scaffold fabrication. One of the most important calcium phosphate bioceramics is β-tricalcium phosphate or β-TCP ($Ca_3(PO_4)_2$); because of its in vitro and in vivo degradation, it is used as a bone graft substitute.

β-TCP has a higher resorption rate than hydroxyapatite (HA); it is normally considered as a biodegradable material allowing bone growth and replacement [9]. Tricalcium phosphate is an ideal material for bone substitution. After implantation, TCP will be resorbed with time; it will be, finally, replaced with the natural bone. This can accelerate the bone regeneration [10], and the porosity of the implanted material will improve this phenomenon.

Different methods have been used by researchers for the porous β-TCP scaffold fabrication. Riberioet al, for instance, reported a new method for the porous β-TCP scaffold fabrication which included about 65 % porosity, by using paraffin microspheres [4]. Hu et al. [6] also used a spongy template method for the HA-TCP composite porous scaffold fabrication. According to the results of their study, the compressive strength of the single phase porous β-TCP scaffold was decreased from about 3 to 1 MPa by increasing the porosity from 50 to 85%. They improved the mechanical properties of the porous β-TCP scaffold via the in situ synthesis of nHA whiskers.

In this study, the porous β-tricalcium phosphate was fabricated using the space holder method, and the effect of the sintering temperature on the mechanical

and structural properties of the scaffold was investigated.

2. Materials and Methods

2.1. Synthesis of β-TCP Powder

β-TCP powder was synthesized using the chemical precipitation method. A solution of 2 M calcium nitrate tetra hydrate $Ca(NO_3)_2 \cdot 4H_2O$ in 1.5 molar ratio of Ca/P (stoichiometric for β-TCP) was added in a drop-wise manner to a solution of 2 M diammonium hydrogen phosphate $(NH_4)_2HPO_4$ under gentle stirring. The pH value of the mixed solution was adjusted in the range of 7.2-7.5 by adding ammonia (NH_3) to precipitate a white precipitate. After washing by water, the precipitate was dried at 80 °C for 24 h; this was followed by calcination at 900 °C for 120 min. The obtained product was milled and sieved to obtain the β-TCP powder.

2.2. Fabrication of the β-TCP Scaffold

Porous β-TCP scaffolds were prepared by using the space holder method. β-TCP powder (as the matrix of scaffold) and NaCl particles (as the spacer agent) were mixed in the 30/70 volume ratio and cold compacted in a cylindrical mold (H=12 mm, D=12 mm) to reach the pressure of 150 MPa. The pellets were heated at 1000 and/or 1100 °C for 120 min to sinter β-TCP scaffolds. Finally, NaCl particles were removed by leaching the sintered samples in deionized water for 24 h.

2.3. Characterization

The microstructure of raw materials and scaffolds was observed using a scanning electron microscopy (SEM: JCM-6000 Plus, JEOL, Japan), and the mean pore size of scaffolds was determined using ImageJ computer program, by measuring the size of 10 pores. The porosity of the scaffolds was measured based on the Archimedes principle (ASTM B962).
The probable contamination during sintering and, new phases were investigated by X-ray diffraction (XRD: Smart Lab, Rigaku Co., Tokyo, Japan); for the radiation source, Cu-Kα (λ=1.5405 A°) at the rate of 2θ= 4°/min and in the range of 2θ=5-70° was used.
The compressive strength of the scaffolds was measured using a Universal Testing Machine (UTM: SHIMADZU, AG- X, 500 N, Japan), according to ASTM-F-451-95, with a testing speed of 1 mm/min.

3. Results and Discussion

3.1. SEM Observation

Results of the SEM observation of NaCl and the synthetized β-TCP particles are presented in Fig. 1. According to this figure, the β-TCP particles with a semi-circular shape were almost smaller than 3 micrometers, and the NaCl particles with a semi-cubic shape were almost smaller than 350 micrometers. The particle size of β-TCP must be much smaller than that of NaCl particles to be able to completely cover NaCl particles and exactly replicate the shape of NaCl particles to be the scaffold pores.

