UNIVERSITI PUTRA MALAYSIA

MORPHOLOGICAL AND PHYSICAL CHARACTERISTICS OF NANO-HYDROXYAPPETITE BIOCOMPOSITES FOR BONE REPAIR

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By

SURYATI BINTI MOHD THANI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

August 2017

DEDICATION

Specially dedicated to …

My loving parents…
My beloved husband…
My wonderful siblings…
My friends…
for their support and encouragements…

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

MORPHOLOGICAL AND PHYSICAL CHARACTERISTICS OF NANO-HYDROXYAPPETITE BIOCOMPOSITES FOR BONE REPAIR

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Chairman : Professor Md Zuki Bin Abu Bakar@Zakaria, PhD
Faculty : Veterinary Medicine

There was 3 phase in this study. The first phase was synthesized of nano-carbonated hydroxy appetite (nano-CHA) using the conventional microwave at 3 level of microwave power (300W, 600W, and 850W). It was found from TEM that all the synthesized powder was in targeted nano size, with insignificant differences in size (0.031-0.03 nm) among all the samples. For 300 W samples, the average size was 10.15±0.78 nm, while for 600 W and 850 W, were 10.19±0.86 nm and 10.18±0.97 nm, respectively. As for FTIR and XRD analysis, the samples exhibit the trend of hydroxyapatite peaks, regardless of level microwave power used. It is concluded that microwave power has no significant effect on nano-CHA produced. Hence, the power of microwave selected was 300 W in view of lower level of microwave power, therefore less electricity used. The second phase was the production of nano-CHA / gelatin scaffolds in three different ratios, 5:5 (50% nano-CHA/50% gelatin), 6:4 (60% nano-CHA/40 % gelatin) and 7:3 (70% nano-CHA/30% gelatin). The mechanical and physical properties of the bone scaffolds were analyzed. An ideal bone scaffold design was later chosen and proceed to *in vitro* study at the third phase. From TEM analysis, 5:5 nano-CHA/gelatin scaffold, the porosity of the scaffold were located mainly in the middle with pore size ranges from 97-639 μm. While for 6:4 the pores were equally scattered. For 7:3 scaffold, large horizontal crack across the scaffold was detected. Pore size for 6:4 and 7:3 ratio was 106-296 μm and 110-295 μm, respectively. As for porosity percentage, scaffold 5:5 have the highest porosity (67%) followed by 6:4 scaffold (60%) and lastly scaffold 7:3 (50%). Mechanical properties analysis of the scaffolds exhibit that, scaffold 6:4 have the highest yield strength (52.36 MPa) and modulus (853.73 MPa), followed by scaffold 5:5, 46.7 MPa and 684.23 MPa, respectively. Scaffold 7:3 has the lowest yield strength (28.46 MPa) and modulus (598.27 MPa). Next, for water absorption analysis, it can be seen that after 24 hours scaffold with 5:5 has the highest water absorption percentage (72%). While for degradation study, bone scaffold 5:5 and 6:4 showed a mild breakage, while sample 7:3 show a more rapid degradation manner, at week 6, all the bone scaffolds started to

disintegrate at the same rate and complete loss of structure was recorded at week 12. Based on these outcomes, scaffold 6:4 (60% nano-CHA: 40% gelatin) was selected as an ideal bone scaffold. For DSC analysis, the onset temperature, $\mathbf{T_0}$ was at 96.31˚C and the melting temperature**,** $\mathbf{T_m}$ of the ideal scaffold was detected at 331.34˚C, compatible with human body temperature. The FTIR trends, all the important functional groups of hydroxyapatite were presence. Next, EDX analysis found that carbon has the highest (w/w) %, 72.8% and calcium was detected with 2.58% (w/w) %. For *in vitro* study, the ideal scaffold shows a higher level of cells viability (0.14±0.03) compared to control culture medium (0.38±0.03), indicating good compatibility on cells viability. To further clarify, the fluorescence staining of acridine orange (AO) / propidium iodide (PI) signals was conducted. The results exhibit all of the cells were stained green representing the live cells with no sign of dead cells. Moreover, images from Environmental Scanning Electron Microscopy (ESEM) display perfect adhesion of the cell into scaffolds both inside and outside after 14 days of culture. Hence, it can be concluded that the ideal bone scaffold (6:4) is biocompatible to act as a bone replacer.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

MORFOLOGI DAN CIRI-CIRI FIZIKAL PERANCAH BIOKOMPOSIT NANO-CARBONATED HYDROXYAPPETITE UNTUK PEMULIHAN TULANG

Oleh

SURYATI BINTI MOHD THANI

Ogos 2017

Pengerusi : Profesor Md Zuki Bin Abu Bakar@Zakaria, PhD
Fakulti : Perubatan Veterinar

