Effect of sintering holding time on the properties of Hydroxyapatite granules fabrication using dripping technique of HA-Alginate sintered at 1000 °C

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Abstract. CaP granules as bone graft are used for open defect of bone, where is not necessary mechanical stability they are proposed for no-load bearing applications. The important feature in the physical structure of a synthetic ceramic bone graft is its porosity, pore size, the volume of porosity and interconnections between the pores are three crucial parameters. The pore structure is of great importance for osteoconduction. The level of porosity, pore size distribution and degree of pore interconnectivity significantly influence the extent of bone ingrowth. According to that, this study investigated the effect of sintering holding time on properties of droplet synthetic ha-alginate using dripping technique sintered at 1000 °C with sintering holding time at 1: 2:3 hours respectively. XRD was used to investigate of phases of the sintered droplet. Scanning Electron Microscope (SEM) was used to investigate the microstructure of beads. In this work, we can conclude that the holding time increased, the crystallinity increased but on the other hand, pore size, porosity, and surface roughness decreased. For all investigated samples. The surface roughness of A102 (0.2–2, μm) which is sintered at 1000 °C 2 hours holding time has a better value offered better cell colonization and has no significant value of porosity (50%) and crystallinity (86.63%) than those of A103 which need more energy for sintering as a consequence.

Keywords: Hydroxyapatite, CaP, sintering, microstructure, surface roughness, granule

1. Introduction

CaP has been known as the material for bone grafting caused by its similarity component with the bone [1], [2]. One of CaP, hydroxyapatite, is accepted as bioactive for guided bone regeneration. On the other hand, hydroxyapatite has a weakness in poor mechanical properties such as low compressive strength and fracture toughness [3]. So that, hydroxyapatite has considered applying in non-load bearing part for implant material [4,5] and applied in the form of powder, granule and porous scaffold where is the porous scaffolds are highly attractive due to good osteoconductivity and fast bone ingrowth [6]. Compared to porous scaffolds, the granule hydroxyapatite is easier to produce. The scaffold has a clearer shape and big macroporosity (typical range 50-500 μm) as a big role in osteoconductivity mechanism compared to granules such as osteoconductivity and fast bone ingrowth, due to high surface area and sufficient blood circulation such as osteoconductivity and fast bone ingrowth, due to high surface area and sufficient blood circulation. However, scaffolds need to cut and
press-fitting into the defect. That is the problem of using scaffolds. So that, for smaller open defects, using granules is more attractive than using scaffolds. Some researcher stated that Microstructure of the bone graft ceramic material has main role of the recovery of bone defect, such as surface roughness is influence to colonization of cell [7] and correlation between surface roughness and osteoconduction has been well described in several systematic reviews [8–10]. So that, the roughness surface was also evaluated in this work.

Granules hydroxyapatite can be produced with some ways: planetary mixers [11], fluidized-bed granulators [12,13], sphezonizers [14,15], pelletizers in forming granules from wet masses containing the desired ingredients [15–17] and recently, droplet extrusion or beads formation [18,19]. This method is based on a gelification mechanism involving natural polysaccharides. Hydrogels are three-dimensional hydrophilic tissue that can absorb large amounts of water or biological fluid, mimics biological tissue [20]. Polymer gel beads are made using a number of natural biodegradable polymers. Because they come from natural sources, do not require organic (toxic) solvents, are easily available and of high quality for a number of chemical modifications [21–23]. Polyelectrolyte polymers are used in the manufacture of hydrogel matrix beads, the most widely used is alginate [24,25] and chitosan [26–29], and also carboxymethyl cellulose [30–32] have been applied.

Ribeiro at al [25] was mixed with sodium alginate solution (3% w/v) at a ratio of 0.2 w/w and well homogenized, they were dried overnight in a vacuum oven at 30°C, and then sintered at 1100°C for 1 h, with a uniform heating rate of 5°C/min from room temperature. This previous research did not declare about the influence of holding time of sintering temperature at 1000°C on its properties. In this work, we investigated the influence of holding time of sintering at 1000°C on its properties of HA granules produced by droplet extrusion technique and determine the properties of sintered granules such as value of crystallinity, pore size, phase identification, and surface roughness as the biological requirement for bone graft material.

2. Materials and Methods

2.1 Materials

1) Materials preparation

Hydroxyapatite powder obtained from process as follow: 1 M solution of calcium oxide and 0.6 gr/l [\((NH_2)_2HPO_4]\) is used as the initial solution. The solution of \((NH_2)_2HPO_4\) added drop-wise in a solution of calcium oxide. Ammonia was used for adjustment of pH values. The suspension is stirred with a magnetic stirrer after the addition of the initial solution for 24 hours at room temperature. Precursor done for 30 minutes at room temperature, then filtered and washed with a vacuum Buchner for five times until a neutral pH is reached. For the first and third phases were washed with distilled water and ammonia, the second phase and the fourth was washed with distilled water to remove residual dirt, and last washed with ethanol to remove water and increase the dispersibility. The end product was dried at room temperature for 24 hours then heated to 160°C for 17 hours for the removal of water. Sintering process was carried out at 650°C with a heating rate of 80 minutes with a time of 2 hours’ detention. Hydroxyapatite samples then characterized by XRD.