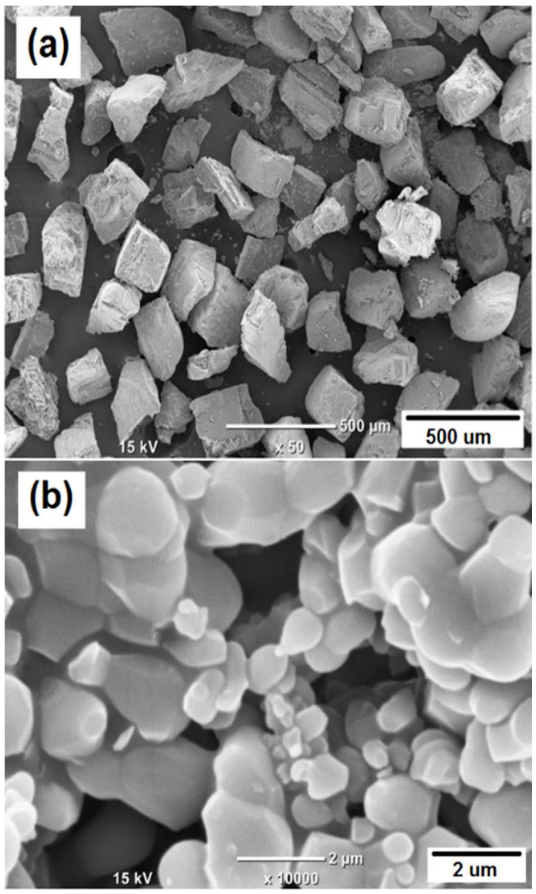

Fig. 1. SEM micrograph of: a) NaCl particles, b) synthetized β-TCP powder.

SEM micrograph of the β-TCP scaffold is shown in Fig. 2., indicating that well distributed pores with the size of almost 340 micrometers were formed in the scaffold, which could improve vascularization and bone ingrowth after implantation. Also, it seemed that pores were interconnected, improving nutrition and the waste removal ability of the scaffold. SEM micrograph of the β-TCP scaffold at the higher magnification indicated that small particles of β-TCP were well sintered to each other and no discontinuity or grain boundary between particles could be observed.
Also, there were some micro pores (smaller than 2 micrometers) at the struts of the scaffold. SEM micrographs of both sintered scaffolds at 1000 and 1100 °C were the same; so just one of them is presented.

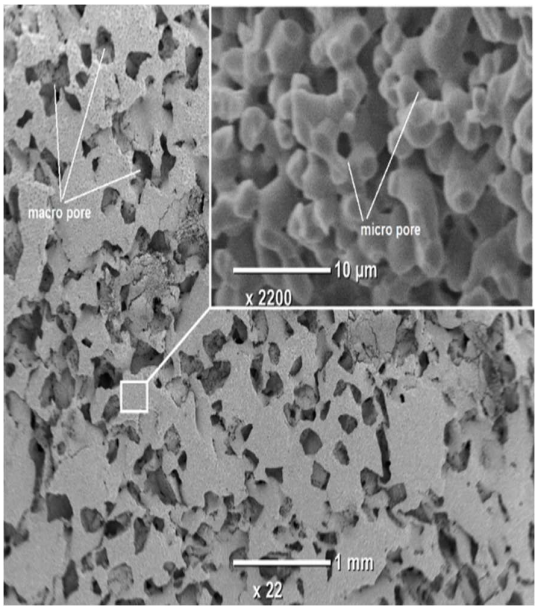

Fig. 2. SEM micrograph of sintered β-TCP scaffold

3.2. Results of Porosity Measurement

According to the results of porosity measurement (average values), it was evident that the temperature of sintering had no significant effect on the apparent porosity of the Scaffolds. With increasing the sintering temperature from 1000 to 1100 ˚C, the apparent porosity decreased from 69% to 68%. In fact, the grain boundary and, micro-isolated pores inside the β-TCP could be removed by increasing the sintering temperature; also, the shrinkage of the samples could be increased [11]. According to the results of Mehdikhani et al. [11], the relative density of the cold compacted β-TCP raised from 68 to 93% by increasing the sintering temperature form 800 to 1100 ˚C; while it was evident that increasing the sintering temperature from 1000 to 1100 ˚C had no significant effect on the porosity of porous samples and more changes in temperature were needed to reveal the mentioned changes.

3.3. Phase Identification

X-ray diffraction analysis used to investigate possible impurity or contamination of materials. The results of X-ray diffraction are presented in Fig. 3., indicating that both the synthetized powder and the sintered scaffold were composed of a major β-TCP phase (JCPDS #09-0169) and no extra contamination was formed during β-TCP powder sintering at both 1000 and 1100 ˚C. X-ray diffraction patterns of both sintered scaffolds at 1000 and 1100 ˚C were the same; only one of them is reported here.

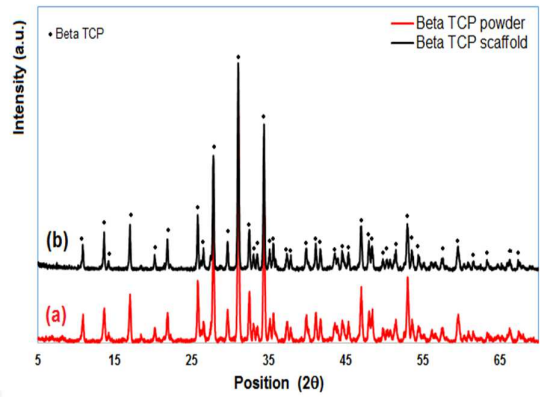

Fig. 3. X-ray diffraction pattern of β-TCP: a) synthetized powder, b) sintered scaffold.

