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Rapid Vacuum Sintering: a novel technique for fabricating fluorapatite ceramic scaffolds for bone tissue engineering

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Abstract

Macroporous bioceramic scaffolds are often fabricated via the foam replica technique, based on polymeric foam impregnation with a glass slurry, followed by slow heat treatment to allow for drying, polymeric burnout, and sintering of the glass particles. As a consequence, the process is time consuming and complicated by concurrent crystallization of the glass, often leading to incomplete sintering. Our goal was to investigate the effect of heating rate on sintering behavior, architecture and mechanical properties of fluorapatite-based glass and glass-ceramic scaffolds. Glass scaffolds were prepared and sintered by rapid vacuum sintering (RVS) at 785°C under vacuum at a fast heating rate (55°C/min.) or without vacuum at a slow heating rate (2°C/min.). Two additional groups were further crystallized at 775°C/1h. XRD confirmed the presence of fluorapatite for crystallized scaffolds. All groups presented interconnected porosity with a pore size in the 500 µm range. Scaffolds produced by RVS exhibited an excellent degree of sintering while scaffolds produced by slow sintering were incompletely sintered. The mean compressive strength was significantly higher for the RVS groups (1.52 ± 0.55 and 1.72 ± 0.61 MPa) compared to the slow-sintered groups (0.54 ± 0.30 and 0.45 ± 0.26 MPa). Meanwhile, the total production time was reduced by more than 12 hours by using the RVS technique.

Keywords

Scaffolds; bioactive glass; calcium phosphate; fluorapatite; porosity

Introduction

Considering their chemistry and biocompatible degradation products, calcium phosphate-based ceramics are prime candidates for the fabrication of bone tissue engineering scaffolds further activated with cells and growth factors^{1–3}. The interest for scaffold substrates with interconnected porosity is supported by the architecture of cancellous bone and the need for efficient vascularization^{4–6}. In addition, the chemistry of calcium phosphate ceramics can be tailored to promote biodegradability with both calcium and phosphate release being beneficial to osteogenesis^{7, 8}. Previous work has shown that fluorapatite glass-ceramics were excellent candidates for the production of ceramic scaffolds for bone regeneration, by

promoting the differentiation of human mesenchymal stem cells and offering a unique nanotopography characterized by flower-shaped fluorapatite crystals^{9–11}. Multiple processing routes are available for the production of highly porous ceramic scaffolds^{12, 13}, including state of the art 3D printing^{14, 15}, inclusion of porogens¹⁶, as well as the well-established foam replica technique^{17–20}. One common feature to these techniques is the sintering step that requires firing at high temperature for long durations^{16, 21–23}. This is true for apatite-based ceramics with sintering temperatures in excess of 1100°C and durations of up to several hours²⁴. We recently demonstrated that by careful adjustment of the chemical composition, adequate sintering of fluorapatite glass-ceramics could be obtained by heat treatment at 775°C for one hour⁹. However, the commonly used foam replica technique, which is our preferred technique for the production of fluorapatite scaffolds, with interconnected porosity and adequate pore size range, also requires extended heat treatment durations^{25, 26}. This is to permit adequate burnout and elimination of the polyurethane sacrificial foam substrate without collapse of the green unconsolidated scaffold structure, consisting of hollow struts with an outer diameter of 100 to 600 µm, depending on the number of pores per inch (ppi) of the template foam²⁷. In practice, heating rates in the order of 1 to 2°C per minute with an extended dwell of 1 to 4 hours at around 400°C are therefore needed to avoid cracking within the struts and produce glass-ceramic scaffolds with mechanical integrity²⁸.

Concurrently, sintering of glass-ceramic scaffolds is complicated by nucleation and crystallization processes that are known to start at lower temperature with slow heating rates. It is well established that crystal formation in most glass-ceramic systems is associated with an increase in glass matrix viscosity, which in turn may prevent adequate sintering^{29, 30}. The concept of a sintering window defined by the temperature range between the glass transition temperature and the onset of crystallization was therefore proposed for glass-ceramic systems. Clearly, the slow heating conditions required for polyurethane template burnout and elimination narrow this sintering window and can potentially hinder the sintering process of fluorapatite glass-ceramic scaffolds by favoring earlier crystallization^{31–35}.

