

# Preparation of macroporous tricalcium phosphate scaffolds for tissue engineering

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## ABSTRACT

There are many types of bone substitute material, such as bioactive glass-ceramic (45S5R), hydroxyapatite (HAp), tricalcium phosphate (TCP), tetracalcium phosphate (TTCP) and so on. Among the above, HAp will transform to  $\beta$  or  $\alpha$  phase of TCP by different heat treatment conditions; the degradation rate of  $\beta$ -TCP is faster, and it also has the good osteoconductive ability, while  $\alpha$ -TCP has the good biocompatibility, and it also has degradation ability, Therefore, they are suitable as a bone graft substitute. In present work, efforts were focused on development of porous calcium phosphate ceramics using stearic acid as pore-forming agent. The results showed that  $\beta$ -TCP,  $\alpha$ -TCP and  $\alpha$ -TCP ceramics could be obtained by sintering at T4, T5 and T7 respectively. The pore structure and pore size of the different crystallization of porous calcium phosphate ceramic were observed by scanning electron microscopy(SEM). Finally, after soaking in distilled water solution, there was no significant change of porous HAp ceramic, the degradation behavior of porous  $\beta$ -TCP/HAp ceramic could be observed, and The surface apatite layer formed on porous  $\beta$ -TCP/  $\alpha$ -TCP/HAp and  $\alpha$ -TCP/HAp ceramics could be observed.

Keywords : Calcium phosphate ceramic、Crystalline phase、Scaffold、Pore structure

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## REFERENCES

參考文獻 [1]Hulbert SF, Bokros JC, Hench LL, Wilson J, Heimke G, Ceramics in clinical applications-past, present and future, in Ceramics, in

Clinical Applications. Edited by Vincenzini P, Elsevier, Amsterdam, Netherlands, pp. 3-27, 1987.

[2]Silver FH, Biomaterials, medical devices, and tissue engineering: an integrated approach. Chapman and Hall, First edition, New York, 1994.

[3]Ratner BD, Biomaterials science: an introduction to materials in medicine. Academic Press, San Diego, 1996.

[4]Kingery WD, Bowen HK, Uhlmann DR, introduction to Ceramics. Second edition Wiley-Interscience, New York, p. 368, 1976.

[5]Park JB, Lakers RS, Biomaterials: An Introduction Second edition Plenum press. New York, 1992.

[6]Lin FH, Lin CC, Liu HC, Huang YY, Wang YY, Sintered porous-bioglass and hydroxyapatite as bone substitute. Biomaterials 15:1087-1098, 1994.

[7]Peltier LE, The use of plaster of paris to fill defect in bone. Orthopedic Clinics of North America 21:10-31, 1961.

[8]Graves GA, Hentrich RL, Stain HG, Baijpai PK, Resorbable ceramic implants in bioceramic. Engineering in Medicine, Interscience Publisher, New York, pp. 91-115, 1972.

[9]Jarcho M, Salsbury RL, Thomas MB, Synthetic and fabrication of  $\beta$ -TCP ceramic for potential prosthesis application. Journal of Materials Science 14:142-150, 1979.

[10]Chohayeb AA, Chow LC, Tsaknins PJ, Evaluation of calcium phosphate as a root canal sealer-filler material. Journal of Endodontics 13:384-387, 1987.

[11]Hong YC, Wang JT, Hong CY, Brown WE, Chow LC, The periapical tissue reactions to a calcium phosphate cement in the teeth of he monkeys. Journal of Biomedical Materials Research 25:485-498, 1991.

[12]Miyamoto Y, Toh T, Ishikawa K, Yuasa T, Nagayama M, Suzuki K, Effect of added  $\text{NaHCO}_3$  on the basic properties of apatite cement. Journal of Biomedical Materials Research Part A 2:147-151, 2001.

[13]Knaack D, Goad MEP, Aiolova M, Rey C, Tofighi A, Chakravarthy P, Lee DD, Resorbable calcium phosphate bone substitute. Journal of Biomedical Materials Research 43:399-409, 1998.

[14]Zhou J, Zhang X, Chen J, Zeng S, de Groot K, High temperature characteristics of synthetic hydroxyapatite. Journal of Materials Science: Materials in Medicine 4:83-85, 1993.

[15]Van Mullen PJ, de Wijn JR, Vaandrager JM, Porous acrylic cement: Evaluation of a novel implant material. Annals of Plastic Surgery 21:576-582, 1988.

[16]De Groot K, Bioceramics of Calcium Phosphate. Journal of Clinical Engineering, pp. 52, 1984.

[17]Bucholz RW, Carlton A, Holmes RE, Hydroxyapatite and tricalcium phosphate bone graft substitutes. Orthopedic Clinics of North America 18: 323-334, 1987.

