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Development and Characterization of Three-Dimensional Lattice Hydroxyapatite Scaffolds Using Aqueous Based Extrusion Fabrication (ABEF) for Biomedical Applications

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Abstract

A solid freeform fabrication technique, aqueous based extrusion fabrication (ABEF), was investigated for the creation of three-dimensional lattice Hydroxyapatite scaffolds with pre-designed pore properties. An aqueous based Hydroxyapatite paste was extruded through a 0.8 mm nozzle, and deposited layer-by-layer at room temperature according to a computer-aided design (CAD) file. The morphology of green body and sintered body were characterized using digital microscope. The phase purity was analyzed using XRD. Fourier transform infrared spectroscopy (FTIR) was performed in order to understand the phase changes upon heating process and to determine HA stoichiometry. The current investigation confirms the possibility of producing three-dimensional lattice Hydroxyapatite scaffolds without any impurities as indicated by XRD and FTIR techniques. The morphology analysis of the structured macroporous Hydroxyapatite bioceramic shows interconnected macro pores and micro pores. It will give possibility for colonization of osteoblast in the pores, fibrovascular ingrowth and finally the deposition of new bone formation.

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1. Introduction

Hydroxyapatite (HA, Ca_{10} $(PO_4)_6$ $(OH)_2$) ceramics has been widely used as bone substitutes. In recent years, particular attention has been paid to the preparation of HA bioceramics with porous morphology. Porous HA exhibits strong bonding to the bone; the pores provide a mechanical interlock leading to a firm fixation of the material. Bone tissue grows well into the pores, increasing strength of the HA implant. It was realized that the dimension and morphology of pores are crucial factors for an excellent osteointegration. The minimum pore size required to enable ingrowth of the surrounding bone together with blood supply, is about 100–150 μm for macropores, but several researchers also state that even at pores of as small as 50 μm osteoconduction is still possible. In addition, the bigger pores size, with diameter around 200–500 μm are very important for colonization of osteoblast in the pores, fibrovascular ingrowth and finally the deposition of new bone. Other important requirements for porous implants are interconnectivity of the pores for the penetration of the osteoblast-like cells inside the pores as well surface roughness for the attachment of cells.

Porous HA can be produced by a number of methods including conversion of natural biological origin material, ceramic foaming technique, polymeric sponge method, gel casting of foams, starch consolidation, microwave processing, slip casting and electrophoretic deposition technique. However, production of three-dimensional lattice HA bioceramics scaffolds with structured or controllable pores properties using solid freeform fabrication technique, aqueous based extrusion fabrication (ABEF) method has not been fully understood and researches in this area are still ongoing. In this research the three-dimensional lattice HA bioceramics will be produced and characterized. The results of the investigation are also expected to shed light on the preparation of structured macroporous HA bioceramics for medical application.

2. Materials and Methods

A. Hydroxyapatite Paste Preparation

Hydroxyapatite powder (Sigma Aldrich 04238) was used to paste preparation. Before making the bioceramic paste, the morphology and particle size were assessed by scanning electron microscopy (SEM, JEOL type JSM-636OLA with acceleration voltage of 20 kV.). The samples were mounted on steel stubs and subsequently coated with palladium (Pd) using a sputter coater (E1030 Ion Sputter, Hitachi). To prepare the HA bioceramic paste, 1 gram HA powder was mixed 1.2 ml distilled water and stirred for 60 minutes to form fine HA paste.

B. Three-Dimensional Lattice HA Development

The experimental setup is shown in Figure 1. The lattice model was prepared using CAD software and generates the G-Code to control ABEF system. The deposition system (Figure 2.) has four axes: X, Y, Z and extrusion. The XY table has precision 0,024 ± 0,003 mm for X-axis, 0,030 ± 0,007 mm for Y-axis and 0,042 ± 0,006 mm for Z-axis. The 60 ml plastic syringe with nozzle diameter of 0.8 mm was mounted on the Extrusion-axis and the sample substrate is deposited on the XY table. The lattice pattern in each layer is defined by the trajectory of the extrusion-axis in relation to the XY table movement and the Z-axis moves in steps of one layer thickness to produce a 3D assembly.