Meanwhile, sintering under vacuum is commonly used for the fabrication of dental ceramic restorations and known to reduce the amount of structural residual porosity by a factor of 10, even though fast heating rates in excess of 50°C per minute and very short dwells at temperature of less than 5 minutes are commonly used^{36, 37}.

The purpose of the present study was therefore to investigate the effect of sintering under vacuum with a fast heating rate and short dwell at temperature, named rapid vacuum sintering (RVS) for simplicity, on the microstructure, overall architecture and compressive strength of fluorapatite glass-ceramic scaffolds.

The hypothesis tested was that RVS would lead to adequate sintering of fluorapatite glass-ceramic scaffolds compared to scaffolds produced using conventional sintering conditions involving slow heating rates and extended dwell times.

Materials and methods

Glass preparation

A fluorapatite-based glass composition (32SiO₂–21MgO–24CaO–2Na₂O–4 K₂O–11CaF₂–5P₂O₅ mol.%, with minor amounts of Nb₂O₅ and Al₂O₃) was prepared as previously described⁹. Briefly, after mixing reagent grade high purity powders of SiO₂, MgCO₃, CaCO₃, Na₂CO₃, K₂CO₃, CaF₂ and P₂O₅ (Sigma-Aldrich, St Louis, MO, USA), batch ingredients were twice melted at 1525°C for 3 h in platinum crucibles. The molten glass was cast into pre-heated cylindrical graphite molds to form rods (10 mm in diameter; 60 mm in length), and furnace-cooled to room temperature. Rods were reduced to powder and sieved to a particle size of 45 µm or less.

Scaffold fabrication

A slurry was prepared by mixing the glass powder with distilled water containing 0.5 wt.% polyvinyl alcohol (PVA) binder, using a liquid to powder ratio of 0.62. Scaffolds were fabricated by immersion of polyurethane (PU) foam cylinders (12 mm in diameter × 6 mm in height) in the glass slurry. The excess of slurry was eliminated by using compressed air under gentle pressure. Scaffolds were dried at room temperature for 15 minutes and the impregnation process was repeated. Scaffolds were then dried at room temperature for 90 minutes prior to sintering. Optimal duration for this particular scaffold volume was determined by monitoring weight loss over time until no further weight loss occurred. Scaffolds were sintered according to heat treatments listed in Table 1. All scaffolds were first sintered either by rapid vacuum sintering (RVS) or by slow sintering without vacuum (SLS) at 785°C, with a dwell time of either 2 or 60 minutes. After this first sintering cycle, scaffolds were immersed in the glass slurry for a second coat, followed by a second sintering cycle at 785°C without vacuum and a dwell time of either 2 (RVS-2-2; SLS-2-2) or 60 minutes (SLS-60-60). An additional group sintered by RVS was subjected to a crystallization heat treatment at 775°C for 60 minutes at a heating rate of 2°C/min (RVS-2-2-60) in order to promote controlled nucleation and crystallization of fluorapatite. One last group was sintered using a high heating rate of 55°C/min. and no vacuum.

Foam, glass and scaffold characterization

Thermogravimetric and differential thermal analyses (DTA) of the polyurethane foam were performed at a heating rate of either 2 or 55°C per minute to determine the temperature range for thermal decomposition of the polymeric scaffold (Q600 DTA/TGA, TA Instruments). Differential thermal analyses were further performed on the powdered glass under nitrogen gas flow to determine glass transition and crystallization temperatures as a function of heating rate. Analyses were performed at 2, 10, 20, 30, 40 and 55 °C/min. up to 1100°C. Aluminum oxide (99.99%) served as reference standard. Glasses or glass-ceramics were analyzed by x-ray diffraction (XRD) on powdered scaffolds. Scans were performed in the two-theta range 10–90° (40 kV; 44 mA) in Bragg-Brentano configuration (SmartLab, Rigaku Americas). Where applicable, peak positions were determined using PDXL-2 analysis software (Rigaku Corporation). Peak position was calibrated using silicon powder standard (NIST, 640d).

