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Effects of microwave sintering on the properties of porous hydroxyapatite scaffolds

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Abstract

Microwave sintering was used to process porous hydroxyapatite scaffolds fabricated by the extrusion deposition technique. The effects of microwave sintering on the microstructure, phase composition, degradation, compressive strength and biological properties of the scaffolds were investigated. After rapid sintering, scaffolds with controlled structure, high densification and fine grains were obtained. A significant increase in mechanical strength was observed relative to conventional sintering. The scaffolds (55–60% porosity) microwave sintered at 1200 °C for 30 min exhibited the highest average compressive strength (45.57 MPa). The degradation was determined by immersing the scaffolds in physiological saline and monitoring the Ca^{2+} concentration. The results indicated that the microwave-sintered scaffolds possessed higher solubility than conventionally sintered scaffolds, as it released more Ca^{2+} at the same temperature. Furthermore, an in vitro MC3T3-E1 cell culturing study showed significant cell adhesion, distribution, and proliferation in the microwave-sintered scaffolds. These results confirm that microwave sintering has a positive effect on the properties of porous hydroxyapatite scaffolds for bone tissue engineering applications.

Keywords: A. Sintering; D. Apatite; Scaffold; Extrusion deposition technique

1. Introduction

Scaffold is a key component of bone tissue engineering, playing an active important role in cell migration and growth, and providing proper mechanical function in both in vitro cell culture and in vivo tissue regeneration. Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), a calcium phosphate bioceramic chemically similar to natural bone mineral, has been widely used as a scaffolding material for bone tissue engineering [1–3]. Sintering is critical for porous HA bioceramic scaffolds, determining microstructural properties such as crystallinity, grain size, density and micro-porosity, which, in turn, influence the performance of the scaffold [4,5]. However, a major concern regarding HA sintering is that lower sintering temperatures are needed to ensure better bioactivity, whereas higher temperature and longer sintering times are needed to ensure higher mechanical strength. The conventional sintering methods, such as muffle sintering, which is characterized by a high temperature, slow heating and a long holding time, cannot solve this contradiction.

Microwave sintering is a rapid volumetric heating process characterized by a high heating rate and short processing time, which offers several advantages over conventional sintering, including a finer microstructure, improved properties, energy and time savings, and cost reduction [6,7]. Microwave sintering has been employed in the sintering of various materials such as oxide ceramics [8–11], metals [12] and composites [13,14] over the last two decades. The application of this technique to HA sintering is still relatively new [15]. At present, most of the studies in this area are focused on improving the densification and mechanical strength of HA bioceramic [6,15–21]. Microwave sintering has been demonstrated to be a viable processing technique to produce dense HA bioceramic with a fine microstructure and improved mechanical strength

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at lower temperatures and in less time [16,17]. As both the mechanical strength and bioactivity of the HA scaffold depend strongly on its microstructure [22], the proper use of this technique may lead to improved mechanical strength and useful biological properties.

Therefore, the aim of this study is to investigate the effects of microwave sintering on the physical and biological properties of porous HA bone scaffolds. Scaffolds with controlled structures were manufactured by extrusion deposition and then microwave sintered at target temperatures. These sintered scaffolds were characterized in terms of their crystalline phases, microstructural morphology, grain size, compressive strength, degradation abilities and cell–material interactions. Conventional sintering was also conducted for comparison.

2. Materials and experiments

2.1. Materials

Commercially available HA powder (MH-HAP, Emperor Nano Material, China) with needle-like particle (20 nm in width and 150 nm in length) was used in this work. HA slurry (30 vol%) was prepared in deionized water using Darvan 821A (R.T. Vanderbilt Co., Norwalk, CT), a 40 wt% ammonium polyacrylate solution as dispersant and hydroxypropyl methylcellulose (Aladdin, Shanghai Jingchun Reagent, China) as viscosifing agent. 1-Octanol (Aladdin, Shanghai Jingchun Reagent, China) was also added to the slurry to prevent foaming, and glycerin (Sinopharm Chemical Reagent, Shanghai, China) was added as humectant.