The hydroxyapatite paste was put into the 60 ml syringe tube (luer lock) in the ABEF system for green body preparation. The prepared green body than was dried at incubator with temperature 100 C for 60 minutes and continued with sintering process in temperature 1250 C for 120 minutes with heating rate 5 C/minutes in air atmosphere. The heat-treated three-dimensional lattice Hydroxyapatite bioceramics then were cooled to room temperature by slow furnace cooling then collected and characterized.

C. Characterization

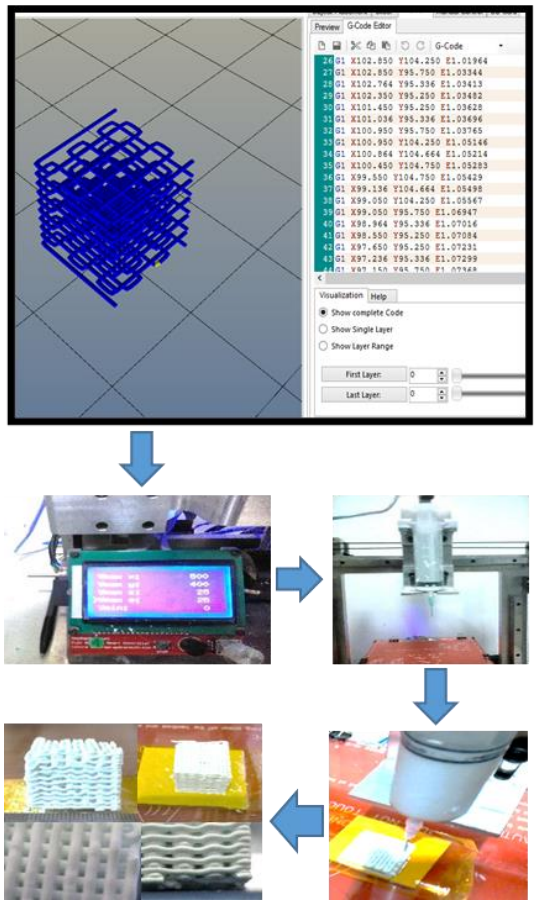

Figure 1. The experimental setup.

Figure 2. The aqueous based extrusion fabrication (ABEF) with RepRap controller

The phases present in the three-dimensional lattice hydroxyapatite bioceramics samples were analyzed using X-ray Diffraction with a monochromated CuKα as the radiation source at 40 kV, 40 mA and a scan speed of 3 /min and step scan of 0.02 . The crystalline phase compositions were identified with reference to standard JCPDS powder diffraction database available in the system software.

The morphology of the three-dimensional lattice hydroxyapatite bioceramics sample was examined using a digital camera and microscope. Fourier transform infrared spectroscopy were performed on the sample in order to understand the phase changes upon heating process and to determine HA stoichiometry deviations, i.e. the presence of anions partially substituting PO_4^{3-} and/or OH- groups. For this measurement, the transmission IR spectra will be recorded using KBr pellets over the range of 400-4000 cm^{-1} with 1 cm^{-1} resolution averaging over 100 scans.

3. Results and Discussion

A. Morphology Analysis

Figure 3(a). shows the morphology of commercial HA that was obtained from chemical synthesis. The HA powder consist of a mixture of fine powder particles ranging from less than 1 - 3 μm in diameter and larger particles of 5 - 10 μm in diameter. The larger particles appear to be large agglomerates of loosely packed smaller particles those are easy broken into smaller particles, resulting in a rough surface shown in Figure 3(b).