Terdapat 3 fasa didalam kajian ini. Fasa yang pertama ialah sintesis nano-carbonated hydroxyappetite (nano-CHA), menggunakan ketuhar gelombang mikro konvensional pada 3 tahap kuasa iaitu (300W,600W dan 850W). Daripada keputusan TEM, kesemua serbuk yang disintesis adalah dalam saiz nano, dengan kepelbagaian saiz yang sangat sedikit (0.031-0.03 nm) di kalangan sampel. Untuk sampel 300 W, purata panjang adalah 10.15±0.78 nm, manakala untuk sampel 600 W dan 850 W, purata panjang adalah 10.19±0.86 nm and 10.18±0.97 nm. Bagi analisis FTIR dan XRD kesemua sampel nano-CHA menunjukkan corak yang sama, dengan puncak hydroxyappetite tanpa mempedulikan kuasa ketuhar gelombang mikro yang digunakan. Oleh yang demikian, kuasa gelombang mikro yang dipilih adalah 300 W untuk penghasilan nano-CHA kerana pengunaan elektrik yang rendah. Untuk fasa kedua, serbuk gelatin telah di campur dengan nano-CHA dalam tiga nisbah berbeza, 5:5 (50% nano-CHA/50% gelatin), 6:4 (60% nano-CHA/40 % gelatin) dan 7:3 (70% nano-CHA/30% gelatin). . Ciri mekanikal dan fizikal perancah tulang telah dikaji. Perancah tulang yang ideal dipilih bedasarkan hasil kajian ini dan diteruskan ke kajian *in vitro* pada fasa ketiga. Untuk kajian TEM keliangan perancah 5:5 nano-CHA/gelatin terletak ditengah-tengah perancah dimana pelbagai saiz liang 97-639 µm. Manakala untuk 6:4, liang didalam perancah disebarkan secara sama. Untuk perancah 7:3, rekahan besar secara melintang disepanjang perancah telah dikesan. Saiz liang untk 6:4 dan 7:3 adalah 106-296 µm and 110-295 µm. Untuk peratusan keliangan, perancah 5:5 mempunyai keliangan yang paling tinggi (67%), diikuti oleh perancah 6:4 (60 %) dan yang terakhir perancah 7:3 (50%). Kajian ciri mekanikal perancah menunjukkan bahawa perancah 6:4 mempunyai kekuatan alah dan modulus yang paling tinggi iaitu (52.36 MPa) dan (853.73 MPa), diikuti oleh perancah 5:5, 46.7 MPa dan 684.23 MPa. Perancah 7:3 mencatatkan kekuatan alah dan modulus yang paling rendah iaitu 28.46 MPa dan 598.27 MPa. Seterusnya, untuk analisis penyerapan air, selepas 24 jam, perancah 5:5 mempunyai peratusan kadar penyerapan air tertinggi

(72%). Manakala untuk kajian degradasi, perancah tulang 5:5 dan 6:4 menunjukkan kadar kepatahan yang perlahan, manakala sampel 7:3 menunjukkan kadar digradasi yang tinggi dimana kadar kepatahan yang sederhana. Pada minggu 6, kesemua perancah tulang mulai digradasi pada kadar yang sama dan kemusnahan keseluruhan struktur dicatatkan pada minggu 12. Bedasarkan keputusan yang diperoleh, perancah 6:4 (60% nano-CHA: 40% gelatin) telah dipilih sebagai perancah tulang yang ideal.

Untuk kajian sifat haba, hasil analisis DSC menunjukkan suhu permulaan, $\mathbf{T_0}$ adalah 96.31˚C dan suhu lebur, $\mathbf{T_m}$ perancah ideal dikesan pada 331.34˚C, serasi dengan suhu badan manusia. Aliran FTIR, menunjukkan kesemua kumpulan kimia hydroxyappetite hadir. Kemudian, analisis EDX menunjukkan unsur karbon (C) telah mencatatkan peratusan (w/w%) tertinggi iaitu 72.8% dan kalsium sebanyak 2.58%(w/w%). Untuk kajian *in vitro*, perancah ideal menunjukkan nilai daya maju sel adalah lebih tinggi (0.14±0.03) berbanding dengan medium kultur kawalan (0.38±0.03), menjelaskan keserasian yang baik dalam daya maju sel. Untuk menerangkan dengan lebih mendalam, pewarnaan pendarfluor daripada acridine orange (AO) / propidium iodida (PI) isyarat telah dijalankan. Keputusan menunjukkan kebanyakan sel diwarnai hijau mewakili sel yang hidup dan tiada sel mati yang diwarnai merah. Selain itu, gambar dari Environmental Scanning Electron Microscopy (ESEM) menunjukkan lekatan sel yang sempurna ke atas perancah dibahagian dalam dan luar. Oleh yang demikian, ini boleh disimpulkan bahawa perancah tulang yang ideal (6:4) adalah bio serasi sebagai pengganti tulang.

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Above all, to the Great Almighty Allah SWT, the ultimate author of knowledge and wisdom, for His countless love.

Thank You,

May ALLAH SWT bless you all…………

I certify that a Thesis Examination Committee has met on 4 August 2017 to conduct the final examination of Suryati bt Mohd Thani on her thesis entitled "Morphological and Physical Characteristics of Nano-Hydroxyappetite Biocomposites for Bone Repair" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

HA	Hydroxyapatite
CHA	Carbonated Hydroxyapatite
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
DSC	Differential Scanning Calorimetric
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-Ray Diffraction Analysis
EDX	Energy Dispersive X-Ray Analyzer

CHAPTER 1

INTRODUCTION

1.1 Introduction

The United Nation via the World Health Authority reinforced, the year of 2000-2015 will be the era of bone and joint development based on justification that almost half of the population, along their life, will develop joint or bone disease either due to osteoporotic fractures or trauma related bone problems, and equally important the children that suffered from bone disease such as osteosarcoma and osteogenesis imperfecta (TMF Orthopedic Decade, 2000) . Some other cases that require bone surgery including trauma cases, bone deformity and aggressive and invasive bone tumour (Boyne *et al*., 2002).

