

Porous hydroxyapatite scaffolds fabricated from nano-sized powder via honeycomb extrusion

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Abstract

In this study, we have developed hydroxyapatite (HA) scaffolds for synthetic bone graft from nano-sized HA particles using ceramic extrusion. We also demonstrate that these HA scaffolds show enhanced compressive strength (29.4 MPa), whilst possessing large pore sizes ($> 600 \mu\text{m}$) that are suitable for bone grafting. The extrusion process involved forming a ceramic paste by mixing the HA powder with a binder and distilled water. The ceramic paste was then fabricated using a ram extruder that was fitted with a honeycomb die to impart large, structured pores. Several green bodies were extruded and then subjected to the same drying and thermal debinding treatment. The samples underwent three different sintering temperatures and two varied dwell times, in order to determine the optimum sintering parameters. The scaffolds were then analysed for their chemical, physical, mechanical and biological properties to elucidate the effects of the sintering parameters on extruded HA scaffolds. The results revealed that the nano-sized particles exhibited a high sinterability, and XRD analysis showed phase purity until 1300°C . At 1300°C , trace amounts of phase impurities were detected, however, scaffolds sintered at this temperature exhibited the highest mean compressive strength. The findings demonstrated that traces of phase impurities were not detrimental to the scaffold's compressive strength. In addition, scanning electron microscopy and density measurements revealed a highly densified solid phase was attained. Copyright © 2017 VBRI Press.

Keywords: Hydroxyapatite, ceramic extrusion, nano-hydroxyapatite, porosity, scaffold.

Introduction

The need to replace defective bone arising from disease, infection and post-traumatic complications, is on the rise, especially among the elder population. Consequently, there is an urgent need for a large number of bone repair substitute materials with good performances [1-4]. Substitute materials used for bone fracture include autograft, allograft and xenograft materials [5]. Among them, autografts are considered the 'gold standard' of bone defect repair, however, the implantation of autografts requires a second invasive surgery, which introduces new complications, and are limited by their availability [5]. Moreover, the implantation of allografts and xenografts may be prone to trigger immunological rejection. Synthetic materials offer an alternative approach to bone repair, as they avoid the aforementioned complications, and can be fabricated in abundance [3].

The bone is comprised of inorganic and organic phases, of which the former provides bones with their compressive strength [6]. The inorganic phase is formed from carbonated hydroxyapatite, and as such the synthetic equivalent (HA) ($\text{Ca}_{10}(\text{PO}_4)(\text{OH})_2$), has been investigated for bone grafting. HA has been found to

demonstrate good biocompatibility and bioactivity [7]. Further to this, HA has demonstrated biological properties that are able to guide and stimulate bone growth, thus forming a secure bond with the surrounding tissue [8].

In addition to biological properties, a synthetic scaffold should possess a porous architecture, to allow for bone ingrowth, and should also exhibit suitable mechanical strength [4]. A survey of the literature shows an inconclusive recommendation of pore sizes, but do range up to $400 \mu\text{m}$ [9, 10]. Nevertheless, the incorporation of porosity is important as it allows nutrient transport and cell attachment, which subsequently enhances the regeneration of natural bone [11]. However, porosity and mechanical strength are mutually exclusive, and hence lies the difficulty in fabricating a synthetic HA scaffold with good performances. Mechanical properties are of equal importance, as the natural bone is under several forms of stress, but principally compression. The limitation in mechanical strength has prompted a search for new methods of improving the property, whilst maintaining porosity and bioactivity [7].

There are several methods that can be used to fabricate a HA scaffold with a porous architecture. Traditional

methods of fabricating porous HA scaffolds have resulted in a typical compressive strength below 10 MPa [12]. Characteristically, such techniques demonstrate random, unpredictable and irreproducible porosity, with poorly interconnected and non-uniform pore distribution [13]. Recently, freeze-casting and facile emulsion templating techniques have been investigated as alternative methods to fabricating a porous HA scaffold, however, with values of 0.67-0.88 MPa [14] and 4.26 MPa [15], respectively, have not provided much of an improvement.

To overcome the low mechanical strength, the present state of the art has demonstrated that an ordered and controlled porosity tends to result in scaffolds with higher mechanical strengths [16, 17]. Solid free form (SFF) techniques are such methods that are able to produce periodic porosity due to their high spatial resolution [17]. The mechanical strength of HA scaffolds fabricated using SFF have produced mixed results, however, Houmard et al. (2013) [18] were able to fashion HA scaffolds with a compressive strength of ~50 MPa. This is a significant improvement with the respect to the aforementioned techniques. Ceramic extrusion is one such technique capable of fabricating a periodic and structured porous HA scaffold. In extrusion, a ceramic paste, which is a highly viscous slurry comprised of a ceramic, organic additives and a solvent, is forced through an orifice. The process does not require high temperatures during the shaping process, and, compared to SFF methods, is a low-cost technique [19]. Using a honeycomb die, similar to that used in the automotive industry for fabricating the catalytic converter substrate, ceramic extrusion can be used to fabricate open pores that span the length of the scaffold.