[18]Laverna C, Schoenung JM, Calcium phosphate ceramics as bone substitutes. American Ceramic Society Bulletin 70:95-100, 1991.

[19]Hench LL, Bioceramics. Journal of the American Ceramic Society 81:1705-1728, 1998.

[20]Baksh D, Davies JE, Kim S, Three-dimensional matrices of calcium polyphosphates support bone growth in vitro and in vivo. Journal of Materials Science: Materials in Medicine 9:743-748, 1998.

[21]Makoto K, Koyama Y, Kazuo T, Miyairi H, Shirahama N, In vitro change in mechanical strength of  $\beta$ -tricalcium phosphate/copolymerized poly-L-lactide composites and their application for guided bone regeneration. Journal of Biomedical Materials Research Part A 62:256-272, 2002.

[22]Tampieri A, Celotti G, Sprio S, Delcogliano A, Franzese S, Porosity-graded hydroxyapatite ceramics to replace natural bone. Biomaterials 22:1365-1370, 2001.

[23]Pilliar RM, Filiaggi MJ, Wells JD, Grynblas MD, Kandel RA, Porous calcium polyphosphate scaffolds for bone substitute applications-in vitro characterization. Biomaterials 22:963-972, 2001.

[24]Shima T, Keller JT, Alvira MM, Mayfield FH, Dunsker SB, Anterior cervical discectomy and interbody fusion. An experimental study using a synthetic tricalcium phosphate. Journal of Neurosurgery 51:533-538, 1979.

[25]Porter NL, Pilliar RM, Grynblas MD, Fabrication of porous calcium polyphosphate implants by solid freeform fabrication: A study of processing parameters and in vitro degradation characteristics. Journal of Biomedical Materials Research 56:504-515, 2001.

[26]Goldstein SA, Moalli MR, Current concepts in tissue engineering: cell, matrices, and genes. Current Opinion in Orthopaedics 12:424-427, 2001.

[27]Lu L, Currier BL, Yaszemski MJ, Synthetic bone substitutes. Current Opinion in Orthopaedics 11:383-390, 2000.

[28]Agrawal CM, Ray RB, Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. Journal of Biomedical Materials Research 55:141-150, 2001.

[29]Lin FH, Hon MH, A study on the bioglass ceramics in the  $\text{NaO-CaO-SiO}_2\text{-P}_2\text{O}_5$  system. Journal of Materials Science 23:4295-4299, 1989.

[30]Feenstra L, de Groot K, Medical use of calcium phosphate ceramics. In Bioceramics of Calcium Phosphate, CRC Press, p. 131-141, 1983.

[31]Hing KA, Best SM, Bonefield W, Characterization of porous hydroxyapatite. Journal of Materials Science: Materials in Medicine 10:135-145, 1999.

[32]De Oliverira JF, De Aguiar PF, Rossi AM, Soares GA, Effect of process parameters on the characteristics of porous calcium phosphate ceramics for bone tissue scaffolds. International Society Artificial Organs 27:406-411, 2003.

[33]Ramay HRR, Zhang M, Preparation of porous hydroxyapatite scaffolds by combination of the gel-casting and polymer sponge methods.

Biomaterials 24:3293-3302, 2003.

[34] Ramay HRR, Zhang M, Biphasic calcium phosphate nanocomposite porous scaffolds for load-bearing bone tissue engineering. *Biomaterials* 25:5171-5180, 2004.

[35] Cai S, Xu GH, Yu XZ, Zhang WJ, Xiao ZY, Yao KD, Fabrication and biological characteristics of  $\beta$ -tricalcium phosphate porous ceramic scaffolds reinforced with calcium phosphate glass. *Journal of Materials Science: Materials in Medicine* 20:351-358, 2009.

[36] Chen QZ, Thompson ID, Boccaccini AR, 45S5 BioglassR-derived glass-ceramic scaffolds for bone tissue engineering. *Biomaterials* 27:2414-2425, 2006.

[37] Juna YK, Kim WH, Kweon OK, Hong SH, The fabrication and biochemical evaluation of alumina reinforced calcium phosphate porous implants. *Biomaterials* 24:3731-3739, 2003.

[38] Callcut S, Knowles JC, Correlation between structure and compressive strength in a reticulate glass-reinforced hydroxyapatite foam. *Journal of Materials Science: Materials in Medicine* 13:485-489, 2002.

[39] Kim HW, Knowles JC, Kim HE, Hydroxyapatite porous scaffold engineered with biological polymer hybrid coating for antibiotic vancomycin release. *Journal of Materials Science: Materials in Medicine* 16:189-195, 2005.

[40] Miao X, Lim G, Loh KH, Boccaccini AR, Preparation and characterization of calcium phosphate bone cement. *Materials Processing for Properties and Performance* 3:319-324, 2004.