In the present time, there are many researchers on manufacturing new biomaterials using a modification of previous biomaterials and chemical which can closely imitate normal bone tissues materials. Dental and orthopedic surgical treatments require sufficient amount of bone tissue in order for a natural bone healing process to take place especially for small defect (Hutmacher, 2001). While for a large defect with bulkiness bone loss that requires an operation, a bone graft is needed, and therefore interest in bone regeneration is increasing. Bone transplantation also is known as bone grafting or bone replacer has a significant growing impact on recovering bone tissue damage patients. It must be biocompatible, nonimmunogenic, well adaptation to surface contour and maintain size when placed (Hutmacher, 2001). It also becomes the second most commonly transplanted human tissue after blood (Boyne, *et al* 2002). At the present times, the ideal solution for bone construction in critical bone defect is scaffold tissue engineering. Its act as the main support system in early phases of the bone healing process, in addition to the fact of its 3D appearances that imitate the extracellular matrix for cell linkage and proliferate (Freed *et al.*, 1994b).

The scaffold substances can be made either from natural or synthetic polymers. The advantage of natural material of scaffolds is the biological similarity that adds to better cell function, conversely due to its limited availability, causing increased in price, extinction of sources and possibility of pathogenic impurities (Liu and Ma, 2004). As different from synthetic polymers, by using natural sources features of the scaffolds such as level of strength, pores sizes, degradation rates and evenness for every batch production could be controlled. (Gunatilake and Adhikari, 2003; Liu and Ma, 2004) .

1.2 Problem Statement

One of the most common fillers that being used for bone replacer and coating material in prosthetic implants is hydroxyapatite (HA). It is a natural mineral of calcium apatite, with the formula of $Ca_{10}(PO_4)_6(OH)_2$, usually found in human bone and teeth (Cameron and Besim, 2013). For this reason, it is frequently used as a bone replacer, but the production of hydroxyapatite required sintering at temperature more than 1000°C. Despite that, it normally used as powder or paste form to replace bone materials in dental and orthopaedic treatment, moreover, it also acts as coating material for metallic prosthesis to enhance better biological function (Samar *et al*. 2007).

The need of nanomaterials is crucial as normal human bone are made up of nanocomposites molecules (Murugan and Ramakrishna, 2004). In the coming decade, the use of nanotechnology is expected to increase to 10%, leading to a rapid expansion of nanotechnology, which will have an impact on daily life; to list out, drug delivery system (Lu and Chen, 2004), tissue engineering (Smith and Ma, 2004) revealing of cancer cell (Ferrrar, 2005) and latest bio imaging technique (Thaalhammer and Heckl,2004 ; Kobayashi and Brechiel, 2005). As such, adequate guidelines for handling nanoparticles and nano-related products are essential. Therefore, it is vital for more research to be carried out to disseminate more information on risks and safety of nanomaterial and nanotechnology.

Sometimes the artificial bone grafts used are not structurally similar to natural bone structure. Insufficient in volume from the donor is the main problem in autogenous bone grafts. A few problems may arise when using biomaterials from the gel and Pluronic F 127 whereby it is unstable, whereas biomaterial from calcium phosphate is difficult to be injected and irresolvable (Brandt *et al*., 2010). Conventionally, autograft acts as a benchmark for bone graft in view of it influences all the important criteria for bone regeneration, on the other hand, it generates a long list of other problems, such as donor site morbidity, less tissue obtainability with possibility of extra complication pre and post operatively (Younger and Chapman, 1989).

Thus, to overcome these problems, the present study was carried out to develop the nanocomposite bio material bone scaffolds, easily replicable, absorbable and has similarity to normal human bone tissue, using minimal chemical and simple methods using the microwave. This research focuses on producing carbonated hydroxyapatite (CHA) with suitable chemical, physical, mechanical and biological properties that can be applied in the biomedical application. Different synthesis methods were performed to obtain a correct chemical composition, particle size, and morphology of carbonated hydroxyapatite. The thermal stability of synthesized CHA powder is also investigated.

1.3 Objectives

The objectives of this research were:

i. to synthesize nano-CHA powder using conventional microwave and characterization of the synthesized powder
ii. to developed nanocomposite carbonated hydroxyapatite (CHA) bone scaffold and characterization of its mechanical and physical properties
iii. to evaluate the nanocomposite CHA bone scaffolds *in vitro* using osteoblast cell line

1.4 Research Scope

Experimental work of this project was carried out in two phase. The first phase was focused on obtaining a nano-carbonated hydroxyapatite (nano-CHA). The synthesis of nano-CHA was carried out by using the microwave at different conditions. Along with this phase, factors affecting the physical properties of CHA were determined. To achieve nano-CHA, numerous preliminary tests for every level of each factor were performed. Under the selected process conditions, nano-CHA samples were characterized using various analyses with the main objective of getting the optimum nano size of CHA powder.

