Fabrication and Characterization of Porous Hydroxyapatite Scaffold and Nano Silver Coated on Scaffold for Tissue Engineering Application

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Abstract

Porous hydroxyapatite scaffold (PHS) was fabricated by replica method, where polyurethane foam was filaments coated with a HAp suspension. A subsequent thermal treatment removed the organic materials, remaining the HAp ceramic in the substratum. And then, microwave processing of the PHS was investigated in a dual frequency microwave sintering furnace. Through the optimization of sintering conditions, such as the sintering temperature and holding time, the microstructure and material properties of the PHS were investigated namely pore sizes, grain size, relative density, and compressive strength. The obtained results were compared to those of the conventional sintering process and also revealed that microwave processing was a promising method to fabricate PHS for tissue engineering application. On the other hand, nano-Ag, which was used as antibacterial effect to prevent infections, was successfully coated on the PHS by the electroless deposition process. The results showed that nano-Ag spots were homogeneously dispersed on the PHS.

Keywords: HAp, scaffold, nano-Ag, compressive strength

1. Introduction

Scaffolds for bone tissue engineering have become a very attractive research field because the scaffold lies at the key of all the new tissue engineering approaches [1]. Various synthetic alternatives such as ceramics, polymers and composites have been tried as scaffolds for many years [2]. Regarding the scaffold, it is generally agreed that a highly porous microstructure with interconnected pores and a large surface area are conducive to hard tissues in growth [3]. But the scaffold should possess not only appropriate porosity but also mechanical properties depending on the application area. Only if it possesses proper mechanical properties, can the scaffold keep its shape and characters after being embedded in the body [4].

Bioceramics have been widely used for the repair and reconstruction of diseased or damaged parts of human body [4-6]. With the great requirements of bioactive materials for orthopaedic as well as maxillofacial surgery, the utilization of hydroxyapatite (HAp, with Ca/P=1.667) as fillers, spacers, and bone graft substitutes has received great attention because of their biocompatibility, bioactivity, and osteoconduction characteristics with respect to host tissue [5, 6]. Recently, there were some reports attempted to fabricate HAp scaffold by replica method using conventional sintering process. However, there were critical limitations in applying the scaffold to real system because of their low compressive strength [7].

On the other hand, microwave heating is a fast sintering process fundamentally different from conventional sintering in that the energy can be deposited volumetrically throughout the material rather than relying on thermal conduction from the surface. The optimization of the microwave sintering condition may lead to a series of benefits, including great microstructural control, improved product properties and reduced manufactural costs due to energy savings and shorter processing times. In this point of view, microwave sintering process shows promising potential in bioceramics processing. During the last 15 years, microwave processing of ceramic materials, which ranged from structural ceramics to functional ceramics, has been widely investigated by various researchers [8, 9], but microwave sintering of bioceramics was seldom reported [10].

Recently, silver (Ag) has received much attention because of its high unique beneficial properties such as antimicrobial activity and corrosion resistance [11]. Its antimi-crobial activity protects the infection of implanted parts [12] and can also removed surrounding pollution.

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In this work, porous hydroxyapatite scaffold (PHS) was fabricated for bone tissue engineering by replica method. And then, microwave processing of the PHS was investigated in a dual frequency microwave sintering furnace. Nano 5 vol.% Ag-coated PHS were synthesized by the electroless deposition process. The primary aim of this research was focused on the microstructure and material properties of PHS depending on sintering conditions and nano Ag coated porous HAp scaffold to prevent infection.

2. Experimental

The porous HAp scaffold was made by a polyurethane foam replica method [13]. The coating slurry was prepared from HAp powder which was synthesized in our laboratory by microwave-hydrothermal method [14]. 50g HAp powder was stirred vigorously in 100ml distilled water for 4h. As a binder, 5g poly vinyl butyl (PVB, Acros, USA) was dissolved in another beaker for 1h, which was subsequently added to the slurry and stirred for an additional 24h. Polyurethane foam templates (Customs Foam Ltd., UK) which were about 300 – 700μm of pore sizes cut to dimensions 15 mm x 15 mm x 15 mm was immersed in the slurry. After blowing with an air gun to disperse the slurry uniformly throughout the porous scaffolds without blocking the pores, the sponge was dried at 80ºC for 8h. These dipping-and-drying steps were repeated three times. The obtained body was heat-treated to burn out the sponge and binder at 600ºC for 3h at a heating rate of 1ºC/min. On the other hand, the microwave sintering was carried-out at a heating rate of 45ºC/min and at different temperatures (1100ºC, 1200ºC, 1300ºC) and holding times (5, 10, 15, 20, 30 minutes). The conventional sintering was carried-out at a heating rate of 5ºC/min and at different temperatures (1100ºC, 1200ºC, 1300ºC) with same holding time of 2 hours.