Aside from the fabrication method, other factors that can influence the mechanical properties of a HA scaffold include the starting raw materials and the thermal treatments applied. For instance, the use of HA powders with nano-sized particles are expected to result in higher bioactivity, due to their more favourable surface topography [20]. In addition, hydroxyapatite scaffolds fabricated using nano-size particles have also been shown to exhibit higher mechanical properties over micro-sized particles [21, 22]. Nano hydroxyapatite powders have been used before for fabricating HA scaffolds, however ceramic extrusion remains an unestablished technique for fabricating porous hydroxyapatite, and thus have not been investigated together. Therefore, the aim of this study was to fabricate hydroxyapatite scaffolds with a structured porous architecture using nano-sized HA powder and ceramic extrusion, and to study their chemical, physical, mechanical and biological properties.

Experimental

Materials

A commercially available hydroxyapatite powder (Purum grade, Sigma Aldrich) was used for this study. The HA powder was plasticised using a cellulose ether-derived organic additive (Dow Chemicals), and distilled water as the solvent.

Fabrication process

Mixing was done in two stages. First, the dry components (ceramic and organic additives) were weighed and then mixed using a high-energy speed mixer. Once mixed, the solvent was added and then mixed. Addition of the solvent was done in increments, typically added after 2 minute mixing segments until a single coalesced paste was obtained. Mixing was continued for a further 5 minutes following coalescence of the ceramic paste. The dry components were mixed first to ensure a homogenous distribution of the ceramic component and polymer. Once a suitable paste was obtained, it was then extruded using a ram extruder and honeycomb die that was designed in-house. A servo-hydraulic press was employed to actuate the piston. The obtained extrudates were then allowed to dry for twenty-four hours before being thermally debound at 400 °C for five hours at rate of 1°C/min in a muffle furnace. The samples were subsequently sintered using a muffle furnace at either 1200, 1250 or 1300 °C and dwell times of either three or five hours. The heating and cooling rate were set to 5 and 2.5 °C/min, respectively. Once a fired body was achieved, the samples were then sectioned and ground until HA scaffolds with a 3x3 cell structure were obtained. Further sectioning of the extrudate was performed to obtain struts for density measurements. The biological tests were performed on HA pellets formed from the same HA paste. The paste was pressed using a 10 mm die. The green pellets were then allowed to dry before sintered at the aforementioned sintering temperatures. All biological samples were held for five hours.

Characterization

Powder morphology: A scanning electron microscope (SEM, Inspect F50, FEI) was used to examine the HA powder morphology. The HA powders were coated with gold prior to analysis.

Optical analysis: Optical microscopy (BA310Met-T, Miotic) was utilised to determine the physical structure of the HA scaffolds (n = 3).

Bulk porosity: A helium pycnometer (Accupyc 1340, Micromeritics) was employed to obtain the bulk porosity of the HA scaffolds (n=3) using the following equation:

$$P = \left[1 - \frac{\rho_a}{\rho_t} \right] \times 100$$

Where P is the bulk porosity; ρ_a is the apparent density, calculated using an electronic scale and a vernier caliper with a resolution of 0.01 mm; and ρ_t is the true density of the scaffold calculated using a helium pycnometer. A balance with a resolution of 0.1 mg was used to weigh the scaffolds. The density of the scaffold struts were measured using the Archimedes' method (n = 5).

Mechanical: The mechanical test was performed using a universal testing machine (Roell, Zwick) in compression mode. A load cell of 20 kN was employed and a displacement rate of 1 mm/min was opted for (n = 10).

Chemical: Chemical analyses of the powder and scaffolds were performed using an XRD (D5000 Changer, Siemens) and FTIR (Frontier, Perkin Elmer). Prior to chemical analyses, the HA scaffolds were ground. In the case of FTIR analysis, the powders were pressed with KBr before readings were taken. Chemical analyses were performed on the samples sintered with a dwell time of three hours. The XRD results were analysed using the PDF-4+ software.

Microstructure: The scaffold microstructure and fracture surface were examined using a SEM. The samples were coated with gold prior to analysis.

Biological: For cell viability investigation, sintered disk samples were fabricated using the HA powder and the binder. The samples were sterilised by soaking once in 70% ethanol for thirty minutes followed by two washes with PBS. hES-MPs cells (Cellartis, Sweden) with a concentration of 100,000 cells/ ml for each well were seeded onto the HA disks. The cells were cultured in medium containing Dulbecco's modified eagle medium (DMEM, Lonza, Belgium), 10% foetal bovine serum (FBS Labtech, UK), 2mM glutamine (Sigma Aldrich, UK), 100 U/ml penicillin (Sigma Aldrich, UK) and 100 µg/ml streptomycin (Sigma Aldrich, UK). Gelatin-coated T75 flasks were used to transfer the cells. The cells were cultured in an incubator at 37 °C under 5% CO₂ and complete medium change was performed every two days. Cells were checked using an optical microscope on a daily basis to ensure they are healthy. After obtaining more than 80% confluency, splitting was performed on two flasks. Again, the aforementioned coating solution was performed on two new T75 flasks. A total number of eight passages were performed. For the MTT assay, the medium was removed and the cells were washed twice with PBS per well. 1 ml of MTT solution (Sigma Aldrich, UK) was added per well and left in an incubator at 37 °C for 40 minutes. The MTT solution was then removed and 300 µl acidified isopropanol was added to elute the stain. The absorbance was read at 540 nm using a spectrophotometer (ELX800, Biotek, USA). All assays were carried out in quadruplicates. The data presented as percent activity of untreated control cells. Statistical analysis on the biological tests were carried out using Graphpad Prism 6 (Graphpad, USA). A parametric one-way Anova with a Tukey post-hoc test multiple comparison was performed to determine statistical significance between the results. The statistical significance threshold was $p < 0.05$.