Understanding that particle size and particle shape of nano-CHA are crucial in designing bone scaffolds, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used for determination of its morphology and differential scanning calorimetric (DSC) was used to determine its thermal properties. Fourier transform infrared spectroscopy (FTIR) and x-ray diffraction analysis (XRD) were finally used to phase characterize the nano-CHA.

After achieving the optimum particle size and shape of the nano-CHA, bone scaffolds were prepared by mixing nano-CHA powder with gelatine powder. As particle size and shape are two main factors in designing effective bone scaffolds. The gelatine powders were also characterized using the same instruments as nano-CHA.

The second phase of this study concentrated on designing and analyze bone scaffolds made from nano-CHA, gelatine powder, and water. The morphology and elemental analysis of bone scaffolds at various level of process conditions were studied using energy dispersive x-ray analyzer (EDX) and scanning electron microscopy (SEM). While XRD and FTIR were used to evaluate the phase characterization of the bone scaffolds. Apart from that, the porosity study, mechanical test, degradation manner and water absorption tests were done to determine the strength of the bone scaffolds. Lastly, in order to study the biocompatibility of bone scaffolds, samples were analyzed using *in vitro* test.

1.5 Hypothesis

The nano-carbonated hydroxy appetite biocomposites bone scaffolds possess physical characteristic as an ideal bone

REFERENCES

A, G. A. (1999). *The concise system of orthopedic and fractures second edition.* The United Kingdom,: Reed Educational and Professional Publishing Ltd.

Agrawal, C. A. (1997). A technique to control pH in the vicinity of bio degrading PLA-PGA implants. *J.Biomed.Mater.Res 38(2),* 105-114.

Andriano, K. T. (1999). In vitro and in vivo comparison of bulk and surface hydrolysis in absorbable polymer scaffolds for tissue engineering. *Journal Biomed. Mater.Res 48(5)*, 602-612.

Arita, I., Castano, V., & Wilkinson, D. (1995). Synthesis and processing of hydroxyapatite ceramic tapes with controlled Porosity. *Journal of Materials Science: Materials in Medicine vol 6 no 1*, 19-23.

Bakar, M., Kaveh, K., Hussein, B., & Mustapha, N. (2011). *Handbook of Bone Engineering.* Universiti Putra Malaysia: UPM Press Publisher.

Bauer, T. M. (2000). Bone Graft Materials: An overview of the basic Science. *Clinical Orthopedic Related Res (371),* 10-27.

Bhat, S. (2007). *Biomaterial 2nd edition.* Harrow UK: Alpha Science International Limited.

Bhatia, S. a. (1999). Tissue engineering at the micro-scale. *Biomedical Microdevices (2),* 131-144.

Bhattacharyya, S. N. (2005). Development of biodegradable polyphosphazene-nanohydroxyapatite composites nano fibers via electrospinning. *Mater.Res.Soc.Symp. (845)* , 91-96.

Bose, S. S. (2003). Synthesis and characterization of hydroxyapatite nanopowder by emulsion technique. *Chemical Materials (15),* 4464-4469.

Bose, S., Roy,M.& Bandyopadhyay,A. (2012) Recent advances in bone tissue engineering scaffolds. *Journal Trend in Biotechnology* 30,(10) , 546-554.

Bourke, S. J. (2003). Polymers derived from ammonia acids L-tyrosine, polycarbonates, polyacrylates and copolymers with poly(ethylene glycol). *Adv. Drug. Delivery. Rev 55(4)* , 447-466.

Boyle, W, J., W, S., & D, L. (2003). Osteoclast differentiation and activation. *Nature,* 283-294.

Boyne, N., Lambrianides, A., & Pollard, C. (2002). An incisional hernia through iliac crest bone graft donor sites. *ANZI Surgical, 72*, 156-157.

Brandt, J., Henning, S., Michler, g., Hein, W., Bernstein, A., & Schulz, M. (2010). Nanocrystalline Hydroxyapatite for bone repair: An animal study. *Journal Material Science Material Medicine, 21*, 283-294.

Byrappa, K., & Yoshimura, M. (2001). *Handbook of Hydrothermal Technology-A Technology for Crystal Growth and Material Processing.* USA: William Andrew Publishing.

Cameron, C., & Besim, B. (2013). Hydroxyapatite-thermal stability of synthetic hydroxy appetite. *Micromaterials: Excellence in Nanomechanics*.

Chai, C. B.-N. (1999). Bioactive nanocrystalline sol-gel hydroxyapatite coating. *Journal Materials Science Mater. Med (10)*, 465-469.

Chen C, Zhou XS, Zhuang YG, Dong LS (2004). Thermal behavior and intermolecular interactions in blends of poly (3-hydroxy-butyrate) and maleated poly(3-hydroxybutyrate) with chito-san. *Journal Application Polym Science*. ;10:35–47.

Chevalier, J. a. (2009). Ceramics for medical applications: a picture for the next 20 years. *Journal European Ceramic Society, 29*, 1245-1255.

Cuneyt, T. (2000). A synthesis of biomimetic ca-hydroxy appetite powder at 37degree Celsius in synthetic body fluids. *Biomaterials (21)* , 1429-1438.

Cypher, T., & Grossman, J. (1996). Biological Principles of Bone Graft Healing. *The Journal of Foot and Ankle Surgery 35(5)*, 413-417.