2.2. Scaffold fabrication and microwave sintering

Porous scaffolds with a lattice-like structure were constructed via the deposition of the pre-prepared HA slurry using a computer controlled extrusion deposition system (MAM-II, Fochif, China), as shown in Fig. 1. The HA slurry was extruded through a conical nozzle with a 350 μm diameter under the force of the stepping motor, and deposited on the platform at a constant volumetric flow rate, which was required to maintain a constant X–Y printing speed. The deposition was repeated layer-by-layer with a nozzle elevation of 350 μm until the construction is completed. In all, 26 layers were deposited to form a cubic scaffold with dimensions of $9 \times 9 \times 9$ mm^3.

The scaffolds were dried at room temperature for 24 h and then sintered in a 1.4 kW, 2.45 GHz microwave furnace (Hamilab-V1500, Changsha Syno-therm. Co., Ltd., China) whose temperature was monitored by an infrared thermometer with an accuracy of $\pm 0.5\%$. The HA scaffolds were heated at 2 °C/min to 400 °C, held for 1 h to enable complete burnout of the organic components, rapidly heated at 40 °C/min to 1000–1200 °C, held for 30 min, and cooled in a furnace to room temperature. The conventional sintering was conducted at a heating rate of approximately 4 °C/min and a duration of 2 h, as shown in Fig. 2.

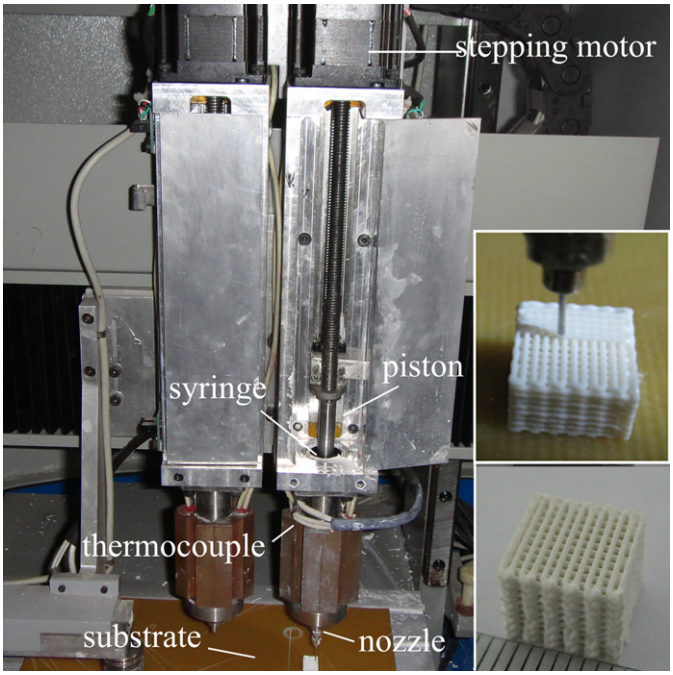

Fig. 1. The HA scaffold fabrication process via a double-nozzles extrusion deposition system and the constructed scaffold before sintering.

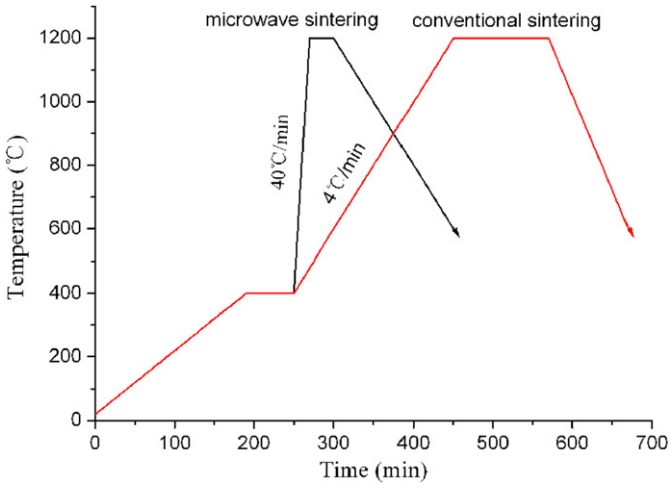

Fig. 2. Schematic of the sintering processes.