2) Droplet formation.

Droplet extrusion method allows the production of spherical drops starting from a bioceramic suspension containing Alginate solution that allows the instantaneous jellification in a crosslinking solution. HA powder has added into composition of microspheres CaP-alginate gelation was mixed with sodium alginate solution (Sigma Aldrich 3% w/v) at a ratio of 0.2 w/w and well homogenized. The paste was extruded drop-wise into a 0.1 M CaCl2 cross-linking solution, where spherical-shaped particles instantaneously formed and were allowed to harden for 30 min. The size of the microspheres was controlled by regulating the extrusion flow rate using a
syringe. At completion of the gelling period, microspheres were recovered and rinsed in water in order to remove the excess CaCl₂. Finally, they were dried overnight in a vacuum-oven at 30ºC, and then sintered at 1100ºC for 1h, 2h and 3 h with a uniform heating rate of 5ºC/min from room temperature.

2.2 Characterization techniques
The sintered Hap granules were investigated with following characterization techniques. To characterize the crystallized solids chemical analyses, powder X-ray diffraction (XRD), FT-IR spectroscopy, thermal analysis and SEM methods were applied. Philips X’pert XRD equipment with a graphite monochromator (PW 1752/00), CuKα, (λ = 0.15418 nm) radiation, a Ni filter in 2× range of 10 to 60 degrees at 30 kV, 30 mA was used.

Thermal decomposition was investigated using a TGA-DTA (simultaneous Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA) fully automated system (Universal V2.3C TA Instruments) with 20 deg/min heating rate. The SEM images were obtained using JEOL JSM 7500F.

The total and apparent density and total porosity of sintered compacts, shrinkage and weight loss during the sintering were also measured. Total density was determined by simple pycnometric method. Hydrostatic weighing (HW) uses the Archimedes principle was used to calculate apparent density of sintered specimens. The procedure of HW involves using the following three measurable values: the weight of the body outside the water, the weight of the completely immersed body and the density of the water. The value of total and apparent density is needed to evaluate the percentage of total material porosity (P) according to the equation:

\[ P = 1 - \frac{\text{apparent density}}{\text{total density}} \times 100\% \]  

3. Results and discussion
3.1. XRD analysis of synthetic HA

Figure 1 shows the result of precipitation method to synthesize hydroxyapatite as describe as above. We used Highscore plus® to identify the pattern of powder sample.

![Figure 1](image)

**Figure 1.** The result of search and Match using high score plus software with Rietveld analysis of the synthetic HA powder sintered at 650 ºC

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Diffraction pattern was match to Carbonate-hydroxyapatite \( / \text{Ca}_{10.00}\text{P}_{6.00}\text{O}_{26.14}\text{H}_{2.60}\text{C}_{0.02} \) (Card. No. 96-900-3554). Table 1 shown the lattice parameter has no significant different with the lattice parameter of the reference. It can conclude that the sample phase was Carbonate-hydroxyapatite. On the sintering temperature at 650°C, the HA powder has 539-696Å and 64% degrees of crystallinity.

3.2. XRD analysis of sintered granules

![Figure 2. XRD pattern of all samples sintering at 1000°C with holding time at 1, 2, 3, hours respectively.](image)

From figure 2. Shown that all of sintered samples has diffraction pattern identically. No phase transformation found compare to HA powder and between all samples. All of pattern had similar to diffraction pattern of Carbonate-hydroxyapatite \( / \text{Ca}_{10.00}\text{P}_{6.00}\text{O}_{26.14}\text{H}_{2.60}\text{C}_{0.02} \) (Card. No. 96-900-3554) which has sharper peak at 32°.

| Sample Code | c | a | Crystallite size, Å | % crystallinity | Intensity at highest peak | FWHM |
|-------------|---|---|--------------------|-----------------|--------------------------|-------|
| HA powder, 650°C | 9.4109 | 6.8777 | 137-212 | 64 | 203 | 0.5088 |
| Reff. Carbonate-hydroxyapatite Card #96-900-3554 (\text{Ca}_{10.00}\text{P}_{6.00}\text{O}_{26.14}\text{H}_{2.60}\text{C}_{0.02}) | 9.4470 | 6.8810 | - | - | - | - |
| A101 | 9.4468 | 6.8806 | 359-762 | 86.34 | 565.06 | 0.1886 |
| A102 | 9.4316 | 6.8760 | 361-745 | 86.63 | 591.77 | 0.1893 |
| A103 | 9.4279 | 6.8742 | 393-869 | 86.08 | 600.15 | 0.1738 |

The crystallography of sintered granules and sintered powder was evaluated and the result are shown at table 1. No phase transformation detected due to the lattice parameter for each sample. The
crystallinity increased when the holding time of sintering increased, but slightly decreased for 3 hours holding time (A103). The linear relationship is shown between the holding time and crystallite size where the holding time increased, the crystallite size increased.