David, R. F. (2003). Harvesting Bone Graft from the Posterior Iliac Crest By Less Traumatic, Midline Approach. *Journal Spinal Disorder Technique 16(1)*, 27-30.

Di Chen, J., Wang, Y., Wei, K., Zhang, S., & Shi, X. (2007). Self-organization of hydroxyapatite nano rods through oriented attachment. *Biomaterials, 28,* 2275-2280.

Doll, B. (2005). Basic Bone Biology and Tissue Engineering in Bone Tissue Engineering. In B. Doll, *Basic Bone Biology and Tissue Engineering in Bone Tissue Engineering* (pp. 3-90). CRC Press LLC.

Dudek, R. (2000). Bone: High -yield Histology (2nd edition). In R. Dudek, *Bone: High -yield Histology (2nd edition* (pp. 33-40). Philidelphia: Lippincott Williams &Wilkins.

Fang, Y., Dinesh, K., Della, M., & Rustum, R. (1994). Microwave Sintering of Hydroxyapatite Ceramics. *Material Research Laboratory, 9* (1), 180-187.

Faraday, M. (1857). Experimental relation of gold and other metals to light.

Feng, W., Mu-sen, L., Yu-peng, L., & Yong-xin, Q. (2005). A simple sol-gel technique for preparing hydroxyapatite nanopowders. *Journal of Materials Letters, 59*, 916-919.

Ferrrar, M. (2005). Cancer nanotechnology:: opportunity and challenges. *Nature Review Cancer, 5* (3), 161-171.

Freed, L., Vunjak-Novakovic, G., Biron, R., Eagles, D., Lesnoy, D., Barlow, S., et al. (1994). Biodegradable polymer scaffolds for tissue engineering. *Biotechnology, 12* (7), 689-693.

Fu, Q. R. (2008). In Vitro study on different cell response to spherical hydroxyapatite nanoparticles. *Journal of Biomaterials Application 23(1),* 37-50.

Geocities. (n.d.). Retrieved from www.Geocities.com: http://www.geocities.com/ctas61/biomim.htm

Getty, R. (1975). *The anatomy of the domestic animals 1:3-1211.* America: W.B.Saunders Company.

Giannoudis, P. D. (2005). Bone substitutes: An updates. *Injury 36(3),* 20-27.

Goldstein, A., Zhu, G., Morris, G., Meszlenyi, R., & Mikos, A. (1999). Effect of osteoblastic culture conditions on the structure of poly(DL-lactic-co-glycolic acid) foam scaffold. *Tissue engineering, 5* (5), 421-433.

Gong, T., Xie, J., Liao, J., Zhang, T., Lin, S., & Lin, Y. (2015). Review articles: Nanomaterial and bone regeneration. *Bone research, 3*, 1-7.

Gopferish, A. T. (2002). Polyanhydride degradation and erosion. *Adv. Drug Delivery Rev. 54(7),* 911-931.

Graham, A., & Louis, S. (2016). *Concise System of Orthopedic and Fractures.* United Kingdom: Morgan Kaufmann Publisher.

Gunatilake, P., & Adhikari, R. (2003). Biodegradable synthetic polymers for tissue engineering. *Eur.Cell.Mater, 20* (5), 1-16.

Hall, S. (2007). Basic Biomechanics. In *Basic biomechanic* (p. 88).

Ham, & A, W. (1974). *Histology 3-1006.* Canada: J.B.Lippincott Company.

Han, J., Song, H., Saito, F., & Lee.B.T. (2006). Synthesis of high purity nano-sized hydroxyapatite powder by microwave hydrothermal method. *Materials Chemistry and Physics, 99*, 235-239.

Han, ,D., Hua, X., Dong,S,J., Shen,G,X., Chai,G., Yu,Z,Y.,Lang,W,J., & Ai,T,S. (2010) Rapid prototyped PGA/PLA scaffolds in the reconstruction of mandibular condyle bone defects. *The International Journal Of Medical Robotics And Computer Assisted Surgery*, 66-72

Hui, G., Qingshan, Z., & Zhao, H. (2005). Mechanochemical-hydrothermal synthesis and characterization of fluoridated hydroxy appetite. *Material Research Bulletin, 40* (8), 1326-1334.

Hutmacher, D. K. (2001). A tissue engineered cell-occlusive device for hard tissue regeneration-A preliminary report. *Int.J.Periodontics Restorative Dent 21 (1)* , 49-59.

Hutmatcher, D. (2000). Scaffolds in tissue engineering bone and cartilage. *Biomaterials 21(24)*,,2529-2543.

Imed corporation. (n.d.). Retrieved from www.imed.co.th: http://www.imed.co.th/medical.htm

Kaplan, F. H. (1994). Form and Function of Bone in Orthopedic Basic Science. In F. H. Kaplan, *Form and Function of Bone in Orthopedic Basic Science* (pp. 127-184). Rosemont, ILL: American Academy of Orthopedic Surgeon.

Keith L. Moore, A. F. (2010). *Clinically Orientated Anatomy*. United States of America: Lippincott Williams & Wilkins.

Kellomaki, M. H. (2000). Processing and properties of two different poly(ortho esters). *Journal Mater.Sci.Mater. Med 11(6)*, 345-355.