Bioactivity

The bioactivity of the glass was assessed by immersion in simulated body fluid (SBF) prepared according to Kokubo's formulation³⁸ revised by Bohner *et al.*³⁹. Glass specimens were immersed in SBF and incubated at 37°C. The solution was replaced every 48h. Grazing incidence x-ray diffraction was performed before and after incubation for 8 days.

Scaffold architecture and degree of sintering

Scaffold architectural characteristics and degree of sintering were assessed by scanning electron microscopy (SEM). Specimens were ultrasonically cleaned and sputter-coated with a thin layer of gold prior to SEM examination (Hitachi S-4800 field emission SEM). Mean pore diameter and strut thickness were measured on digital micrographs using NIH image analysis software. The degree of sintering was also assessed by x-ray microtomography (μ CT - Skyscan 1272, Bruker). Scaffolds from all groups were scanned using a source voltage of 70 kV and a source current of 142 μ A, with a 0.5mm aluminum filter. The samples were rotated to 180° with a rotation step of 0.25° and a frame averaging of 10. The pixel size was 6 μ m. The acquisition images were reconstructed using NRecon 1.6.9.18 without corrections, except for the specific misalignment for each acquisition. 3D reconstructions were visualized using CT-Vox software while mean pore diameter and pore size distribution were measured using CT-An software²⁵.

The density of the scaffolds ($d_{scaffold}$) was calculated from the measured mass and dimensions ($n=10$). The density of the scaffold struts (d_{strut}) was determined by helium pycnometry ($n=10$) (AccuPyc II 1340, Micromeritics) and compared to the known theoretical density of the glass⁹ to assess the quality of the sintering process. The overall macroporosity P of the scaffolds was calculated according to the following equation (1):

$$P=1-\frac{d_{scaffold}}{d_{strut}} \quad (1)$$

Compressive strength

The mean compressive strength of sintered cylindrical scaffolds (10 mm in diameter; 5 mm in height; $n=10$) was investigated using a Universal Testing machine at a crosshead speed of 0.5 mm/min. according to ASTM D1621-10⁴⁰. Due to the step-wise failure mode characteristic of scaffolds, the mean maximum breaking load was calculated by averaging the first five highest peaks corresponding to a compression level leading to a deflection equal to or lower than 30% of the height of each specimen. Peaks occurring beyond this point were discarded to eliminate bias associated with crumbling and subsequent densification of scaffold specimens.

Statistical analyses

Results were analyzed by ANOVA and Tukey's test to detect statistically significant differences. A p -value of less than 0.05 was considered statistically significant.

Results

Thermal behavior

Results from thermogravimetric and differential thermal analyses of the polyurethane foam are summarized in Table 2. Characteristic temperatures increased significantly with heating rate, with completion of thermal decomposition at 385°C at a heating rate of 2°C per minute and 485°C at a heating rate of 55°C/min. Results from differential analyses of the glass as a function of heating rate are displayed in Figure 1. Characteristic temperatures are summarized in Table 3. The glass transition temperature increased linearly with heating rate ($R^2=0.98$). DTA traces were characterized by the presence of two exotherms, except for the glass heated at 55 °C/min., which exhibited only one large exotherm. Previous work has shown that the first exotherm, occurring between 818 and 910 °C, was associated with the formation of fluorapatite ($\text{Ca}_5(\text{PO}_4)_3\text{F}$), while the second exotherm was attributed to the concurrent crystallization of akermanite ($\text{Ca}_2\text{MgSi}_2\text{O}_7$) and forsterite (Mg_2SiO_4)⁹. There was a linear relationship between the temperature of the first exotherm and heating rate up to 40°C per minute ($R^2=0.96$). Meanwhile, the processing window, classically defined as the temperature difference between the glass transition and the onset of crystallization, increased with heating rate, however, this relationship was not linear (Table 3).