3.4. Mechanical Properties

The engineering stress-strain curves of β-TCP scaffolds sintered at 1000 and 1100 ˚C are presented in Fig. 4. According to this figure, the average compression strength of β-TCP scaffolds sintered at 1000 and 1100 ˚C was 0.8 and 1.2 MPa respectively, which would be inadequate for load bearing applications [5]. These values were the calculated averages of stress after the strain of 0.4 (yield point) of the stress- strain curves.

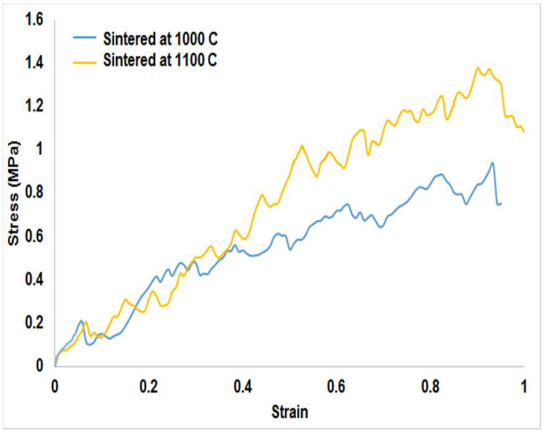

Fig. 4. The engineering stress-strain curves for β-TCP scaffolds

The obtained values for the strength of scaffolds were much smaller than those of the cortical bone (180-220 MPa [12]), but they were in range of the cancellous bone strength (0.2-80 MPa [13]). Also, the strength of the obtained scaffolds was sufficient for care handling. It seemed that the fabricated scaffolds could not be used in the load bearing bones, and they might be suitable for non-load bearing sites of human body such as maxillofacial and skull bone.

4. Conclusions

1. In the presented study, a β-TCP scaffold was fabricated by using the space holder method successfully.
2. By raising the sintering temperature from 1000 to 1100 ◦C, the compression strength of scaffold was increased by 15% without any phase transformation.
3. Because of the β-TCP good biodegradability and also, the interconnected porosity of scaffolds, it could be a suitable candidate for bone substitution.

References

[1] K. Yang, J. Zhang, X. Ma, Y. Ma, C. Kan, H. Ma, Yulin Li, Y. Yuan and C. Liu, Mater. Sci. Eng. C, 56(2015), 37.
[2] M. Yamamoto, A. Hokugo, Y. Takahashi, T. Nakano, M. Hiraoka and Y. Tabata, Biomater. , 56(2015), 18.
[3] D. Bellucci, A. Sola, V. Cannillo, A revised replication method for Bioceramic scaffolds, Bioceram. Dev. Appl., 1(2011), 1.
[4] G. B. M. Ribeiro, R. M. Trommer, L. A. dos Santos and C. P. Bergmann, Mat. Lett., 65(2011), 275.
[5] T. Tanaka, H. Komaki, M. Chazono, S. Kitasato, A. Kakuta, S. Akiyama and K. Marumo, Morphologie, 101 (2017), 164.
[6] H. Hu, G. Xu, Q. Zan, J. Liu, R. Liu, Z. Shen and X. Ye, Microelectro. Eng., 98(2012), 566.
[7] I. S. Muhammad, X. Xu and L. Li, J. Mater. Sci., 44(21) (2009), 5713.
[8] S. C. Wu, H. C. Hsu, S. K. Hsu, F. W. Lin, W. F. Ho, Ceram. Int., 41(2015), 7596.
[9] S. T. AbdulQader, T. P. Kannan, I. A. Rahman, H. Ismail, Z. Mahmood, Mater. Sci. Eng. C, 49(2015), 225.
[10] B. Mehdikhani, B. Mirhadi and N. Askari, J. Ceram. Processing Res., 13(2012), 486.
[11] B. Mehdikhani, B. Mirhadi and Na. Askari, J. Ceram. Processing Res., 13(4) (2012), 486.
[12] C. Öhman, M. Baleani, C. Pani, F. Taddei, M. Alberghini, M. Viceconti and M. Manfrini, Bone 49(2011), 769.
[13] H. C. Hsu, S. C. Wu, S. K. Hsu, T. Y. Chang, W. F. Ho, J. Alloys Compd., 582(2014), 793.