Kempen, D. L. (2006). Controlled drug release from a novel injectable bio degradable micro sphere/scaffolds composites based on poly(propylene fumarate). *Journal Biomed.Mater.Res A. 77(1)*, 103-111.

Kim, H. S. (2005). Nanofiber generation of gelatin-hydroxy appetite biomimetics for guided tissue regeneration. *Adv.Funct.Mater (15)* , 1988-1994.

Kobayashi, H., & Brechiel, M. (2005). Nano sized MRI contrast agents with dendrimers cores. *Advanced Drug Delivery Review, 57*, 2271-2286.

Koegler, W. G. (2004). Osteoblast response to PLGA tissue engineering scaffolds with PEO modified surface chemistries and demonstration of patterned cell response. *Biomaterials 25(14)* , 2819-2830.

Komlev, V., Barinov, S., Orlovskii, V., & Kurdyumov, S. (2001). Porous Ceramic granules of hydroxyapatite. *Refractories and Industrial Ceramics vol 42*, 195-197.

Kumar, T., Manjubala, I., & Gunasekaran, J. (2000). Synthesis of carbonated calcium phosphate ceramic using microwave irradiation. *Biomaterials, 21*, 1623-1629.

Lakers, R. (1993). Materials with structural hierarchy. *Nature (361)* , 511-515.

Lakshmi, S. K. (2003). Biodegradable polyphosphazene for drug delivery application. *Adv.Drug.Delivery Rev. 55(4)*, 467-482.

Langer, R. V. (1993). Tissue Engineering. *Science 260 (5110)* , 920-926.

Laurencin, C. K.-A. (2006). Bone Graft Substitutes. *Expert Rev Med Devices 3(1)* , 49-57.

Lazic, S. Z. (2001). The effect of temperature on properties of hydroxy appetite precipitated from calcium hydroxide and phosphoric acid. *Thermochim.Acta 374(1)*,13-22.

Lee, I. H. (2001). Nanoparticle- directed crystallization of calcium carbonate. *Advanced Materials 13(21)*, 1617.

Lee, K. W. (2006). Fabrication and characterization of poly(propylene fumarate) scaffold with controlled pore structure using 3-D printing and injection molding. *Tissue Eng. 12(10)*, 2801-2811.

Leong, K. C. (2003). Solid freeform fabrication of three dimensional scaffolds for engineering replacement tissues and organs. *Biomaterials 20(13)* , 2363-2378.

Lewandroski, K. B. (2003). Enhanced bioactivity of a poly(propylene fumarate) bone graft substitute by augmentation with nano-hydroxy appetite. *Biomed.Mater.Eng 13(2)*, 115-124.

Liu, C. H. (2001). Kinetics of hydroxyapatite precipitation at pH 10-11. *Biomaterials (22)* , 301-306.

Liu, J. L. (2004). Rapid formation of hydroxyapatite nanostructures by microwave irradiation. *Chemical Physics Letters 396 (4-6)*, 429-432.

Liu, X. W. (2006). Porogen induced surface modification of nanofibrous poly(L-lactic acid)scaffolds for tissue engineering. *Biomaterials 27(21)* , 3980-3987.

Liu, X., & Ma, P. (2004). polymeric scaffolds for bone tissue engineering. *Annals of Biomedical Engineering*, *32* (3), 477-486.

Lu, Y., & Chen, S. (2004). Micro and nano fabrication of biodegradable polymers for drug delivery. *Advanced Drug Delivery Review, 56* (11), 1621-1633.

Luten, J. V.-N. (2003). Water soluble biodegradable cationic polyphosphazene for gene delivery. *Journal Control Release 89(3)*, 483-497.

Mann, S. W. (1989). *Biomeneralization: Chemical and Biochemical Perspectives.* Vancouver USA: Eds.

Maquet, V. B. (2004). Porous poly (alpha-hydroxy acid)/ bio glass composites scaffolds for bone tissue engineering: Preparation and in-vitro characterization. *Biomaterials 25(8)*, 4185-4194.

Marieb, E. (2016). *Human Anatomy & Physiology 10th Edition.* Menlo Park, California: Benjamin/Cummings Science Publishing.

Meejo, S., Maneeprakorn, W., & Winotai, P. (2006). Phase and thermal stability of nanocrystalline hydroxyapatite prepared via microwave heating. *Thermichimica Acta*, *447*, 115-120.

Meerloo,V,J., Kaspers,G,J,L., & Cloos,J (2011) Cell Sensitivity Assays: The MTT Assay. Cancer Cell Culture Journal. 731, 237-245.

Moisenovish,M,M., Akhipova,A,Y., Orlova,A,A., Drutskava,M,S., Volkova,S,V., Zacharov,S,E., Agapov,I,I., & Kirpichnikov,M,P. (2014). Composite Scaffolds Containing Silk Fibroin, Gelatin, Hydroxyappetite For Bone Tissue Regenration And 3D Cell Culturing, *Acta Naturae Journal* 6(1),96-101.

Murugan, R., & Ramakrishna, S. (2004). Bioresorbable composite bone paste using polysaccharides based nano hydroxy appetite. *Biomaterials , 25* (17), 3829-3835.

Muschler, G. N. (2004). engineering principles of clinical cell-based tissue engineering. *Journal Bone Joint Surgery 86-A(7)*, 1541-1558.