Crystalline phases and bioactivity

X-ray diffraction analyses (Figure 2) confirmed the presence of a glassy phase, together with traces of fluorapatite for the scaffolds heat treated for 2 minutes, regardless of heating rate (Figure 2A and 2C). Fluorapatite ($\text{Ca}_5(\text{PO}_4)_3\text{F}$; PDF 15-876), was present after heat treatment for one hour (Figure 2B and 2D) and associated with a higher degree of crystallinity with a slow heating rate (Figure 2D), together with the presence of traces of akermanite ($\text{Ca}_2\text{MgSi}_2\text{O}_7$; PDF 35-592). Grazing incidence x-ray diffraction (GIXRD) revealed surface crystallization of both akermanite and fluorapatite (Figure 3A). Meanwhile, high resolution SEM images of scaffold surfaces showed the presence of flower-shaped crystals identified as fluorapatite on all scaffolds (Figure 3B). In addition, scaffolds produced by slow sintering exhibited islands in darker contrast (Figure 3C), surrounded by severe microcracking (indicated by arrows) and identified as akermanite crystals. GIXRD patterns of the base glass before and after incubation in SBF for one week are displayed in Figure 4A and B, respectively. The major reflection corresponding to carbonated hydroxyapatite is clearly present after incubation (Figure 4B).

Sintering behavior

The macrostructure of scaffolds sintered under various experimental conditions is shown in Figure 5(A through D). There was no usable specimen in the group sintered at 55°C/min. with no vacuum due to the lack of mechanical integrity that led to spontaneous crumbling. Scaffolds in all other groups presented interconnected macroporosity. Smooth strut surfaces were present for scaffolds produced by rapid vacuum sintering, independently of heat treatment duration (Figure 5A and 5B). Exposed strut sections (Figure 5A) confirmed the excellent quality of the sintering process with only a few micropores remaining after sintering. Triangular pores corresponding to internal strut defects remaining after burnout of the polyurethane template were also observed. The surface texture present after subsequent

heat treatment at 775°C for one hour is consistent with surface crystallization (Figure 5B). In contrast, the macrostructure and strut architecture associated with slow sintering schedules was characterized by very rough surfaces (Figure 5C and 5D). Although the glass particles were clearly rounded in both cases, there was no or minimal particle coalescence after slow sintering for 2 minutes (5A). Meanwhile, although strut surfaces still appeared very rough, a greater degree of particle coalescence was observed after slow sintering for a total of 2 hours (5D). Scaffold architectural characteristics including mean strut thickness and pore diameter as determined from both SEM and micro-CT images are listed in Table 4, together with the mean percent porosity calculated from density measurements. When measured from SEM micrographs, mean strut diameter ranged from 169 to 209 μm , while mean pore diameter ranged from 496 to 570 μm . Mean pore diameter determined from micro-CT analyses were slightly lower than those measured from SEM images but followed the same trends (Table 4). Meanwhile pore size distributions were comparable with a minimum pore size of 339 μm and a maximum of 644 μm across all experimental groups. The mean percent porosity of the groups subjected to RVS with or without crystallization heat treatment (73.4 ± 3.8 and $76.1 \pm 2.9\%$, respectively) was significantly lower than that of the groups processed by slow sintering, regardless of duration (82.9 ± 3.0 for SLS-2-2 and 83.0 ± 3.4 for SLS 60-60). Meanwhile, the mean strut density was significantly higher for the group sintered by RVS ($99.8 \pm 0.6\%$) compared to the groups produced by slow sintering (94.0 ± 1.0 and $95.4 \pm 0.4\%$).

Characteristic 2D images from microcomputed tomography analyses of the scaffolds (Figure 6A–D), confirmed adequate sintering for the scaffolds produced by RVS (6A and 6B), clearly revealing fully dense struts and minimal residual porosity or defects. Meanwhile numerous defects including triangular pores with sharp apices were present in the scaffolds prepared by the conventional slow sintering technique (Figure 6C and 6D). Representative 3D images used to establish pore size distributions are displayed in Figure 7A–D.

Compressive strength

The mean compressive strength as a function of heat treatment schedule is listed in Table 4. Rapid vacuum sintering led to a three-fold increase in mean compressive strength compared to conventional slow sintering, regardless of total heat treatment duration.