[41] Sepulveda P, Binner JGP, Rogero SO, Higa OZ, Bressiani JC, Production of porous hydroxyapatite by the gel-casting of foams and cytotoxic evaluation. *Journal of Biomedical Materials Research Part A* 50:27-34, 2000.

[42] Barralet JE, Grover L, Gaunt T, Wright AJ, Gibson IR, Preparation of macroporous calcium phosphate cement tissue engineering scaffold. *Biomaterials* 23:3063-3072, 2002.

[43] Almirall A, Larreq G, Delgado JA, Martinez S, Planell JA, Ginebra MP, Fabrication of low temperature macroporous hydroxyapatite scaffolds by foaming and hydrolysis of an  $\alpha$ -TCP paste. *Biomaterials* 25:3671-3680, 2004.

[44] Frayssinet P, Mathon D, Lerch A, Autefage A, Collard P, Rouquet N, Osseointegration of composite calcium phosphate bioceramics. *Journal of Biomedical Materials Research Part A* 50:125-130, 2000.

[45] Miao X, Hu Y, Liu J, Chen Y, Transactions-7th World Biomaterials Congress, p. 317, 2004.

[46] Miyazaki T, Ohtsuki C, Iwasaki H, Ogata S, Tanihara M, Organic modification of porous  $\beta$ -Tricalcium phosphate to improve chemical durability. *Materials Science Forum* 426-432:3201-3206, 2003.

[47] Prado da Silva MH, Lemons AF, Gibson IR, Ferreira JMF, Santos JD, Porous glass reinforced hydroxyapatite materials produced with different organic additions. *Journal of Non-Crystalline Solids* 304:286-292, 2002.

[48] Liu DM, Fabrication and characterization of porous hydroxyapatite granules. *Biomaterials* 17:1955-1957, 1996.

[49] Flautre B, Descamps M, Delecourt C, Blary MC, Hardouin P, Porous HA ceramic for bone replacement: Role of the pores and interconnections-experimental study in the rabbit. *Journal of Materials Science: Materials in Medicine* 12:679-682, 2001.

[50] Schwartzwalder K, Somers H, Somers AV, Method of making porous ceramic articles. United States Patent Office No.3090094, 1963.

[51] 朱新文, 江東亮. 有機泡沫浸漬工藝一種經濟實用得多孔陶瓷製作工藝. 矽酸鹽通報. p. 45-51, 2000.

[52] Falamaki C, Naimi M, Aghaie A, Dual behavior of  $\text{CaCO}_3$  as a porosifier and sintering aid in the manufacture of alumina membrane/catalyst supports. *Journal of European Ceramic Society* 24:3195-3201, 2004.

[53] Zu LJ, Luo SJ, Study on the powder mixing and semi-solid extrusion forming process of SiCp/2024Al composites. *Journal of Materials Processing Technology* 114:189-193, 2001.

[54] Kim T, Goto T, Lee B, Microstructure control and mechanical properties of fibrous  $\text{Al}_2\text{O}_3/\text{ZrO}_2$  composites fabricated by extrusion process. *Scripta Materialia* 52:725-729, 2005.

[55] Qiao GJ, Ma R, Jin ZH, Microstructure transmissibility in preparing SiC ceramics from natural wood. *Journal of Materials Processing Technology* 120:107-110, 2002.

[56] Qian JM, Wang JP, Qiao GJ, Jin ZH, Preparation of porous SiC ceramic with a woodlike microstructure by sol-gel and carbothermal reduction processing. *Journal of European Ceramic Society* 24:3251-3259, 2004.

[57] Luhlrich H, Dias J, Nickel H, The coat-mix procedure using carbon fillers. *Carbon* 35:95-102, 1997.

[58] Simwonis D, Thulen H, Dias FJ, Naoumidis A, Stover D, Properties of Ni/YSZ porous cermets for SOFC by tape casting and coat-mix process. *Journal of Materials Processing Technology* 92:107-111, 1999.

[59] Middleton H, Diethelm S, Ihinger R, Co-casting and co-sintering of porous MgO support plates with thin dense perovskite layers of  $\text{LaSrFeCoO}_3$ . *Journal of European Ceramic Society* 24:1083-1086, 2004.

[60] 伍祖璁, 黃錦鐘. 粉末冶金. 高立圖書有限公司. p. 193, 1996 [61] LeGeros RZ, Lin S, Rohanizadeh R, Mijares D, LeGeros JP, Biphasic calcium phosphate bioceramics: preparation, properties and applications. *Journal of Materials Science: Materials in Medicine* 14:201-209, 2003.