The physical properties of the sintered scaffolds were studied as follows. The porosity of each sintered sample was determined by the Archimedes method using distilled water. The lengths before and after sintering were measured to calculate the linear shrinkage. The phases of the original powder and sintered scaffolds were studied by X-ray diffraction (XRD, X'Pert PRO, PANalytical B.V., Netherlands). The microstructure was characterized using scanning electron microscopy (SEM, Quanta 200, FEI, Netherlands), and the grain size was determined by the

linear intercept method based on the SEM images. The compressive strength was measured using a mechanical testing machine (WDW2000, Harbin Kexin ins., China) at a cross-head speed of 1 mm/min in the direction perpendicular to the printing plane. Six scaffolds were tested for each condition.

2.3. Degradation in vitro

To study the degradation, both microwave-sintered and conventionally sintered scaffolds were soaked in physiological saline (0.9% NaCl) for 1 to 12 days, without refreshing the solutions. The solid–liquid ratio of the weight of HA to the volume of solution was 1/200 (g/ml). The scaffolds were suspended in polystyrene bottles containing saline solution in a 2 Hz shaking bath at 37 °C. At each time point, the Ca^{2+} concentration in the solution was measured by atomic absorption spectrophotometer (AAS, Perkin Elmer AAnalyst 300, USA).

2.4. Cell seeding and observation

MC3T3-E1 pre-osteoblasts (ATCC, USA) were used to study cell–scaffold interactions in vitro. Scaffolds were sterilized by autoclaving at 121 °C for 20 min and placed in a 24-well plate. The scaffolds were pre-wetted by a-MEM and then seeded with cells at a density of 1×10^4 per well. Next, 1 ml of a-MEM containing 10% fetal bovine serum (FBS; HyClone, Logan, UT) was added to each well. The cell–scaffold constructs were cultured in a humidified atmosphere of 5% CO_2 at 37 °C for a pre-specified number of days. The culture medium was changed every two days.

A CCK-8 assay (Dojindo, Japan) was performed to assess cell proliferation on the sintered HA scaffolds. After cell culturing for one, three and five day, the scaffolds were rinsed by phosphate-buffered saline (PBS) solution to remove the unattached cells. Next, 500 μl CCK-8 solution diluted by a-MEM (1:9) was added to each sample in a 24-well plate. After 3 h of incubation, 100 μl of the resulting supernatant was transferred into a 96-well plate, and read by a microplate reader (ELx808, BIO-TEK Instruments Inc., USA) at 450 nm. The results were presented as the mean ± standard deviation. Comparative analysis was performed using Student's *t*-test and a P value < 0.05 was considered significant.

The morphologies of the cell adhesion were characterized by SEM. After 24 h of incubation, the scaffolds were removed from the plate, rinsed with PBS, fixed in 2.5% glutaraldehyde for 1 h and rinsed with PBS again. Next, the scaffolds with fixed cells were dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90% and 95%; 20 min each), immersed in 50% alcohol–HMDS (hexamethyldisilazane) solution for 30 min and then immersed in pure HMDS for 30 min. Finally, the scaffolds were critical point dried and sputter-coated with gold before observation.

3. Results and discussion

3.1. Fabricated scaffolds

Fig. 3 shows images of the fabricated scaffold. After microwave sintering at 1200 °C for 30 min, the scaffold dimensions were reduced to $6.3 \times 6.3 \times 6.2$ mm^3 (Fig. 3(a)). The cross-section image (Fig. 3(b)) illustrates that the pores in the scaffold are interconnected with a size of approximately 250×250 μm^2, within the suggested range for cell and tissue growth [23]. The rod diameter was measured to be 350 ± 20 μm, and there was a 50 ± 10 μm rod overlap between adjacent layers. Furthermore, the microwave-sintered scaffold possesses much finer and more uniform grains. The grain size of the microwave-sintered scaffold was 1.12 ± 0.23 μm compared to 4.76 ± 2.30 μm for conventional sintering, as shown in Fig. 3(c and d). The porosity of the scaffolds that had been microwave sintered at 1200 °C for 30 min was 55–60% as measured by liquid displacement.