Results and discussion

Starting powder

Despite its promising potential, ceramic extrusion continues to be an untested method for the fabrication of bioceramic scaffolds. The aim of the present work was to realise its potential by utilising nano-sized hydroxyapatite particles for the fabrication of HA scaffolds, and to investigate the structure-property relationship of the fabricated scaffolds. **Fig. 1** is a collection of

SEM micrographs used to illustrate the particle morphology of the HA powder used for this study. The image alludes to the size of the primary particles, which were evidently in the nano-range (**Fig. 1(d)**). It was difficult to discern the exact size of the primary particles due to their tendency to agglomerate, as well as the limitations of the SEM instrument and the preparation method used. The agglomeration observed by the primary particles occurred to a large extent, due to the high surface energy of nano-sized ceramic particles, and had a tendency to form a near-spherical shape, as illustrated in **Fig. 1(b)**. The primary particle size was believed to be below 100 nm (**Fig. 1(d)**), and produced agglomerates of less than 20 µm (**Fig. 1(b)**).

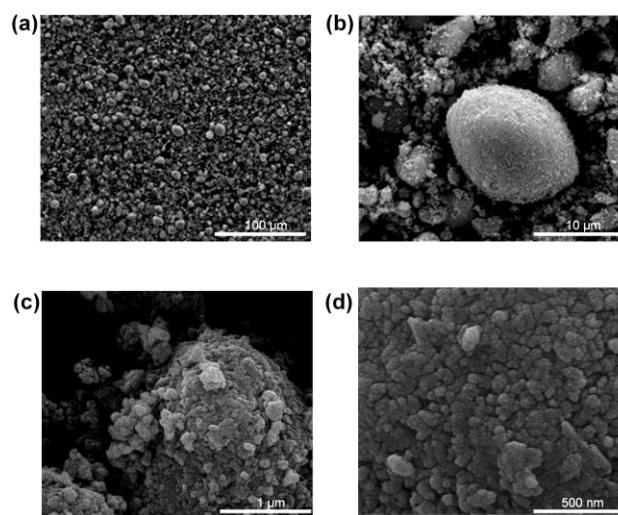


Fig. 1. SEM micrographs of the as received hydroxyapatite powder with magnifications at (a) 1,000, (b) 10,000, (c) 100,000 and (d) 200,000.

Scaffold properties

Using the nano-HA powder and ceramic extrusion, porous scaffolds were fabricated using sintering temperatures of 1200, 1250 and 1300 °C. The role of sintering is to densify the HA scaffolds and form one coalesced body from the starting raw powder. The higher the sintering temperature, the higher the densification the particles will undergo, which consequently leads to higher mechanical properties.

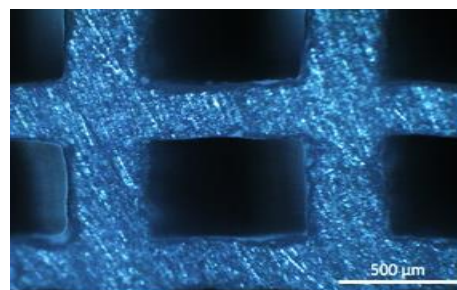


Fig. 2. An optical microscope image of a scaffold, displaying the physical characteristics. The original magnification of the optical microscope was 5x.

However, at elevated sintering temperatures, hydroxyapatite decomposes into other calcium phosphates that are inherently mechanically weaker. Previous work has elucidated hydroxyapatite's tendency to decompose above 1300 °C, and hence it was the maximum temperature that was investigated in this study. In addition, coarsening of the grains can be caused as the sintering temperature is increased, which has been shown to negatively affect the mechanical properties of ceramics. Therefore, a balance between densification and grain growth needs to be determined. In this study, three temperatures were investigated to ascertain the optimal sintering range with regards to compressive strength. **Fig. 2** highlights the symmetrical square pore shapes that were fabricated, which spanned the length of the scaffold.

Chemical structure

The chemical properties of a HA scaffold determine both the mechanical and biological properties, and hence chemical analyses were performed to elucidate the effects of the sintering parameters on the nano-sized HA powder. Both XRD and FTIR were employed and the results are portrayed in **Fig. 3**. The results of the as received powder were also included in the respective graphs for comparison. The XRD pattern for the as received powder exhibits poor crystallinity, comprising of poorly defined and broad peaks. The latter two characteristic are indicative of nanosized particles [23]. Sintering the HA scaffolds at 1200 °C was found to improve the crystallinity of the material, where the scaffolds exhibited larger and sharper peaks with respect to the XRD pattern for the as received powder. The pattern was confirmed to be hydroxyapatite (PDF card number: 01-089-4405).

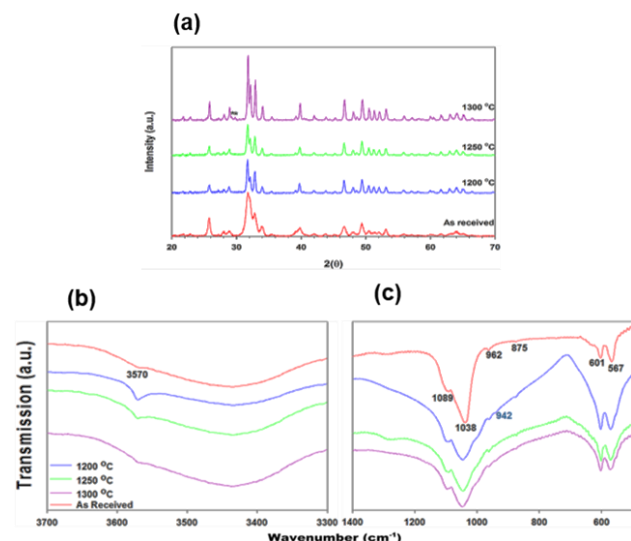
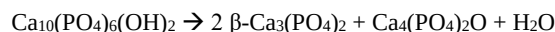


Fig. 3. Chemical analyses of the HA scaffolds, displaying the (a) XRD patterns at varying sintering temperatures and the (b) & (c) FTIR spectra.