[62] Livingston Arinze T, Tran T, Mcalary J, Daculsi G, A comparative study of biphasic calcium phosphate ceramics for human mesenchymal stem cell-induced bone formation. *Biomaterials* 26:3631-3638, 2005.

- [63]Bouler JM, Trecant M, Delecrin J, Royer J, Passuti N, Daculsi G, Macroporous biphasic calcium phosphate ceramics: Influence of five synthesis parameters on compressive strength. *Journal of Biomedical Materials Research* 32:603-609, 1996.
- [64]Posner AS, Betts F, Synthetic amorphous calcium phosphate and its relation to bone mineral structure. *Accounts of Chemical Research* 8:273-281, 1975.
- [65]Hench LL, Wilson J, Surface-active biomaterials. *Science* 226:630-636, 1984.
- [66]Kivrak N, Cuneyt Tas A, Synthesis of calcium hydroxyapatite-tricalcium phosphate (HA-TCP) composite bioceramic powders and their sintering behavior. *Journal of the American Ceramic Society* 81:2245-2252, 1998.
- [67]Hardouin P, Chopin D, Devyver B, Flautre B, Blary MC, Guigui P, Anselme K, Quantitative histomorphometric evaluation of spinal arthrodesis after biphasic calcium phosphate ceramic implantation in sheep. *Journal of Materials Science: Materials in Medicine* 3:212-218, 1991.
- [68]Ryu HS, Youn HJ, Hong KS, Chang BS, Lee CK, Chung SS, An improvement in sintering property of  $\beta$ -tricalcium phosphate by addition of calcium pyrophosphate. *Biomaterials* 23:909-914, 2002.
- [69]De Groot K, Klein C, Wolke J, Blicek-Hogervorst J, Chemistry of calcium phosphate bioceramics, *CRC Handbook of Bioactive Ceramics, Calcium Phosphate and Hydroxylapatite Ceramics*, vol. II, CRC press, Boca Raton, FL, 1990.
- [70]Billotte WG, Park JB, Bronzino JD, *Ceramic Materials in Biomaterials: Principles and Applications*. CRC Press, Boca Raton, FL, 2002.
- [71]Enderle R, Gotz-Neunhoeffer F, Gobbels M, Muller FA, Greil P, Influence of magnesium doping on the phase transformation temperature of  $\beta$ -TCP ceramics examined by rietveld refinement. *Biomaterials* 26:3379-3384, 2005.
- [72]LeGeros RZ, Calcium phosphates in oral biology and medicine. in: H.M. Myers, Editor, *Monographs in Oral Science*, Karger, Basel, p. 31, 1991.
- [73]Ishikawa K, Ducheyne P, Radin S, Determination of the Ca/P ratio in calcium-deficient hydroxyapatite using X-ray diffraction analysis. *Journal of Materials Science: Materials in Medicine* 4:165-168, 1993.
- [74]Elliott JC, *Structure and chemistry of the apatites and other calcium orthophosphates*. Amsterdam: Elsevier Science, New York, p. 34, 1994.
- [75]Famery R, Richard N, Boch P, Preparation of  $\beta$ - and  $\alpha$ -tricalcium phosphate ceramics, with and without magnesium addition. *Ceramics International* 20:327-336, 1994.
- [76]Dorozhkin SV, A review on the dissolution models of calcium apatites. *Progress in Crystal Growth and Characterization of Materials* 44:45-61, 2002.
- [77]Ding SJ, Wang CW, Chen DCH, Chang HC, In vitro degradation behavior of porous calcium phosphates under diametral compression loading. *Ceramics International* 31:691-696, 2005.
- [78]Michael J, Calcium phosphate ceramics as hard tissue prosthetics. *Clinical Orthopaedics and Related Research* 157:259-278, 1981.
- [79]Ducheyne P, Radin SR, Heughebaert M, Heughebaert JC, Calcium phosphate ceramic coatings on porous titanium: effect of structure and composition on electrophoretic deposition, vacuum sintering and in vitro dissolution. *Biomaterials* 11:224-254, 1990.
- [80]Ducheyne P, Radin S, King L, The effect of calcium phosphate ceramic composition and structure on in vitro behavior. I. Dissolution. *Journal of Biomedical Materials Research* 27:25-34, 1993.
- [81]Case ED, Smith IO, Baumann MJ, Microcracking and porosity in calcium phosphates and the implications for bone tissue engineering. *Materials Science and Engineering* 390:246-254, 2005.
- [82]Ozguur EN, Cuneyt TA, Manufacture of Macroporous Calcium Hydroxyapatite Bioceramics. *Journal of European Ceramic Society* 19:2569-2572, 1999.
- [83]Koc N, Timucin M, Korkusuz F, Fabrication and characterization of porous tricalcium phosphate ceramics. *Ceramics International* 30:205-211, 2004.