To coated 5 vol.% nano Ag to PHS bodies by Ag electroless deposition process, 1.71 g of silver nitrate as the silver source was dissolved in ammonia water and then 97 ml of ethanol was added. As a reducing agent, 1.1–1.4 ml of formaldehyde was dissolved in ethanol. Twenty grams of Al_2O_3 powder were added to the mixed solution of silver and reducing solutions and then reacted at 70ºC for 30 min. And then, PHS body was immersed in the solution and keep in there about 5 min. The Ag-coated PHS were dried at 100ºC for 24 h.

The relative density of PHS was measured by the Archimedes method. The pore size and microstructure of the PHS were investigated by scanning electron microscopy (SEM, JSM-635F, JEOL). To identify the crystal structure and phases, an X-ray diffractometer (XRD, D/MAX-250, Rigaku, Japan) was used. The specimens with a dimension of 12x8x8 mm^3 were used for a compression test using a universal testing machine (UnitechTM, R&B, Korea) with a crosshead speed of 0.5 mm/min in ambient conditions. The stress–strain curve obtained was used to determine mechanical properties. The compressive strength was determined from the maximum load recorded and from the slope at the initial stage, respectively. Five specimens were tested for each condition.

3. Results and disscution

Fig. 1 shows (a) low magnification and (b) enlarged SEM micrographs of PHS and (c) cross sectional view of pore frame. These figures revealed that the structure of obtained scaffold is similar to cancellous bone, its pore sizes was about 300 – 700μm and the highly interconnected pores were homogeneously dispersed in the scaffold. In general, the optimized pore sizes were recognized with about over 200μm facilitates the new bone formation. Especially, Fig. 1(c) confirmed that PU foam was completely removed due to the burn-out and sintering processes as indicated with an arrow.

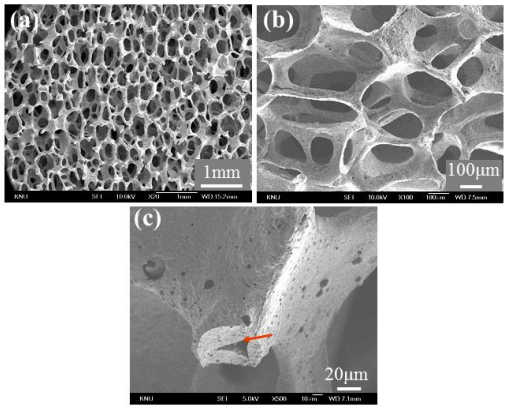

Fig. 1. SEM micrographs of porous HAp scaffold (a) low magnification, (b) enlarged image and (c) cross sectional view of pore frame

Fig. 2 shows SEM micrographs of pore frame of PHS depending on the sintering approaches and sintering temperatures, conventional sintering at (a) 1100ºC and (b) 1300ºC for 3h, microwave sintering at (c) 1100ºC and (d) 1300ºC with 30 min. of holding time. In the microwave sintering at 1100ºC (Fig. 2(c)), densification of pore frame is better and the particle sizes are (about 500nm) smaller than those of conventional sintering (about 1μm, Fig. 2(a)), and small amounts of fine pores of less than 700nm were observed in the Fig. 2(c). However, at 1300ºC of sintering temperature (Fig. 2(d)), it is hardly to observe fine pores due to the densification. In this case, particle sizes were about 1 – 3μm in diameter.

On the other hand, in the conventional sintering with 3h of holding time at 1100°C (Fig. 2(a)), so many pores of about 1 - 3μm were observed. However, when the sintering temperature increased to 1300°C (Fig. 2(b)), a few pores remained due to the growth of grain sizes as well as improve the densification of pore frame. Their grain sizes are about 2 – 6μm, respectively.

Fig. 3 shows XRD profiles of PHS depending on the sintering temperature at 30 min of holding time. There was no new phase appearance and they were all HAp phases due to the change of sintering temperature at 1100°C (a), 1200°C (b) and 1300°C (c). However, in the Fig. 3(d), using conventional sintering at 1300°C also showed perfectly HAp phase.