The same was true for samples sintered at 1250 °C, where a further increase in crystallinity was observed and narrowing of the peaks. Sintering at 1300 °C produced two additional trace peaks with respect to the 1200 and 1250 °C XRD patterns, as highlighted by the two asterisks

in **Fig. 3(a)**. The newly developed peaks were found to belong to tetra calcium phosphate (TTCP) (PDF card number: 00-025-1137). Thus, sintering of up to 1300 °C produced relatively homogenous HA scaffolds according to the XRD analysis.

The FTIR spectrum of the as received powder also corroborated with the XRD pattern in that a nonstoichiometric HA powder was used, as attested by the broadly-defined spectrum with respect to other hydroxyapatite powders used in another study, as well as used by other researchers. The bands at 567, 601, 962, 1038 and 1089 cm^{-1} were attributed to the PO_4^{3-} group [24-26]; the band at 875 cm^{-1} has been suggested as HPO_4^{2-} group [27], or equally it could be CO_3^{2-} group [28, 29] that could have originated from the binder during thermal debinding and dissolved into the HA lattice [30-32]; The band at 3570 cm^{-1} was attributed to the stretch-vibration of the hydroxyl group, [24], but a second hydroxyl group at $\sim 630 \text{ cm}^{-1}$ was not detected [24]. FTIR analysis of the crushed scaffolds showed no change in trough positions, however trough broadening was observed as the sintering temperature was increased. In **Fig. 3(b)**, a minute OH^- trough was observed in the as received powder, however, at 1200 °C it can be seen to increase in sharpness. Increasing the sintering temperature thereon reduced the prominence of the OH^- trough at $\sim 3570 \text{ cm}^{-1}$. Furthermore, the FTIR spectra demonstrated the presence of a minute band at 942 cm^{-1} that appeared in the sintered samples, which was attributed to the PO_4^{3-} group pertaining to β -TCP and not hydroxyapatite [33, 34]. The XRD pattern did not detect a secondary phase at sintering temperatures of 1200 and 1250 °C, but the FTIR spectra highlighted a chemical structural change had occurred as early as 1200 °C. This was confirmed by SEM analyses that also alluded to the development of secondary phases occurring at 1200 °C onwards (see below). Therefore, from both the chemical analyses, it can be inferred that the HA was undergoing the following decompositional reaction:



Physical and mechanical properties

As with chemical properties, the physical properties also influence both the mechanical and cellular activity. Optical microscopy was used to examine the cell lengths, as well as the strut thickness of the sintered scaffolds. It was discovered that increasing the sintering temperature resulted in a decrease in the overall scaffold size, and that the ratio of solid to porous phase was maintained across all three sintering temperatures. The cell lengths were found to range from 655 to 763 μm , which exceeded the required pore size detailed in the introduction. Furthermore, although the overall scaffold shrunk with increasing temperature, the strut thicknesses were found to be of varying sizes, indicating that the scaffold's struts were inconsistent in their shrinking behavior. Poor paste homogeneity, or the development of a thermal gradient across the scaffolds during sintering could have caused this effect. However, one would expect to eliminate such

undesirable properties through the use of a high-energy mixer and isothermal heating treatment, respectively. The varying strut thickness could also be due to the heavy agglomeration of the starting particles that are prone to differential shrinkage, however, the employment of a high-energy shear mixer was expected to eliminate the agglomeration. Moreover, previous work by the author has found that ram extrusion is capable of breaking the agglomerates, producing compacted and uni-directional primary particles parallel to the extrusion direction. It is also possible that the nonstoichiometric nature of the starting hydroxyapatite powder could have caused the variation in strut thickness, which in turn resulted in the discrepancies in physical and mechanical properties increasing as the sintering temperatures were raised. The use of high purity HA powder could improve the consistency of the strut shrinkage.

The bulk porosity of the HA scaffolds was examined, and are displayed in **Table 1**. It was found that increasing the sintering temperature resulted in a decrease in bulk porosity. At 1200 °C, the bulk porosity was found to be ~ 50%, but this decreased to ~33% at 1300 °C. This was expected as the ceramic body becomes denser, and the pore sizes are reduced. The holding times of three and five hours for the individual sintering temperatures were found to have no significant influence on the bulk porosity. The reduction in bulk porosity due to increase in temperature was higher when compared to other studies that used similar sintering temperatures and with similar temperature increments [15, 35-38], but certainly not the largest decreases recorded [39].

Table 1. The bulk porosity and compressive strength of the fabricated HA scaffolds as a function of sintering temperature and dwell time.