3.3. SEM analysis
Results for SEM data were presented on Fig. 3 to Fig. 5 below

Figure 3. SEM of sintered granules (A101). Sintering Temperature at 1000 °C, holding time 2 hour with magnifications 45x, 500x, 5k, and 10k respectively

Figure 4. SEM of sintered granule (A102). Sintering Temperature at 1000 °C, holding time 2 hour with magnifications 45x, 500x, 5k, and 10k respectively
Figure 5. SEM of sintered granule (A103). Sintering Temperature at 1000 °C, holding time 2 hour with magnifications 45x, 500x, 5k, and 10k respectively

| Sample Code | Surface roughness, µm | Pore size, µm | Porosity, % |
|-------------|-----------------------|--------------|-------------|
| A101        | 0.25-2.1              | 0.12 - 0.4   | 51          |
| A102        | 0.2-2                 | 0.13-0.4     | 50          |
| A103        | 1-28                  | 0.2 – 3.2    | 50          |

Based on the Table 2 it can declared that the holding time on sintering process had influenced on pore size, degrees of porosity and surface roughness where surface roughness and porosity decreased relatively when the holding time increased caused by densification. On the other hand, pore size decreased when holding time increased. The surface roughness become smoother and less of porosity. It is in agreement with Patel, 2001 [33]. At the contrast, in A103 we found a large pore size and we considered it could be effect of non-homogenous mixing processes between alginate and HA slurry for investigated sample.

4. Conclusions
Hydroxyapatite sintering holding time has a significant impact on surface roughness, on microstructure including crystallinity and micro porosity were shown to be of great importance in promoting protein adsorption on the material surface and capillary effect, and would consequently influence cell adhesion, proliferation, and bone tissue growth. So that, the fulfillment of properties of microstructure of bone graft for that biological requirement is depending of sintering process such as degree of temperature and
holding time. This work shown that at the same degree of sintering temperature, the holding time effected on surface roughness, crystallinity, pore size, and porosity. It is known that smooth surfaces are less expected to conduct cell colonization; for that reasons, the holding time for 2 hours at 1000 °C is better than other and not significant different of pore size and degrees of porosity of A101. Besides, the crystallinity is better than holding time at 1 hour. The crystallinity of a biomaterial is important caused by high crystallinity materials display lower biodegradation rates and better volume stability. It means that dissolution of material is depend of degree of crystallinity. In 2 hours holding time at 1000 °C sintering temperature, we obtained 86.63% crystallinity compare to 3 hours holding time (86.08%) but with the less energy for sintering process.

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References
[1] S. Shahgoli and M. H. Levine, “Introduction and overview of bone grafting,” N. Y. State Dent. J., 2011.
[2] N. Eliaz and N. Metoki, “Calcium phosphate bioceramics: A review of their history, structure, properties, coating technologies and biomedical applications,” Materials (Basel), 2017.
[3] H. A. Siddiqui, K. L. Pickering, and M. R. Mucalo, “A review on the use of hydroxyapatite-carbonaceous structure composites in bone replacement materials for strengthening purposes,” Materials. 2018.
[4] M. R. Mucalo, Hydroxyapatite (HAp) for Biomedical Applications. 2015.
[5] “The Chemistry of Medical and Dental Materials,” Oral Dis., 2003.
[6] P. Chocholata, V. Kulda, and V. Babuska, “Fabrication of scaffolds for bone-tissue regeneration,” Materials. 2019.
[7] D. D. Deligianni, N. D. Katsala, P. G. Koutsokoukos, and Y. F. Missirlis, “Effect of surface roughness of hydroxyapatite on human bone marrow cell adhesion, proliferation, differentiation and detachment strength,” Biomaterials, 2000.
[8] A. Wennerberg and T. Albrektsson, “Effects of titanium surface topography on bone integration: A systematic review,” Clinical Oral Implants Research. 2009.
[9] H. H. Hessam, S. I. S. Izman, and S. H. S. Hamtaeipour, “Evaluating the surface properties of hydroxyapatite coating on titanium alloy substrate,” J. Mek., vol. 36, no. 1, 2013.
[10] M. M. Shalabi, A. Gortemaker, M. A. Van’t Hof, J. A. Jansen, and N. H. J. Creugers, “Implant surface roughness and bone healing: A systematic review,” Journal of Dental Research. 2006.
[11] A. Faure, I. M. Grimsey, R. C. Rowe, P. York, and M. J. Cliff, “A methodology for the optimization of wet granulation in a model planetary mixer,” Pharm. Dev. Technol., 1998.
[12] M. Dosta, S. Heinrich, and J. Werther, “Fluidized bed spray granulation: Analysis of the system behaviour by means of dynamic flowsheet simulation,” Powder Technol., 2010.
[13] P. Roy, R. Khanna, and D. Subbarao, “Granulation time in fluidized bed granulators,” Powder Technol., 2010.
[14] M. Thommes and P. Kleinebudde, “The Science and Practice of Extrusion-Spheronization,” 2017.
[15] M. Evers, D. Weis, S. Antonyuk, and M. Thommes, “Scale-up of the rounding process in pelletization by extrusion-spheronization,” Pharm. Dev. Technol., 2019.
[16] P. Nold, R. Löbe, and M. Müller, “Granule production - Easy and cost-effective,” InterCeram Int. Ceram. Rev., 2003.
[17] S. Shanmugam, “Granulation techniques and technologies: Recent progresses,” BioImpacts, 2015.
[18] B. V. Parakhonskiy et al., “Size controlled hydroxyapatite and calcium carbonate particles:
Synthesis and their application as templates for SERS platform,” *Colloids Surfaces B: Biointerfaces*, 2014.