The XRD patterns of original HA powder and sintered scaffolds are illustrated in Fig. 4. The results show that all of the organic components in the scaffold are burned out during sintering; both the microwave-sintered and conventionally sintered scaffolds contain pure HA phase as well as the original powder (JCPDS #09-0432). However, the sintered HA exhibits larger and sharper peaks, which imply higher crystallinity and larger crystals in the scaffolds.

3.2. Mechanical properties

The average compressive strength and linear shrinkage of the scaffolds after sintering are shown in Fig. 5. The compressive strength and linear shrinkage increase with increasing temperature from 1000 °C to 1200 °C for both microwave sintering and conventional sintering. The microwave-sintered scaffolds exhibit much higher strength and shrinkage. The scaffolds microwave sintered at 1200 °C show the highest average compressive strength of 45.57 MPa and the highest shrinkage of 31.8%. In contrast, the average compressive strength and shrinkage for conventional sintering at that temperature are 20.70 MPa and 24.2%, respectively. Fig. 6 shows the fracture surfaces of the sintered scaffolds. We can find that all the necks between particles disappear, grains merge together and little micropores remain in the rods when sintered at 1200 °C, thus leading to a much higher degree of densification and, therefore, higher mechanical strength.

Three main factors provide the microwave-processed scaffolds with a better mechanical strength relative to the conventionally sintered scaffolds, higher densification, finer microstructure and uniform shrinkage. When thermal energy is applied to a powder compact, the compact is densified and the average grain size increases. Hence, we