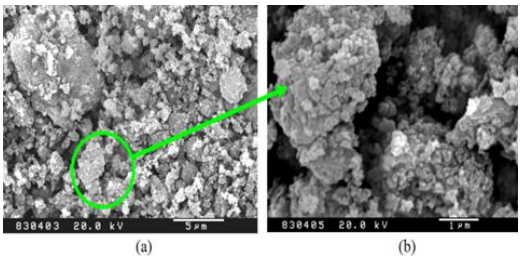

Figure 3. The scanning electron micrograph of commercially available HA (Sigma Aldrich) exhibit the presence of loosely packed particles.

A direct observation made after the green body preparation shows the sample has structured macroporous with interconnected pores as shown in Figure. 4. This structure was similar with the pre-design structure that was made in the CAD software.

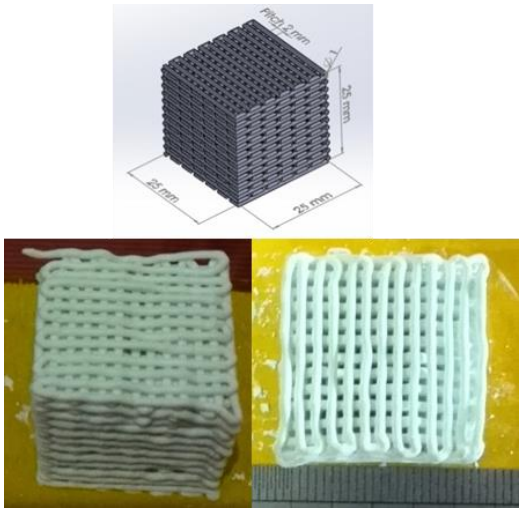

Figure 4. General morphology the macroporous HA bioceramics green body (before sintering process).

Before sintering, the HA powder and macroporous HA green body are white in color as shown in Figure 4. But after sintering using electric box furnace up to 1250ºC for 120 minutes in air atmosphere, the macroporous HA bioceramics became shrinkage 35% and the color become light blue. Generally, the color of the mineral is related to some impurities or defects. Furthermore, the crystal structure of HA powder also determines the color change.

The commercial HA powder that was used in this research is considered as a synthetic HA material. It has hexagonal space group $P6_3/m$ with a molecule of $Ca_{10}(PO_4)_6(OH)_2$ in the unit cell. The light blue color appears at the structured macroporous HA bioceramics samples are believed due to the existence of manganese content. Previous research also confirmed that synthetic HA containing manganese shows a blue color after sintering at high temperature in an oxidizing atmosphere.

Figure 4. also shown the macroporous HA bioceramic that was produced in this research show macro porous with structured and interconnecting pore. Porous bone graft as a temporary matrix for bone growth must possess an open pore, fully interconnected geometry in a highly porous structure with large surface area to volume ratios that will allow cell in-growth and an accurate cell distribution throughout the porous structure, and will facilitate the neovascularization of the construct from the surrounding tissue. Furthermore, the porous implant materials should also exhibit adequate microporosity, in order to allow capillary in-growth.

B. Phase Structure Analysis

The XRD patterns of the as received commercial HA powder and macroporous HA bioceramics are shown in Figure 5. The well resolved XRD both samples could be easily indexed on the basis of hexagonal crystal system of space group $P6_3/m$ with respect to JCPDS File No. 9-432. The Bragg peaks at approximately 21, 22, 25, 26, 28, 31, 32, 34, 35, 39, 40, 42, 43, 45, 46, 48 and 49 (2θ) corresponds to the characteristic peaks of pure HA phase (JCPDS File No. 9-432). XRD analysis also indicates the absence of secondary phases, such as TCP or calcium oxide (CaO).

The as received HA powder peaks in Figure 5. also exhibited a low or semicrystalline crystallinity as indicated by low intensity and wide diffraction peak. But, after was developed into structured macroporous HA bioceramic by manually extrution method and sintering process in 1250ºC, peaks of the sample become narrow and high intensity compare to previous peaks of commercially available HA from Sigma Aldrich as shown in Figure 5. The high crystallinity will improve the mechanical strength of HA material, but if the crystallinity is too high it will more difficult for it to be absorbed into the body compared to bone apatite.