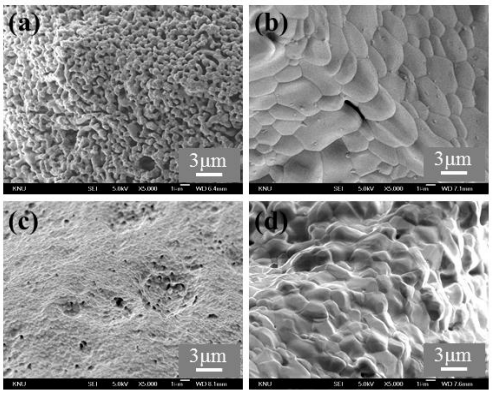

Fig. 2. SEM micrographs of pore frame of porous HAp scaffold depending on the sintering method, conventional sintering at (a) 1100°C and (b) 1300°C, microwave sintering at (c) 1100°C and (d) 1300°C

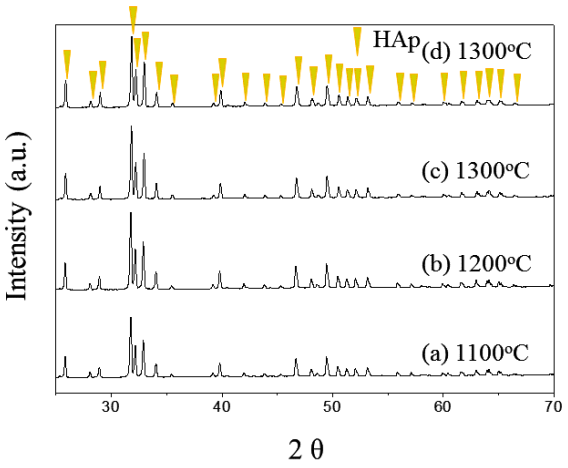

Fig. 3. XRD profiles of porous HAP scaffold sintered by (a, b, c) microwave sintering depending on the sintering temperature with 30 min of holding time and by (d) conventional sintering

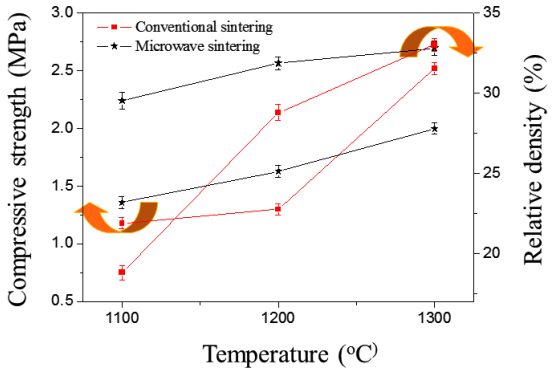

Fig. 4. Relative density and compressive strength of porous HAp scaffold depending on the sintering temperature and sintering method

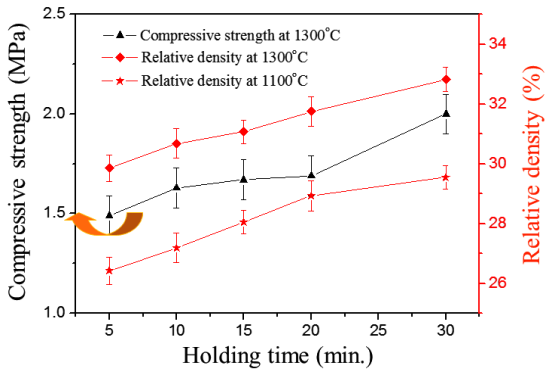

Fig. 5. Relative density and compressive strength of porous HAp scaffold depending on the holding time

Fig. 4 shows relative density and compressive strength of PHS depending on the sintering temperature and sintering method. The relative density of PHS increased as sintering temperature increased. At low sintering temperature, the values of relative density of microwave sintering were higher than those of conventional sintering. However, at 1300°C of sintering temperature, the values of two sintering methods were the same about 33%, respectively. On the other hand, the values of compressive strength of PHS increased as increasing of sintering temperature. At 1100°C and 1200°C, the values compressive strength of microwave sintering method was higher than those of conventional sintering method. However, at 1300°C, its value was lower than that of conventional sintering. At high sintering temperature, relative density of conventional sintering sample increased and obtained the same value with relative density of microwave sintering sample. Therefore, compressive strength of conventional sintering sample increased and a little bit higher than that of microwave sintering sample. The compressive strength values of PHS, which were

sintered by microwave sintering and conventional sintering, were 2 MPa and 2.6 MPa, respectively.