Sintering temperature (°C)	Dwell Time (h)	Bulk Porosity (%)	Compressive Strength (MPa)
1200	3	48.1 ± 3.9	10.6 ± 2.3
	5	50.9 ± 5.3	8.5 ± 1.2
1250	3	37.9 ± 1.2	22.2 ± 5.0
	5	40.2 ± 2.3	19.6 ± 6.3
1300	3	35.2 ± 3.2	29.8 ± 8.6
	5	32.5 ± 0.1	29.4 ± 8.9

Mechanical testing was performed on the Hydroxyapatite scaffolds, and the results are also displayed in **Table 1**. All samples were found to fail in a brittle manner, which was denoted by an initial linear elastic region that was succeeded by a large drop once the maximum compressive strength was reached. The values obtained at the peak of the curve were recorded and are enumerated in **Table 1** as the compressive strength. The table also shows, once again, that the sintering temperature influenced the strength of the scaffold. The compressive strength increased from ~ 10 MPa at 1200 °C, to ~30 MPa at 1300 °C. Thus, the increase in sintering temperature by 100 °C had approximately tripled the compressive strength of the scaffolds. Similar temperature increments that produced comparable increases in compressive strength were found by others who used nano-sized HA particles [40, 41]. On the other hand, Aminzare et al. (2013) found that the mechanical

strength peaked at 1250 °C, and decreased when sintered at 1300 °C [42]. The presence of TCP was much more pronounced at 1300 °C than what was determined in this study, which is the probable cause for the decrease in mechanical property.

Ceramic extrusion was successful in producing HA scaffolds from nano-sized particles. The HA scaffolds demonstrated compressive strengths that exceeded those fabricated via traditional methods. The results are similar to that of the SFF method used by Houmard et al. (2013) [18], who revealed their HA scaffolds with a ~ 33 vol% porosity to possess a compressive strength of ~35 MPa. However, the macro-pore sizes recorded were at 150 µm, which is markedly lower than those fashioned in this study, and macro-pores of comparable size were found to reduce their compressive strength to ~ 5 MPa. Therefore, the scaffolds fashioned via extrusion are advantageous in that they are expected to have a faster cell adhesion rate, due to having larger macro-pore sizes. In addition, the compressive strength is an improvement on the commercially available Endobon® grafts, with the pores being comparable in size [43]. Endobon®, which are hydroxyapatite grafts, have a compressive strength that ranges between 1 to 20 MPa, with an intended application for replacing bone defect below load-bearing joints (e.g. tibial head fractures). Hence, due to their improved compressive strength, the scaffolds fabricated in this study can be used for a similar application, and provide improved mechanical stability.

Table 2. The relative density of HA scaffold struts as a function of sintering temperature and dwell time.

Dwell Time (h)	Relative Density (%)		
	1200 °C	1250 °C	1300 °C
3	82.4 ± 0.8	89.3 ± 1.5	95.3 ± 3.0
5	89.0 ± 2.7	90.2 ± 1.4	93.3 ± 2.9

Further to the aforementioned physical properties, the density of the scaffold struts (i.e. the solid phase) were also measured to determine the densification behaviour of the selected sintering parameters on the solid phase of the scaffolds. The measured densities were compared to the theoretical density of HA (3.156 g/cm³) to obtain the relative density, and are listed in **Table 2**. The results indicate a high level of densification had occurred, with a general proportional relationship between sintering temperature and density. The relative densities can be seen to range from 82.4 ± 0.8, to 95.3 ± 3.0%. Relative densities of HA sintered in air of over 98% have been previously obtained [44]. Cho and Rhee (2013) have demonstrated that nano-sized HA are highly sinterable, and in their study a theoretical density of 99.8% was achieved [45]. Thus, the nanosized HA particles used in this study can also be said to have exhibited enhanced sintering, and despite the use of the organic additive and solvent, the solid phase of the scaffolds exhibited high densification. The non-ceramic components, which totaled approximately 30% of the ceramic paste, were expected to hinder the densification of the scaffold solid

phase, however, the slow drying and thermal debinding steps used in this study could have mitigated their impact.

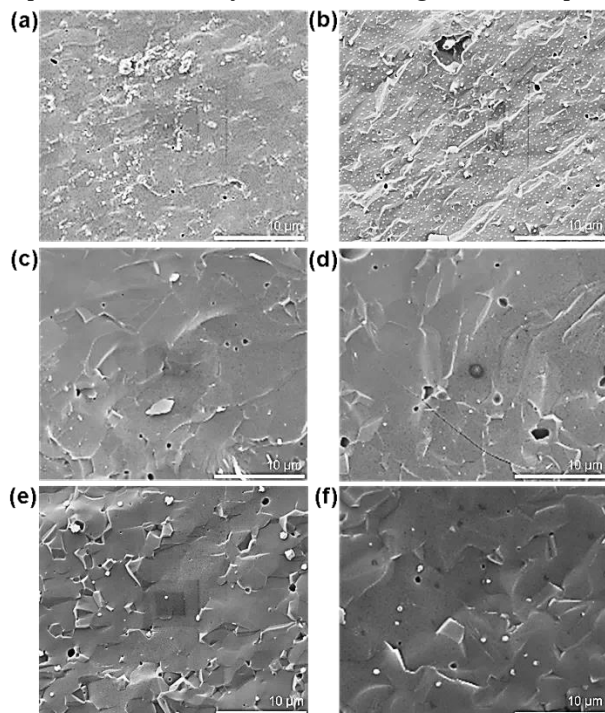


Fig. 4. SEM (SE) micrographs of the fractured surface at 1200 (a) & (b), 1250 (c) & (d) and 1300 °C (e) & (f), with a dwell time of three (left) and five (right) hours. (original magnification $\times 10,000$).