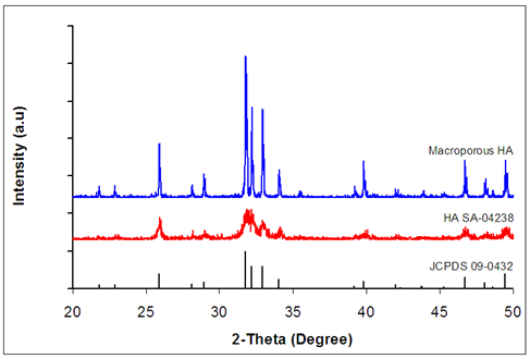

Figure 5. X-ray diffraction pattern of as received commercial HA powder in comparison with sintered macroporous HA and XRD data of HA standard JCPDS No. 09-0432.

C. Chemical Compounds Analysis

The infra red spectrum of as received commercial HA powder (Sigma Aldrich 04238) and the structured macroporous Hydroxyapatite bioceramic are shown in Figure 6. and Figure 7. The infra red spectrum of commercial HA powder and the structured macroporous Hydroxyapatite bioceramic exhibit only the characteristic absorption peaks of HA. A large number of bands in the spectra matches the bands in the HA reference spectrum and are in close agreement with reported data on HA [28]. There are no significant differences observed in the FTIR spectra of both samples.

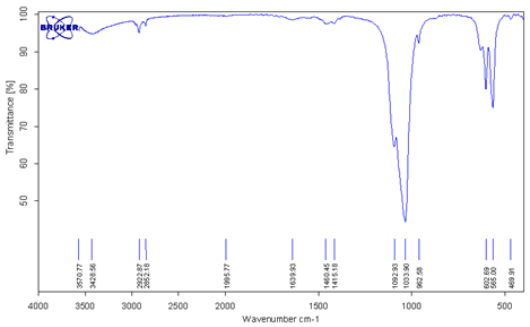

Figure 6. FTIR spectra of as commercial HA (Sigma Aldrich 04238)

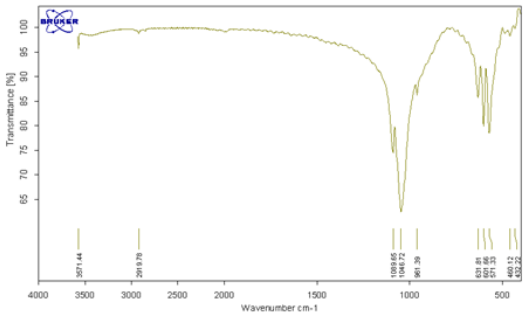

Figure 7. FTIR spectra of structured macroporous HA bioceramics from commercial HA powder (Sigma Aldrich 04238) after sintering at 1250oC in air atmosphere

In general, the FTIR spectra from both HA powder and the structured macroporous Hydroxyapatite bioceramic indicated the presence of phosphate (PO_4^{3-}), hydroxyl (OH^-) and carbonate (CO_3^{2-}) ions. The 1033-1090 cm^{-1} band arise from ν_3 PO_4^{3-}, the ~962 cm^{-1} arise from ν_1 PO_4^{3-} and the ~570 cm^{-1} bands arise from ν_4 PO_4^{3-}.

The FTIR spectra from all samples in this research exhibit a pronounce peak at ~601 cm^{-1} and ~3570 cm^{-1} due to the presence of hydroxyl groups. The band of OH^- at 3570 cm^{-1} increases in intensity due to the sintering process in 1250°C. This condition can be attributed to the increase in HA crystallinity with increasing sintering temperature as observed from XRD pattern of these samples in Figure. 5.