Discussion

The observed thermal behavior of the glass as a function of heating rate is in excellent agreement with classical literature analyzing the effect of heating rate on glass transition and thermal characteristics of glasses^{41–44}. More specifically, the dependence of the glass transition temperature on the heating rate in differential thermal analysis experiments is classically described by Kissinger's equation⁴⁵ relating heating rate and activation energy to glass transition temperature. Our results fit this analysis, with the widening of the processing window associated with higher heating rates as a direct consequence of higher crystallization temperatures. Meanwhile, thermogravimetric analyses of the PU foam indicated that higher heating rates also retarded decomposition of the foam, permitting retainment of a structural support at higher temperatures during the sintering of the glass with particle coalescence

prior to disintegration of the PU support. This may have played an important role in achieving adequate sintering under RVS conditions. Concurrently, sintering under vacuum further enhanced pore elimination and final density. As mentioned earlier, vacuum sintering is routinely used in the fabrication of ceramic dental restorations^{46–48}. The vacuum is released at high temperature and the associated relative increase in pressure, while the ceramic is in a softened state, permits adequate pore elimination and densification. Our results show that the combination of high heating rates and vacuum sintering led to an excellent degree of sintering, as indicated by the mean density of the struts after RVS, compared to slow sintering and corresponding to a degree of sintering of 99.8% vs. 95%. Meanwhile sintering at high heating rates without vacuum led to spontaneous crumbling of the scaffolds (results not shown). The quality of the sintering process under vacuum is well reflected in the mean compressive strength values with a significant three-fold increase compared to slow sintering without vacuum. Although the compressive strength values remained modest, results compare well with previously published data for bioactive glass scaffolds^{25, 49}. It is interesting to note that groups sintered by RVS also exhibited a significantly lower amount of porosity (73.4 to 76.1%) and slightly reduced pore diameter compared to scaffolds sintered by slow sintering without vacuum (82.9 to 83.0%). This can be explained by greater shrinkage of the strut architecture as a consequence of enhanced sintering and could have also contributed to the observed increase in compressive strength. However, in combination with this level of interconnected porosity and large pores in the 500 micrometer range^{50, 51}, the compressive strength values appear adequate for bone tissue regeneration in non load-bearing defects where significant biodegradation is expected within a 12 to 24-week window, depending on defect size and model^{1, 52}.

The crystalline phases and microstructure observed in the present work are in line with results from previous work. The bioactivity of this glass composition was confirmed by GIXRD, detecting the presence of carbonated hydroxyapatite at the glass surface after incubation in SBF⁵³. Bioactivity ensures a chemical bond between scaffold material and surrounding tissues and is known to promote *de novo* bone formation^{54, 55}. Flower-shaped fluorapatite crystals crystallize readily in this system and akermanite was expected to crystallize at higher temperatures as a secondary silicate phase rich in calcium and magnesium⁵⁶. However, surface crystallization of akermanite was also associated with significant microcracking within and around the crystals. This is indicative of a thermal expansion mismatch between akermanite crystals and surrounding glassy matrix. The thermal expansion of melilite phases, including akermanite has been well studied, with linear values comprised between 10 and $11 \times 10^{-6}/^{\circ}\text{C}$ ⁵⁷. Crystallization of akermanite is likely to induce compositional changes within the surrounding glassy matrix, possibly associated with a decrease in the coefficient of thermal expansion. This would explain the observed macrocracking due to a greater contraction of the akermanite crystals upon cooling within the glassy matrix. This also indicates that the crystallization of akermanite is not desirable in this particular glass system, as it may weaken the strut structure.

Conclusions

Rapid vacuum sintering led to the production of fully sintered fluorapatite-based glass and glass-ceramic scaffolds with interconnected porosity and high strut density, while permitting significant timesaving in excess of 12 hours for the full sintering process.

Scaffolds produced by RVS also exhibited a three-fold increase or more in mean compressive strength, attributed to an increase in degree of sintering from 94.0 to 99.8%.

Fluorapatite crystallization occurred upon heat treatment of the scaffolds together with traces of akermanite regardless of heating rate. Combined with bioactivity, the presence of submicrometer flower-shaped fluorapatite crystals at the scaffold surfaces is expected to be beneficial to local cell anchorage and bone regeneration.

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Figure 1.
Differential thermal analysis thermograms of the base glass as a function of heating rate.

Figure 2.

X-ray diffraction patterns of the various groups. A: RVS-2-2; B: RVS-2-2-60; C: SLS-2-2; D: SLS-60-60.