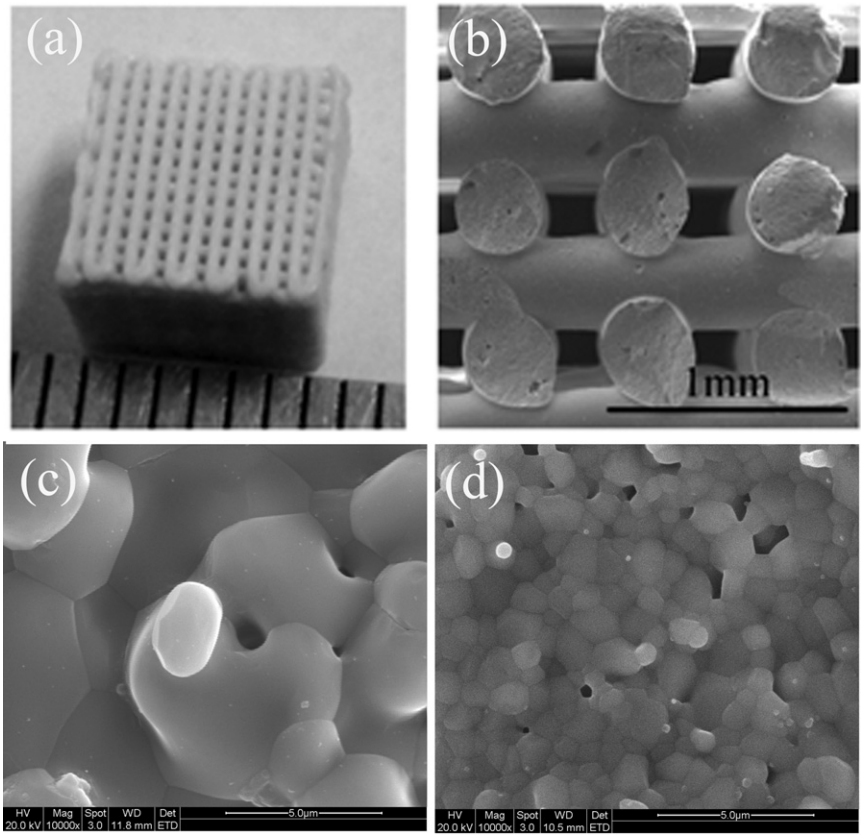

Fig. 3. Images of scaffold after sintering: (a) optical image of a porous HA scaffold microwave sintered at 1200 °C, (b) scaffold cross-section, (c) microstructures of a conventionally sintered scaffold (1200 °C, 2 h), and (d) microstructures of microwave-sintered scaffold (1200 °C, 30 min).

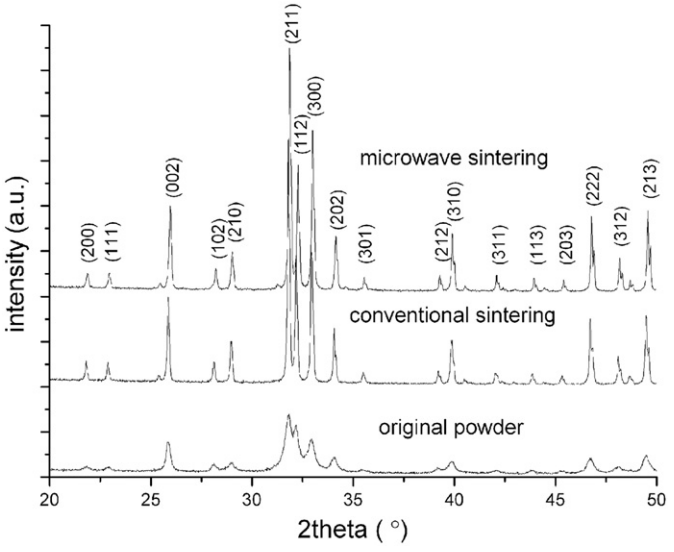

Fig. 4. XRD patterns of original HA powders and sintered scaffolds (1200 °C, 30 min for microwave sintering, 2 h for conventional sintering). Peaks were identified using JCPDS # 09-0432.

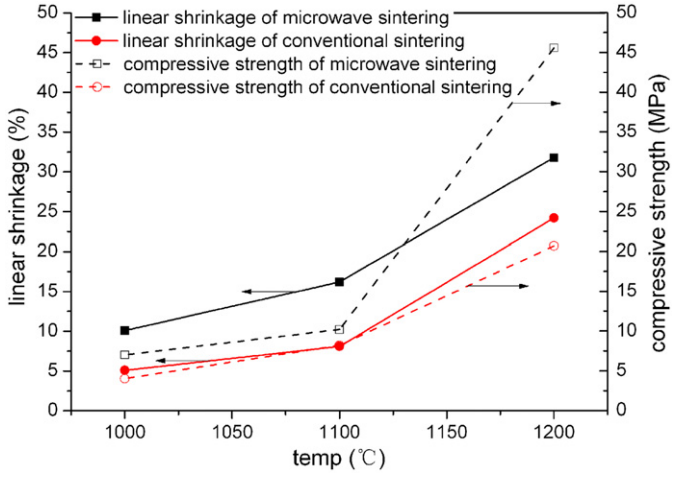

Fig. 5. Compressive strength and linear shrinkage of scaffolds sintered at different temperatures. The holding times for microwave sintering and conventional sintering are 30 min and 2 h, respectively.

can conclude that,

Sintring process = densification + grain growth

The conditions of these two factors will determine the properties of the products. Essentially, densification is a process of particles packing and merging together and pore elimination under the driving force of temperature, which shows shrinkage outside. HA is a microwave-sensitive iono-covalent ceramic [15]. When microwave energy is applied to the scaffolds, the internal molecular kinetic energy increases, which reduces the sintering activation energy and enhances the diffusion power. As a result,