The results from this study give the evidences the possibility of producing the three-dimensional lattice Hydroxyapatite bioceramic from commercial HA powder using ABEF technique. The interconnected pore and pore distribution of the sample give a shed light on the preparation of structured macroporous HA bioceramics for medical application.

4. Conclusion

In the present work, three-dimensional lattice Hydroxyapatite bioceramic was prepared and characterized. The following conclusions can be drawn:

1. The current investigation confirms the possibility of producing the structured

macroporous Hydroxyapatite bioceramic from commercial HA powder (Sigma Aldrich 04238) without any important impurities as indicated by XRD and FT-IR techniques.

2. The morphology analysis of the structured macroporous Hydroxyapatite bioceramic shows interconnected macro pores and micro pores.

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6. References

A. J. Salgado, O. P. Coutinho, and R. L. Reis, Bone tissue engineering: state of the art and future trends, Macromol. Biosci., vol. 4, pp. 743-765, 2004

B.S. Chang, C.K. Lee, K.S. Hong, H.J. Youn, H.S. Ryu, S.S. Chung and K.W. Park, Osteoconduction at porous hydroxyapatite with various pore configurations, Biomaterials, vol. 21, pp. 1291-1298, 2000

H. Yoshikawa and A. Myoui, Bone tissue engineering with porous hydroxyapatite ceramics, J. Artif. Organs., vol. 8, pp. 131-136, 2005

I. Sopyan, M. Mel, Ramesh S. and K.A. Khalid, Porous hydroxyapatite for artificial bone applications, Science and Technology of Advanced Materials, vol. 8, pp. 116-123, 2007

J. Ma, C. Wang and K.W. Peng, Electrophoretic deposition of porous hydroxyapatite scaffold, Biomaterials vol. 24, pp. 3505-3510, 2003

J.A. Toque, M.K. Herliansyah, M. Hamdi, A. Ide-Ektessabi and I. Sopyan, Adhesion failure behavior of sputtered calcium phosphate thin film coatings evaluated using microscratch testing, Journal of the Mechanical Behavior of Biomedical Materials, 3, pp. 324-330, 2010

J.C. LeHuec, T. Schaeverbeke, D. Clement, J. Faber and A. LeRebeller, Influence of porosity on the mechanical resistance of hydroxyapatite ceramics under compressive stress, Biomaterials, vol. 16, pp. 113-118, 1995

J.H.G. Rocha, A.F. Lemos, S. Kannan, S. Agathopoulos and J.M.F. Ferreira, Hydroxyapatite scaffolds hydrothermally grown from aragonitic cuttlefish bones, J. Mater. Chem., vol. 15, pp. 5007–5011, 2005

J.S. Woyansky, C.E. Scott and W.P. Minnear, Processing of porous ceramics, Am. Ceram. Soc. Bull. Vol. 71, pp. 1674-1682, 1992

Karimzadeh, M.R. Ayatollahi, A.R. Bushroa and M.K. Herliansyah, Effect Of Sintering Temperature on Mechanical And Tribological Properties of Hydroxyapatite Measured by Nanoindentation and Nanoscratch Experiments, Ceramics International,vol 40, pp. 9159-9164, 2014

L.M. Rodriguez-Lorenzo and J.M.F. Ferreira, Development of porous ceramic bodies for application in tissue engineering and drug delivery systems. Mater. Res. Bull., vol. 39, pp. 83-91, 2004

L.M. Rodriguez-Lorenzo, M. Vallet-Regi and J.M.F. Ferreira, Fabrication of porous hydroxyapatite bodies by a new direct consolidation method: starch consolidation, J. Biomed. Mater. Res., vol. 60, pp. 232-240, 2002a

L.M. Rodriguez-Lorenzo, M. Vallet-Regi and J.M.F. Ferreira, Fabrication of Hydroxyapatite Bodies by Uniaxial Pressing from a Precipitated Powder. Biomaterials, vol. 22, pp. 583-588, 2001