Figure 3.

Surface characterization of group D (SLS-60-60). A: Grazing incidence XRD pattern demonstrating surface crystallization of akermanite and fluorapatite. B: Characteristic scanning electron micrograph confirming the presence of flower-shaped fluorapatite crystals. C: Scanning electron micrograph revealing the presence of darker contrast akermanite crystals surrounded by significant microcracking (arrows).

Figure 4. Grazing incidence XRD patterns of the glass before (A) and after (B) incubation in SBF.

Figure 5.
Scanning electron micrographs of the glass and glass-ceramic scaffolds. A: RVS-2-2; B: RVS-2-2-60; C: SLS-2-2; D: SLS-60-60.

Figure 6. Micro-CT images of the sintered glass and glass-ceramic scaffolds. A: RVS-2-2; B: RVS-2-2-60; C: SLS-2-2; D: SLS-60-60.

Figure 7. 3D visualization from micro-CT images of the sintered glass and glass-ceramic scaffolds. A: RVS-2-2; B: RVS-2-2-60; C: SLS-2-2; D: SLS-60-60. (Scale bar: 500 µm)

Table 1

Heat treatment schedules for the various experimental groups.

Group	RVS-2-2		RVS-2-2-60			SLS-2-2		SLS-60-60	
Heat treatment	1	2	1	2	3	1	2	1	2
Heating rate (°C/min)	55	55	55	55	2	2	2	2	2
Vac. level (Atm)	0.1	1	0.1	1	1	1	1	1	1
High temp (°C)	785	785	785	785	775	785	785	785	775
Hold time (min.)	2	2	2	2	60	2	2	60	60

Table 2

Polyurethane foam thermal characteristic temperatures as a function of heating rate.

Heating rate (°C/min.)	Onset of weight loss (°C)	End of weight loss (°C)	Endotherm 1 (°C)	Endotherm 2 (°C)
2	249	385	261	347
55	290	485	325	445

Table 3

Thermal behavior characteristic temperatures as a function of heating rate.

Heating rate (°C/min.)	Glass transition T_G (°C)	Onset of crystallization (°C)	Processing window (°C)	Exotherm 1 (°C)	Exotherm 2 (°C)
2	646	792	146	818	925
10	659	830	171	848	965
20	665	853	188	873	995
30	676	870	194	892	1015
40	681	882	201	906	1038
55	698	877	179	910	N/A

Table 4

Architectural characteristics and mean compressive strength for the various experimental groups.

TREATMENT GROUP	RVS-2-2	RVS-2-2-60	SLS-2-2	SLS-60-60
Strut thickness (μm)	$187 \pm 52^{\text{ab}}$	$192 \pm 26^{\text{ab}}$	$209 \pm 31^{\text{b}}$	$169 \pm 42^{\text{a}}$
Strut density (g/cm^3)	$2.923 \pm 0.017^{\text{a}}$	-	$2.753 \pm 0.030^{\text{b}}$	$2.792 \pm 0.011^{\text{b}}$
Mean pore diameter (SEM) (μm)	$496 \pm 133^{\text{a}}$	$502 \pm 92^{\text{a}}$	$514 \pm 85^{\text{a}}$	$570 \pm 124^{\text{a}}$
Mean pore diameter (Micro-CT) (μm)	$469 \pm 57^{\text{a}}$	$471 \pm 58^{\text{a}}$	$498 \pm 44^{\text{a}}$	$494 \pm 43^{\text{a}}$
Pore diameter range (Micro-CT) (μm)	339 – 621	339 – 644	395 – 643	395 – 597
Porosity (%)	$73.4 \pm 3.8^{\text{a}}$	$76.1 \pm 2.9^{\text{a}}$	$82.9 \pm 3.0^{\text{b}}$	$83.0 \pm 3.4^{\text{b}}$
Mean compressive strength (MPa)	$1.52 \pm 0.55^{\text{a}}$	$1.72 \pm 0.61^{\text{a}}$	$0.54 \pm 0.30^{\text{b}}$	$0.45 \pm 0.26^{\text{b}}$

* Identical letters denote no statistically significant difference ($P > 0.05$)