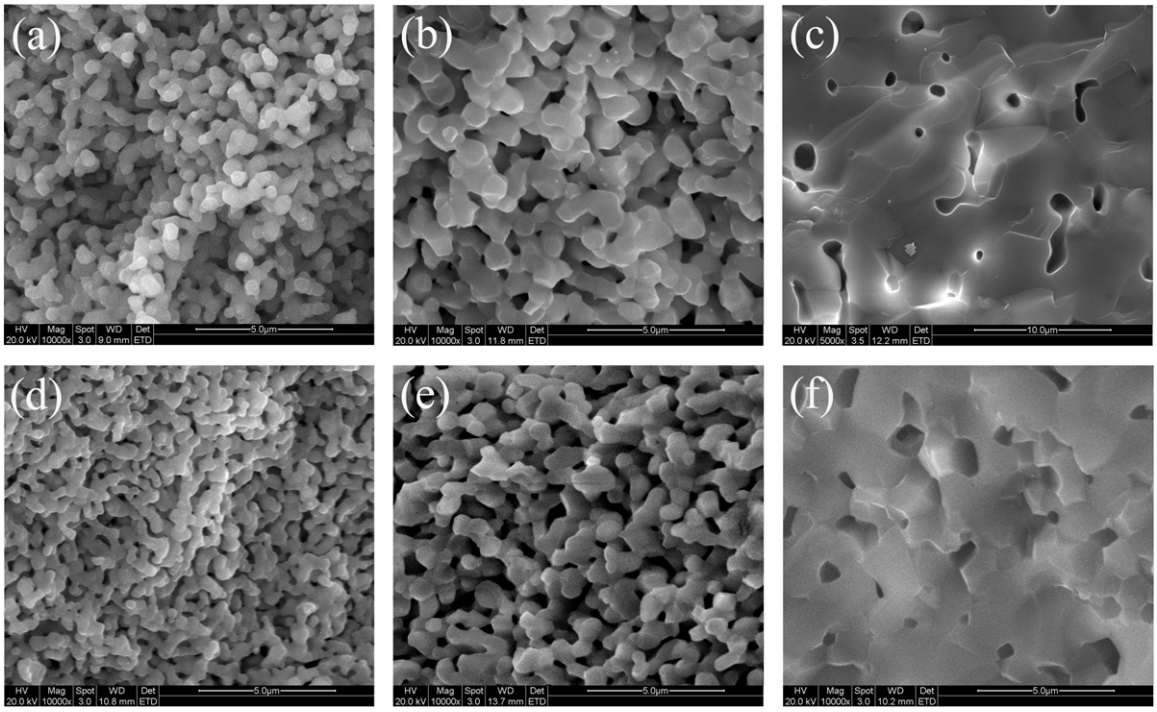

Fig. 6. Fractured surface of scaffolds conventionally sintered for 2 h at (a) 1000 °C, (b) 1100 °C, and (c) 1200 °C; and microwave sintered for 30 min at (d) 1000 °C, (e) 1100 °C, and (f) 1200 °C.

higher densification can be achieved at lower temperatures and in less time. As verified by Fig. 6(a and d), the sintering neck between particles appears in microwave sintering at 1000 °C; in contrast, the particles in conventional sintering are merely collected. Similarly, the high heating rate and short holding time of microwave sintering greatly restrain grain growth. According to the Hall–Petch relation, an inverse relationship exists between strength and grain size. Therefore, the higher densification and finer grains produced by microwave sintering will increase the compressive strength. Furthermore, the volumetric heating of microwave sintering ensures uniform heating (with almost no thermal gradient in the scaffolds) that leads to a uniform shrinkage in all directions. Thus no thermal stress is retained in the structure, which also contributes to improving the strength.