L.M. Rodriguez-Lorenzo, M. Vallet-Regi, J.M.F. Ferreira, M.P. Ginebra, C. Aparicio and J. Planell, A Hydroxyapatite Ceramic Bodies with Tailored Mechanical Properties for Different Applications, J. Biomed. Mat. Res., vol. 60, pp. 159-166, 2002

M. Itokazu, Y. Wenyi, T. Aoki, A. Ohara and N. Kato, Synthesis of antibiotic-loaded interporous hydroxyapatite blocks by vacuum method and in vitro drug release testing, Biomaterials, vol. 19, no. 7-9, pp. 817-819, 1998

M. Otsuka, Y. Nakahigashi, Y. Matsuda, J.L. Fox, W.I. Higuchi and Y. Sugiyama, A novel skeletal drug delivery system using self-setting calcium phosphate cement VIII: the relationship between in vitro and in vivo drug release from indomethacin-containing cement, Journal of Controlled Release, vol. 43, pp. 115–122, 1997

M.K. Herliansyah, M. Hamdi, A. Ide-Ektessabi, M.W. Wildan and J.A. Toque, The Influence of Sintering Temperature on the Properties of Compacted Bovine Hydroxyapatite, Materials Science And Engineering: C, vol. 29, pp. 1674-1680, 2009

M.K. Herliansyah, Suyitno, P. Dewo, M. Hamdi, and A. Ide-Ektessabi, Development And Characterization of Bovine Hydroxyapatite Porous Bone Graft For Biomedical Applications, Advanced Materials Research Vol. 277, pp 59-65, 2011

N. Tamai, A. Myoui, T. Tomita, T. Nakase, J. Tanaka, T. Ochi and H. Yoshikawa, Novel hydroxyapatite ceramics with an interconnective porous structure exhibit superior osteoconduction in vivo, J. Biomed. Mater. Res. Vol. 59, pp. 110-117, 2002

NIST, Certificate of Analysis – Standard Reference Material 2910: Calcium Hydroxyapatite, National Institute of Standard & Technology. 1997

O. Gauthier, J. Bouler, E. Aguado, P. Pilet and G. Daculsi, Macroporous biphasic calcium phosphate ceramics: influence of macropore diameter and macroporosity percentage on bone ingrowth, Biomaterials, vol. 19, pp. 133-139, 1998

S. Guicciardi, C. Galassi, E. Landi, A. Tampieri, K. Satou and G. Pezzoti, Rheological characteristics of slurry controlling the microstructure and the compressive strength behavior of biomimetic hydroxyapatite, J. Mater. Res. Vol.16, pp. 163-170, 2001

S. Joschek, B. Nies, R. Krotz and A. Gopfrerich, Chemical and physicochemical characterization of porous hydroxyapatite ceramics made of natural bone, Biomaterials vol. 21, pp. 1645-1658, 2000

S. Pramanik, A.K. Agarwaly and K.N. Rai, Development of High Strength Hydroxyapatite for Hard Tissue Replacement, Trends Biomater. Artif. Organs, vol. 19 no. 1, pp. 46-51, 2005

S.F. Hulbert, S.J. Morisson and J.J. Klawitter, Tissue reaction to three ceramics of porous and non-porous structures, J. Biomed. Mater. Res., vol. 6, pp. 347-374, 1972

T.J. Flatley, K.L. Lynch and M. Benson, Tissue Response to Implants of Calcium Phosphate Ceramic in the Rabbit Spine, Clin. Orthop., vol 179, pp. 246-252, 1983

Y. Fang, D.K. Agarwal, D.M. Roy and R. Roy, Fabrication of porous hydroxyapatite ceramics by microwave processing, J. Mater. Res. Vol. 7, pp. 490-494, 1992

Y. Suzuki, S. Matsuya, K. Udoh and M. Nakagawa, Fabrication of hydroxyapatite block from gypsum block based on (NH4)2HPO4 treatment, Dental Materials Journal,vol. 24,pp. 515-521, 2005