Microstructure

SEM micrographs in **Fig. 4** are of the fracture surfaces of the sintered HA scaffolds. The micrographs (**Fig. 4 (a-f)**) display the mix-mode fractures that the HA scaffolds exhibited, as a result of compression testing. Moreover, the images also illustrate that neighbouring grains grew and merged together, thus eliminating necking which is a possible source of stress concentration. The lack of necking observed suggests that sintering was at its final stage [46]. The micrographs reaffirm that the sintering temperatures resulted in scaffolds with a highly dense solid phase. The voids observed in the micrographs are likely to be as a result of fragmentation that occurred during the mechanical testing.

Fig. 5 displays the grain structure of the HA scaffolds. Grain sizes were found to increase in size as the sintering temperature was increased, but holding time had less of a pronounced affect. Nanosized particles are known to have an enhanced sintering rate, consequently resulting in the densification of structures that are susceptible to rapid grain growth [46]. The grain sizes are similar to what others have found when using starting powders of submicron particle size [47, 48]. Moreover, as portrayed in the micrographs, abnormal grain growth was observed, which indicated that a sufficiently high sintering temperature was used. Secondary grain structures can be found nucleating on the grain boundaries.

Given the propensity of HA to decompose into other calcium phosphate phases at elevated temperatures, it is likely that the secondary grains are secondary phases. Their preferential nucleation on the grain boundary was a result of the atoms found along the boundary that have a

higher energy than those within the grains. Hence, the grain boundaries were the primary site for their nucleation. HA is known to decompose to β -TCP and TTCP at temperatures above 1200 and 1250 °C, respectively, and together the XRD and FTIR analyses have detected both phases. Thus, it can be inferred that the nucleates are those of β -TCP and TTCP. Such nucleates can be seen to increase in size and frequency as the sintering temperature was increased. However, as illustrated by the subfracture micrograph in **Fig. S 1**, the nucleates are less prominent below the surface, and thus only superficial decomposition had occurred [49]. Furthermore, this can explain why the secondary phases were not detected by XRD, due to the instrument's limitations.

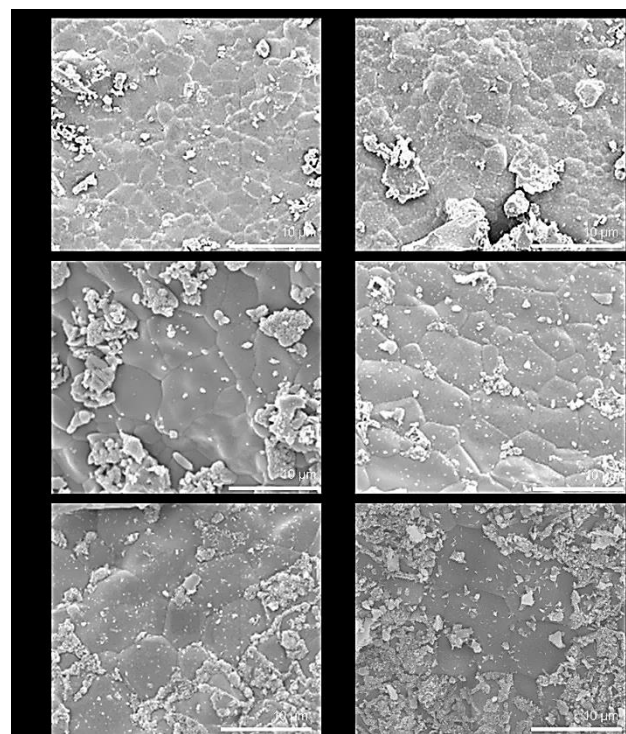


Fig. 5. SEM (SE) micrographs depicting the grain structure of the HA scaffolds at sintering temperatures of 1200 (a) (& b), 1250 (c) & (d) and 1300 °C (e) & (f), and dwell times of three (left) and five (right) hours.

The effects of the secondary phases on the mechanical strength of the scaffolds are yet to be determined. The presence of secondary phases produced at grain boundaries have been found to improve the mechanical properties of the ceramic matrix by deflecting crack propagation into the grain, where more energy is required to continue propagating [50]. However, the same study concluded that not all nanoparticles can achieve this feat, and only those that form a strong interfacial bond at the grain boundary can [51]. In this study, it was difficult to ascertain the decomposed products' influence on both the physical and mechanical properties of the fabricated HA scaffolds. However, it has to be noted that the decomposed products of hydroxyapatite have a larger volume than HA, and possess lower theoretical densities. Therefore, it is possible that their presence may have influenced the relative density of the scaffold struts. Further to this, the larger volume that accompanies the

decomposed products produces internal pressure that obstructs densification, consequently compromising the scaffolds mechanical integrity at the microstructural level [52]. Moreover, Abnormal grain growth also obstructs densification [53]. Wang et al. (1998) have postulated that the much larger difference in coefficient of thermal expansion between HA and its decomposed products results in internal defects that aid crack propagation [54].

Fig. 6 is a plot depicting the grain sizes (

Fig. 6(a)) and the derived Arrhenius plot for the activation energy of grain growth (

Fig. 6(b)). The trend displayed by the grain growth as a function of sintering time is similar to that of others [8]. The activation energy for the HA scaffolds were determined from the slope of the line of best fit, and were found to be 53.4 and 59.1 kcal/mol for three and five hour dwell times, respectively, across the three sintering temperatures. The values obtained are similar to that found in other studies, which ranged from 47 to 58 kcal/mol [55, 56].