3.3. Degradation in vitro

The degradation of the sintered scaffolds was studied in vitro by immersion in physiological saline. Fig. 7 illustrates the Ca^{2+} concentrations of microwave-sintered and conventionally sintered scaffolds after immersion for 1 to 12 days. It shows that the Ca^{2+} concentration of each type of scaffold tends to increase with immersion time. The microwave-sintered scaffolds produce much higher Ca^{2+} concentrations than the conventionally sintered scaffolds under the same temperature. Furthermore, an increase in the sintering temperature decreases the Ca^{2+} concentration. For example, after 12 days of immersion, the Ca^{2+} concentrations are 5.98 ppm and 4.12 ppm for the scaffolds

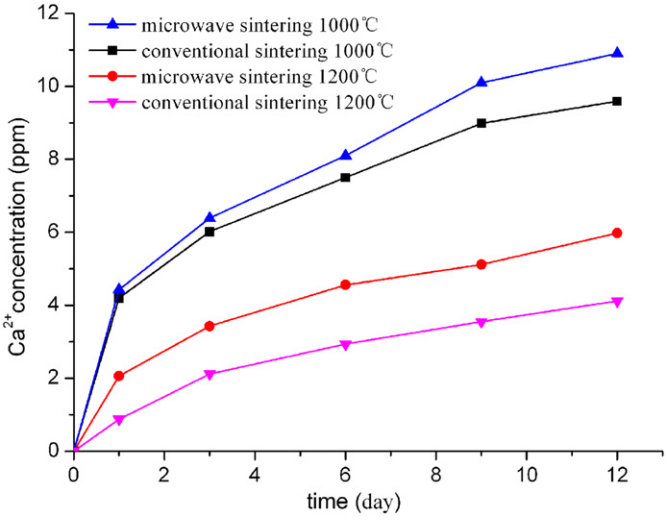

Fig. 7. Ca^{2+} concentrations for sintered scaffolds immersed in physiological saline for 1 to 12 days.

microwave sintered and conventionally sintered at 1200 °C, but 10.90 ppm and 9.59 ppm for the scaffolds sintered at 1000 °C, respectively.

The degradation mechanism of the CaP bioceramics with good crystallization is mainly driven by dissolution [24], which occurs easily at the pore and grain boundaries. As shown in Figs. 3 and 6, finer grains are obtained after microwave sintering due to the prevention of serious grain growth in the scaffolds by the use of a rapid heating process. These finer grains increase the boundaries exposed

to the solution and more Ca^{2+} is released from microwave-sintered scaffolds. With increasing temperature, both densification and grain size increase, resulting in a reduction of contact area when immersed in the solution. Therefore, the scaffolds sintered at higher temperature exhibit lower Ca^{2+}concentrations. The higher solubility of the scaffolds leads to a higher local Ca^{2+} concentration, which can be beneficial in bone growth. Local supersaturation of the constituent ions of the bone mineral phase, arising from an enhanced solution of calcium phosphate, may enhance bone growth [25]. Thus, the microwave-sintered scaffolds could present an improved biological response.

3.4. Cell–scaffold interactions

To evaluate the effect of microwave sintering on cell–scaffold interactions, we have studied the cell proliferation and the early stage of cell attachment using CCK-8 solution and SEM, respectively. Fig. 8 compares cell proliferation on the sintered scaffolds for one, three and five day, showing that the cells proliferate continuously with increasing culture time in all samples. The O.D values of microwave-sintered scaffolds are visibly higher than those of conventionally sintered scaffolds for all days within the cell culture experiments ($p < 0.05$). The attachment and spreading of the cells on the scaffolds are important processes of the cell–scaffold interactions. Fig. 9 shows SEM micrographs of cells attached on the microwave-sintered scaffold after seeding for 24 h. Fusiform-shaped cells are distributed on the top surface and sidewalls of the rod in the scaffold. Most cells are adhered tightly on the surface and spread well. The results indicate that microwave-sintered scaffolds have better bioactivity to promote cell proliferation and adhesion.