Nagai,A., Yamazaki, Y., Ma,C., Nozaki,K., Toyama,T., & Yamashita,K. (2012) Response of osteoblast-like MG63 cells to TiO_2 layer prepared by micro-arc oxidation and electric polarization, *Journal of the European Ceramic Society*, 32, (11), 2647–2652, Nair, L. L. (2006). Polymers as bio materials for tissue engineering and controlled drug delivery. *Adv.Biochem.Eng / Biotechnology (102)* , 47-90.

Ng, S. V. (1997). Synthesis and erosion studies of self-catalyzed poly(ortho esters). *Macromolecules 30(4)*, 770-772.

Oh, S., Park, I., Kim, J., & Lee, J. (2007). in vitro and in vivo characteristics of PCL scaffolds with pores size gradient fabricated by centrifugation method. *Biomaterials* , *28* (9), 1664-1671.

Oliverira, A., & Reis, R. (2004). Pre-mineralisation of starch/polycaprolactone bone tissue engineering scaffolds by a calcium silicate-based process. *Journal of materials science: Materials in Medicine, 15*, 533-540.

Palsson, B. a. (2004). *Tissue engineering.* USA: Pearson Prentice Hall.

Pandit, A. F. (1998). In vivo wound healing response to a modified degradable fibrin scaffolds. *Journal of Biomaterials Applications:12*, 222-236.

Parhi, P., Ramanan, A., & Ray, A. (2004). A convenient route for the synthesis of hydroxyapatite through a novel microwave mediated metathesis reaction. *Materials Letters, 58*, 3610-3612.

Payne, R. M. (2002). Development of an injectable, in situ cross-linkable, degradable polymeric carrier for the osteogenic cell population.Part 3: Proliferation and differentiation of encapsulated marrow stromal osteoblast cultures on cross-linking poly(propylene fumarate). *Biomaterials 23(22)* , 4381-4387.

Porter, B., Oldham, J., He, S., Zobitz, M., Payne, R., & An, K. (2000). Engineering material properties of biodegradable bone regeneration scaffold. *Journal Biomechanical, 122*, 268-288.

Pulapura, S. L. (1990). Structure property relationships for the design of poly imino carbonates. *Biomaterials 11(9)* , 666-678.

RajabiZamani, A. A., & Kazemzadeh, A. (2008). Synthesis of Nanocrystalline Carbonated Hydroxyapatite Powder Nonalkoxide Sol-Gel Method. *Material Science and Engineering C, 28*, 1326-1329.

Rameshbabu, N., Kumar, T., & Kalvala, P. (2006). Synthesis of nanocrystalline fluorinated hydroxyapatite by microwave processing and it's in vitro dissolution study. *Bulletins of Materials Science, 29* (6), 611-615.

Rickert, D. L. (2006). Biocompatibility testing of novel multifunctional polymeric biomaterials for tissue engineering application in head & neck surgery: An Overview. *Eur.Arch.Otorhinolaryngol (263)*, 215-222.

RIman, R., Suchanek, W., Byrappa, K., Chen, C., Shuk, P., & Oakes, C. (2002). Solution synthesis of hydroxy appetite designer particulates. *Journal of Solid State Ionics*, *151*, 393-402.

Robertson, P. W. (2001). Natural history of posterior iliac crest bone graft donation for spinal surgery: A prospective analysis of morbidity. *Spine 26(13)*, 1473-1476.

Roco, M. (2003). Nanotechnology: Convergence with modern biology and medicine. *Current Opinion In Biotechnology (14)*, 337-346.

RZLegeros. (1991). Calcium Phosphate in Oral Biology and Medicine. In RZLeGeros, *Calcium Phosphate in Oral Biology and Medicine* (pp. 154-157). H.M.Myers (Education).

Samar, J., Abhilasha, B., & Himesh, A. (2007). Nanocrystalline calcium phosphate ceramics in biomedical engineering. *Material Science and Engineering, 27* (3), 441-449.

Sarig, S. K. (2002). Rapid formation of nanocrystalline apatite. *journal Crystal Growth (237-239)*, 55-59.

Sen, M. M. (2007). Autologous iliac crest bone graft: should it still be the gold standard for treating nonunions? *Injury (38)*, 75-80.

Sengupta, D. T. (2006). The outcome of local bone versus autogenous iliac crest bone graft in the instrumented posterolateral fusion of the lumbar spine. *Spine 31(9)*, 985-991.

ServiceRF. (2000). Tissue Engineering build New bone. *Science 289*, 1498-1500.

Siddhartan, A., Seshadri, S., & Kumar, T. (2006). Influence of microwave power on nano-sized hydroxyapatite particles. *Scripta Materialia, 55* (2), 175-178.

Silva, C. G. (2006). MIcrowave preparation, structure and electrical properties of the calcium-sodium-phosphate biosystem. *Journal Non-Cryst Solids (352)*, 3512.

Silva, C. P. (2003). Structural properties of hydroxyapatite obtained by mechanosynthesis. *Solid State Sci. (5)*, 553-558.

Silva, V. L. (2001). Microstructural and mechanical study of zirconia-hydroxyappetite (ZH) composites ceramic for biomedical applications. *Composites Science and Technology 61(2)* , 301-310.