As a whole, the SEM analysis of the microstructure produced in the HA scaffolds provided valuable information, with respect to grain morphology, and with secondary phase nucleation and growth. Firstly, the micrographs, along with the FTIR analysis, exposed the limitations of the XRD, as the XRD was unable to detect phase changes at lower sintering temperatures. Secondly, it was concluded that the sintering temperatures of 1250 and 1300 °C were sufficient for significant abnormal grain growth. This in turn limited the final density that can be achieved by the scaffolds. Nevertheless, high densities of the solid phase were obtained as demonstrated by the SEM micrographs in **Fig. 4** and **Fig. 5**, which were further corroborated by the density measurements of the struts. Isothermal heating was purposely chosen to avoid a heating gradient within the scaffolds and thus producing a homogenous grain size distribution. Instead, the isothermal heating resulted in abnormal grain growth, which was more noticeable with increasing dwell time. Therefore, the isothermal heat treatment chosen only further compounded the problem by extending the time for the enhanced driving force to continue with its undesirable effects.

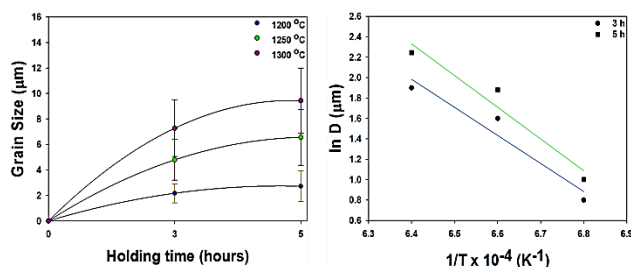


Fig. 6. (a) Depicting the effects of sintering temperature on the average grain size of the HA scaffolds, and (b) is an Arrhenius plot of log mean grain size against the reciprocal of sintering temperature used. ($n = 20$).

According to the Hall-Petch relation, an inverse relationship exists between mechanical strength and grain size [57]. Previous studies have elucidated the negative correlation between mechanical strength and grain growth [58-61]. Different works have shown that reducing the grain boundary phase in samples resulted in higher mechanical properties [62], as it was postulated that grain boundaries are mechanically the weakest link in microwave sintered samples [61].

In this study, the main influence on compressive strength was the bulk porosity, which was reduced as a result of densification. Although the sintering improved the densification, which subsequently resulted in increased compressive strength, microstructural features that have been regarded as detrimental to the compressive strength of HA scaffolds were found to increase in prevalence. Further studies will need to be performed to elucidate the effects of the microstructural features on mechanical properties of scaffolds fabricated via extrusion. Furthermore, the presence of the secondary phases and abnormal grain growth could have affected the thickness of the struts. Increasing the sintering temperature from 1200 to 1300 °C resulted in an increase in grain size from 2 to 7.5 μm for a three hour dwell time, and from 2 to 9 μm for a dwell of five hours. Despite the increase in grain size, the mechanical strength was not compromised. Therefore, it can be concluded that the preponderant determinant of the compressive strength was bulk porosity and not the microstructural features.

One possible explanation for the increase in compressive strength in extruded HA scaffolds could be due to the types of load the struts (i.e. the solid phase) were subjected to. The compression test in this study was performed parallel to the strut alignment, and as a result the struts were subjected to only compressive stresses. Whereas, foams and other stochastic-oriented scaffolds have struts of varying thickness and orientation, which are consequently subjected to multiple forms of stress during compression loading (e.g. flexural stress) that leads to earlier failures. Furthermore, extrusion does not require large volumes of polymers to impart porosity, as is the case with traditional methods. Therefore, in using a markedly lower polymer content, the defects caused by the difference in drying shrinkage and thermal expansion of the polymer and ceramic, were mitigated [63]. It has to be noted, however, that the scaffolds fabricated by ceramic extrusion are almost exclusively anisotropic in their mechanical properties [63]. Further studies, such as subjecting the scaffolds to a compressive load perpendicular to the strut alignment, will need to be performed in order to determine the full mechanical properties of extruded HA scaffolds. In addition, no conclusive microporosity (0.1 – 10 μm) was observed due to the densification characteristics of the starting powder. Radiographic and histological findings have demonstrated the clinical significance of microporosity for a successful bone implant [11] by offering a faster rate of implant degradation that expedites the restoration of normal bone function.

Cytocompatibility

The cell viability tests were performed on HA pellets fashioned using the cellulose ether-derived organic additive at days one and seven (**Fig. S2. (a,b)**). The tests were performed to determine the compatibility of the starting materials (HA and organic additive) at 1200, 1250 and 1300 °C, as well as the presence of the secondary phases. No cytotoxicity was observed in all samples, and cell viability was maintained until day seven (**Fig. S2. (b)**). The mean results of the samples on day one far exceed that of the control, with samples sintered at 1200 °C and 1250 °C found to be statistically significantly higher. The reverse occurred at day seven where all of the sintered samples exhibited a lower mean than that of the control. Only the data at 1300 °C was found to be statistically significantly lower than that of the control. It should be noted that, although not determined to be significant, both graphs displayed a similar decreasing trend in cell viability with increasing sintering temperature, with respect to the mean. Whether this is due to microstructural properties, for example the grain size or the presence of decomposed products, is uncertain. Further tests will need to be conducted to determine the cause. Nevertheless, the cell viability tests demonstrate that the raw materials used to fabricate the HA scaffolds, as well as the presence of the secondary phases, are not cytotoxic.