[19] M. Descamps, J. C. Hornez, and A. Leriche, “Manufacture of hydroxyapatite beads for medical applications,” *J. Eur. Ceram. Soc.*, 2009.

[20] Q. Chai, Y. Jiao, and X. Yu, “Hydrogels for Biomedical Applications: Their Characteristics and the Mechanisms behind Them,” *Gels*, 2017.

[21] M. R. Singh, S. Patel, and D. Singh, “Natural polymer-based hydrogels as scaffolds for tissue engineering,” in *Nanobiomaterials in Soft Tissue Engineering: Applications of Nanobiomaterials*, 2016.

[22] L. Gasperini, J. F. Mano, and R. L. Reis, “Natural polymers for the microencapsulation of cells,” *Journal of the Royal Society Interface*. 2014.

[23] M. C. Catoira, L. Fusaro, D. Di Francesco, M. Ramella, and F. Boccafoschi, “Overview of natural hydrogels for regenerative medicine applications,” *J. Mater. Sci. Mater. Med.*., 2019.

[24] J. Sun and H. Tan, “Alginate-based biomaterials for regenerative medicine applications,” *Materials*. 2013.

[25] C. C. Ribeiro, C. C. Barrias, and M. A. Barbosa, “Calcium phosphate-alginate microspheres as enzyme delivery matrices,” *Biomaterials*, 2004.

[26] M. D. Effendi, D. Gustonio, D. Ayu, and F. Kurniawati, “Comparison on mechanical properties of single layered and bilayered chitosan-gelatin coated porous hydroxyapatite scaffold prepared through freeze drying method,” *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 172, p. 12031, Feb. 2017.

[27] N. Bhattarai, J. Gunn, and M. Zhang, “Chitosan-based hydrogels for controlled, localized drug delivery,” *Advanced Drug Delivery Reviews*. 2010.

[28] M. C. G. Pellá, M. K. Lima-Tenório, E. T. Tenório-Neto, M. R. Guilherme, E. C. Muniz, and A. F. Rubira, “Chitosan-based hydrogels: From preparation to biomedical applications,” *Carbohydrate Polymers*. 2018.

[29] A. Anitha et al., “Chitin and chitosan in selected biomedical applications,” *Progress in Polymer Science*. 2014.

[30] N. A. Zakharov, Z. A. Ezhova, E. M. Koval, V. T. Kalinnikov, and A. E. Chalykh, “Hydroxyapatite-carboxymethyl cellulose nanocomposite biomaterial,” *Inorg. Mater.*, 2005.

[31] D. Pasqui, P. Torricelli, M. De Cagna, M. Fini, and R. Barbucci, “Carboxymethyl cellulose - Hydroxyapatite hybrid hydrogel as a composite material for bone tissue engineering applications,” *J. Biomed. Mater. Res. - Part A*, 2014.

[32] M. Sayed, H. F. El-Maghraby, F. Bondioli, and S. M. Naga, “3D carboxymethyl cellulose/hydroxyapatite (CMC/HA) scaffold composites based on recycled eggshell,” *J. Appl. Pharm. Sci.*, 2018.

[33] N. Patel, I. R. Gibson, S. Ke, S. M. Best, and W. Bonfield, “Calcining influence on the powder properties of hydroxyapatite,” *J. Mater. Sci. Mater. Med.*, 2001.