Slosarczyk, A., Pasziewicz, Z., & Paluszkiewicz, C. (2005). FTIR and XRD evaluation of Carbonated Hydroxyapatite Powders Synthesized By Wet Method. *Journal of Molecular Structure, 744-747*, 657-661.

Smith, L., & Ma, P. (2004). Nanofibrous scaffolds for tissue engineering colloid and surfaces. *Biointerfaces, 39*, 125-131.

Sonju, C. R. (1997). Quantitative determination of type A and type B carbonate in human deciduous and permanent enamel by means of Fourier transformation infrared spectroscopy. *Advance Dental Res. (11)*, 523-527.

Stadler, A. (2015, August 13). *How Microwave Synthesis Can Help to Change Nanoparticles Materials Properties.* Retrieved from Chemical Industry, Cosmetic and Personal Care, Materials Science and Nanotechnology, Microwave Synthesis: http://blog.anton-paar.com/how-microwave-synthesis-can-help-to-change-nanoparticles-material-properties

Suchanek, W., Byrappa, K., Shuk, P., Riman, R., Janas, V., & Tenhuisen, K. (2004). Preparation of magnesium substituted hydroxyapatite powders by the mechanochemical-hydrothermal method. *Journal of Solid State Chemical, 177*, 793-799.

Takeyama, K., Chatani, M., Takano, Y., & Kudo, A. (2014). In-vivo imaging of the fracture healing in medaka revealed. *Developmental Biology, 394*, 292-304.

Temenoff, J. M. (2000). Injectable biodegradable materials for orthopedic tissue engineering. *Biomaterials 21(23)* , 2405-2412.

Thaalhammer, S., & Heckl, W. (2004). Nanotechnology and medicine. *4th IEEE Conference on Nanotechnology*, (pp. 577-579).

The basic composition of bone (https://upload.wikimedia.org/wikipedia/commons/b/b4/Composition_of_bone.png

Thompson, R., Yaszemski, M., Powers, J., & Mikos, A. (1995). Fabrication of biodegradable polymer scaffolds to engineer trabecular bone. *Journal Biomaterial Science Polymer, 7*, 23.

TMF Orthopedic Decade, I. O. (2000). Retrieved from http://www.devicelink.com/news/100/2/27291.html.

Tortora, G. D. (2006). The Skeletal System: Bone tissue Principles of anatomy and physiology. In G. D. Tortora, *The Skeletal System: Bone tissue Principles of anatomy and physiology* (pp. 171-193). Massachusetts: JohnWiley & Sons, Inc.

Vaccaro, A., Chiba, K., Heller, J., Patel, T., Thalgott, J., Truumees, E., et al. (2002). Bone Grafting Alternatives in Spinal Surgery. *The Spine Journal 2(3)*, 206-215.

Vallet-Regi, M. F.-L. (2001). Colloidal Processing of Hydroxyapatite. *Biomaterials (22)* , 1847-1852.

Velema, J. K. (2006). Biopolymer based biomaterials as scaffolds for tissue engineering. *Advances in Biochemical Engineering/Biotechnology (102)*, 187-238.

Venugopal, J. P. (2010). Biomimetic hydroxyapatite containing a composite nano fibrous substrate for bone tissue engineering. *Philosophical Transactions of the Royal Society. A mathematical, Physical and Engineering Sciences 368(1917)*, 2065-2081.

Vijayan, S., & Varma, H. (2002). Microwave sintering of nano-sized hydroxyapatite powder compacts. *Materials Letters, 86*, 827-831.

Wan, P., Li, C., Gong, H., Jiang, X., Wang, H., & Li, K. (2010). Effects of synthesis conditions on the morphology of hydroxyapatite nanoparticles produced by the wet chemical process. *powder technology, 203*, 315-321.

Webster, T. A. (2007). Nanostructured biomaterials for tissue engineering bone. *Adv.Biochem.Eng/Biotechnology (103)* , 275-308.

Webster, T. (2001). Nanophase Ceramics: The Future orthopedics and dental implant materials. *Advances in Chemical Engineering (27)*, 125-166.

Xiang, L. X. (2004). Formation of CaCo3 nanoparticles in the presence of terpineol. *Materials Letters 58(6)*, 959-965.

Yarlagadda, P. S. (2005). Recent advances and current development in tissue scaffolding. *Biomed.Mater.Eng. 15(3)*, 159-177.

Yoshikawa, H. T. (2009). Interconnected porous hydroxyapatite ceramics for bone tissue engineering. *J R Soc Interface 6 Suppl 3*, 341-348.

Younger, E., & Chapman, M. (1989). Morbidity at bone graft donor sites. *Journal of Orthopedic Trauma, 3* (3), 192-195.

Yunzhi, Y., & Joo, L. (2002). Rapid sintering of hydroxyapatite by microwave processing. *Journal of materials science letters, 21*, 67-69.

Zhang, R. M. (1999). Poly(alpha-hydroxy acids)/ hydroxyapatite porous composites for bone tissue engineering; Preparation and morphology. *Journal Biomed.Mater.Res 44(4)*, 446-455.

Zhensheng, L., Ramay, H., Hauch, K., Xiao, D., & Zhang, M. (2005). Chitosan-alginate hybrid scaffolds for bone tissue engineering. *Biomaterials, 26*, 3919-3928.