Conclusion

The present study demonstrates that nano-HA particles can be used to fabricate HA scaffolds using ceramic extrusion, and that the compressive strengths greater than traditional fabrication can be achieved. In this work, we used ceramic extrusion to fabricate a porous hydroxyapatite scaffold using nano-sized HA particles that had a tendency to agglomerate. Scaffolds sintered at 1200, 1250 and 1300 °C were examined to determine a suitable temperature, where it was found that the temperature ranges opted for exceeded the threshold for high sinterability of the starting material. As a result, the HA scaffold exhibited abnormal grain growth, nucleation and growth of secondary phases, and the elimination of micro-porosity. A combination of XRD and FTIR analyses inferred that it was TCP and TTCP phases that were forming on the grain boundaries. Such microstructural features have been known to compromise the mechanical strength of HA scaffolds, however, this was found not to be the case in this study. It is possible that the undesirable microstructural features were superficial, and thus not significant enough to impact the mechanical strength of the HA scaffolds, or to be detected by XRD analysis. The maximum compressive strength obtained was 29.8 ± 8.6 MPa, which is an improvement on HA scaffolds fabricated using traditional methods. A further benefit to the extruded HA scaffolds was that porosity of above 600 μm was obtained, which exceeded the recommended size for a synthetic bone graft. The study herein adds further credence to the use of techniques that are capable of fabricating a HA scaffolds with structured porosity for improved mechanical strength. Further studies will involve addressing the agglomeration issue with the aim of improving the compressive strength of extruded HA scaffolds.

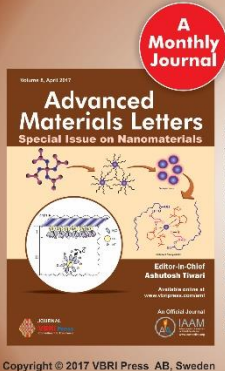
Author's contributions

Conceived the plan: JM, IMR; Performed the experiments: ME; Data analysis: ME, MM; Wrote the paper: ME, MM.

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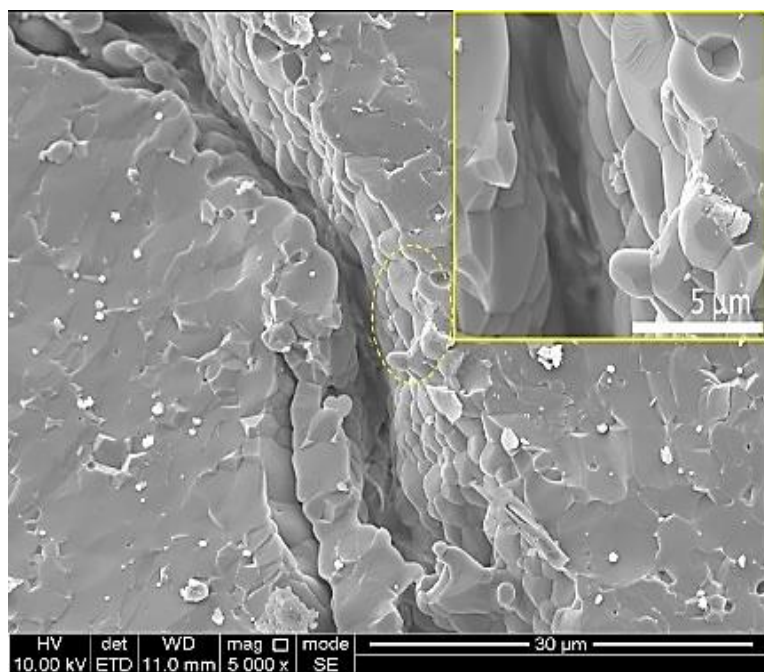


Fig. S1. SEM (SE) micrograph of a HA fractured surface sintered at 1250 °C for five hours. The micrograph is intended to demonstrate the lack of decomposed products growing below the surface. The inset is a higher magnification of the dotted region.

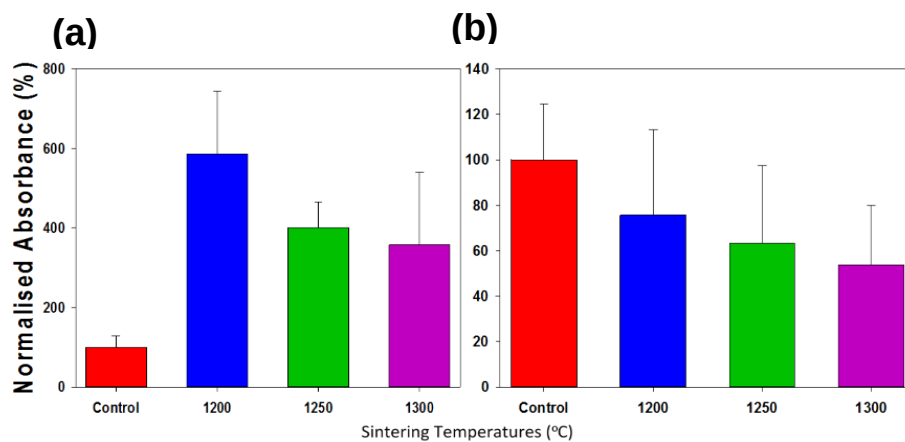


Fig. S2. Illustrating the cell viability results of day 1 (a) and day 7 (b) measured by MTT assay.