Fig. 5 shows relative density and compressive strength of PHS depending on the holding time. At 1100ºC, the value of relative density of PHS increased as increasing of holding time, maximum value was about 29%. However, its value was improved when PHS was sintered at 1300ºC and was confirmed as indicated in Fig. 3. On the other hand, the value of compressive strength of PHS also increased as holding time increased and obtained maximum value at 30 min of holding time.

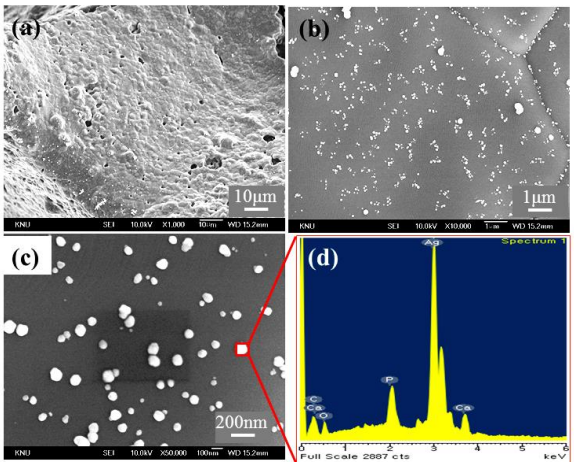

Fig. 6. SEM micrographs and EDS profile of nano-Ag coated porous HAp scaffold (a) pore frame, (b) enlarged image, (c) high magnification and (d) EDS profile

Fig. 6 shows SEM micrographs and EDS profile of nano-Ag coated porous HAp scaffold. Obtained results showed that the nano-Ag was homogeneously coated on the surface of porous HAp scaffold by electroless deposition process. The size of Ag coated PHS was narrow distribution; i.e., the fine, spherical Ag particles and their particle size were about 50-100 nm such as indicated in Fig. 6(c, d).

4. Conclusion

Porous HAp scaffold was successfully fabricated by microwave sintering using replica method. The PHS showed spherical, interconnected pores of about 300 – 700 μm in diameter and the densification of the pore frame was improved as the sintering temperature and holding time increased. No new phase was found during the increase of sintering temperature and holding time. The values of relative density, compressive strength of PHS sintered at 1300oC and 30 min. of holding time were 33%, 2 MPa, respectively. Moreover, the nano-Ag was homogeneously coated on the surface of porous HAp scaffold by electroless deposition process and their particle size were about 50-100 nm. The obtained results were compared to those of the conventional sintering process and also revealed that microwave processing was a promising method to fabricate PHS for tissue engineering application.

References

[1] Yongnian Y, Zhuo X, Yunyu Hu, Shenguo W, Renji Z, Chao Z., Mater. Lett. 2003; 57:2623-5.

[2] Kose GT, Kenar H, Hasirci N, Hasirci V., Biomaterials 2003; 24:1949-9.

[3] Susan LI, Genevieve MC, Michael JM, Alan WY, Michael JY, Antonios GM., J. Biomed. Mater. Res. 1997; 36:17-11.

[4] Takashi N, Francoise MW., Biomaterials 2000; 21:415-4.

[5] K. De Groot., Biomaterials 1980; 1: 47-3.

[6] Christopher JD, Parsons JR., J. Appl. Biomaterials 1991; 2:187-21.

[7] Kim HW, Lee SY, Bae CJ, Noh YJ, Kim HE, Kim HM, Ko JS., Biomaterials 24 (2003) 3277.

[8] Sutton WH., Am. Ceram. Soc. Bull. 1989; 68:376-5.

[9] Monika AWP., MRS Bull. 1993; 18:51-6.

[10] Nath S, Sinha N, Basu B., Ceram. Inter. 2008; 34:1509-11.

[11] Shi GM, Han JK, Zhang ZD, Song HY, Lee BT., Surf. Coat. Technol. 2005; 195:333–4.

[12] Feng QL, Kim TN, Wu J, Park ES, Kim JO, Lim DY, Cui FZ., Thin Solid Films 1998; 335:214–5.

[13] Moreira EA, Innocentini MDM, Coury JR., J. Euro. Ceram. Soc. 2004; 24:3209-9.

[14] Han JK, Song HY, Saito F, Lee BT., Mater. Chem. Phy. 2006; 99:235-4.