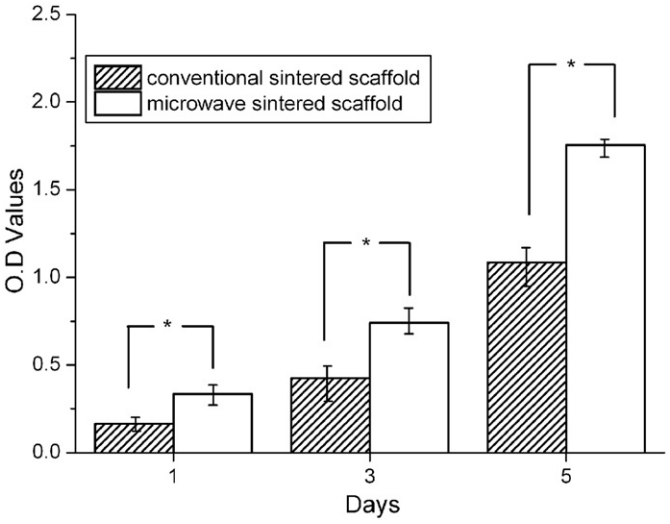

Fig. 8. Proliferation of MC3T3-E1 pre-osteoblast in the sintered scaffolds (1200 °C, 30 min and 2 h for microwave and conventional sintering, respectively).

After different sintering processes, the porous scaffolds show different biological performances, even if there is no difference in composition. A previous study shows that the surface properties of biomaterials will influence the adhesion, proliferation and growth of cells [26]. The different sintering methods produce different surface properties. The microwave-sintered scaffolds possess much finer grains. With the decrease of grain size, the surface energy of scaffolds increases. Higher surface energy encourages bone cell attachment and growth in the scaffolds. Therefore, better cell–scaffold interactions are observed in microwave-sintered scaffolds.

4. Conclusions

Porous HA scaffolds with controlled structures were fabricated by extrusion deposition technique and microwave sintering. Scaffolds with $250 \times 250\ \mu m^2$ macropores, high densification and fine grains (1200 °C, 1.12 μm) were obtained. The effect of microwave sintering on the properties of HA scaffolds was investigated through phase analysis, microstructure observation, compressive strength testing, in vitro degradation testing and cell–scaffold interaction studies and compared to that of conventional sintering. The results show significantly greater compressive strength for the microwave-sintered scaffolds due to

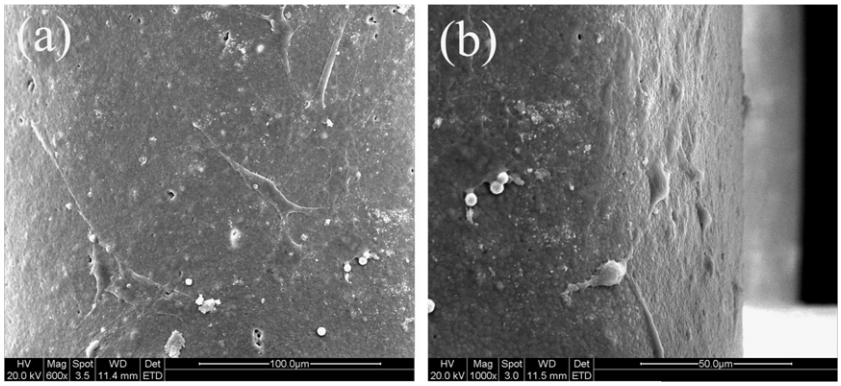

Fig. 9. SEM micrographs of cells attached to the microwave-sintered scaffold (1200 °C, 30 min): (a) fusiform-shaped cells attached to the top surface of the scaffold, and (b) cells attached to the pore walls of the scaffold.

their higher densification and finer grains. The scaffolds (with porosity of 55–60%) that were microwave sintered at 1200 °C for 30 min possessed an average compressive strength of 45.57 MPa, much higher than that of the conventionally sintered scaffolds (20.70 MPa). The much higher solubility of microwave-processed scaffolds releases more Ca^{2+} during degradation in physiological saline, which can be beneficial in bone growth. As expected, the in vitro MC3T3-E1 cell culturing study shows significant cell adhesion, spread, and growth on the scaffold surface.

Acknowledgments

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