CALCIUM PHOSPHATE BONE FILLING MATERIALS: SYNTHESIS, HARDENING AND CHARACTERIZATION

BY

ASEP SOFWAN FATUROHMAN ALQAP

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Kulliyah of Engineering
International Islamic University
Malaysia

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ABSTRACT

Calcium phosphate based materials are clinically accepted ceramics and have been widely used in biomedical application. Apart from brittleness, calcium phosphate ceramics are introduced in the forms of coating, porous, granule, dense, powder and paste. Various methods have been developed to produce calcium phosphate ceramics for bone filler application. This work developed a novel technique which straightforwardly provided the calcium phosphate as bone filling materials from one pot low temperature hydrothermal synthesis. Calcium oxide, CaO, and ammonium dihydrogen phosphate, $NH_4H_2PO_4$, were used as calcium and phosphorus precursors respectively with the media of stirred distilled water at 80-100°C. The amount of CaO was varied at 0, 1, 2, 10 and 20 mol-% excess. Synthesis condition has shown remarkable effects on phase, crystal physics, mechanical strength, hardening by aging in moist and simulated body fluid (SBF) environments, water washout resistance, cohesivity, injectability, bioactivity and Vero cell proliferation capacity. The characterization involved X-ray diffraction (XRD), energy dispersive X-ray (EDX), thermogravimetry/differential thermal analyses (TGA/DTA), Fourier transform infra red spectroscopy (FTIR), scanning electron microscopy (SEM) and Brunnauer-Emmet-Teller (BET) methods. Bone fillers were produced through two types of strategies: mixing between the precipitated powder, p, and water, w, (called as Mixture) at variation of p/w ratios and direct synthesis (called as Paste). The syntheses resulted in non crystalline apatite or Ca-deficient hydoxyapatite (CDHA) as the main phase of the non sintered product in all the excess CaO variations. The maximum compression strength after aging for Mixture was 2.0 MPa in the moist and 3.4 MPa in SBF, while for Paste, it was 2.3 MPa in the moist and 2.7 MPa in SBF. Paste showed as better performance in aspects of mechanical strength, resistance to watering, less fluctuation of the lattice crystal after aging and being injectable after 60 min waiting time. The precursors regulated the products to be single, non-single phase or potential as the candidate of antibacterial material. The apatite cell forming capacity that was affected by the pressure of the pellet compaction was also reported. The study showed that the filler materials are mechanically and biochemically suitable for non-load bearing bone implant applications and Paste is excellent in flexible handling time.

ملخص البحث

المواد المرتكزة على فوسفات الكالسيوم مسلم بكونها سيراميك معتمد سريرياً ويستخدم على نطاق واسع في تطبيقات الطب الحيوي. و بصرف النظر عن هشاشتها، فمادة سيراميك فوسفات الكالسيوم قدمت في أشكال طلاء ،مسامية ، حبيبية ، مشكلة وغيرمشكلة الجزئيات ، أو بشكل عجينة. وقد تم تطوير أساليب متعددة لإنتاج سيراميك فوسفات الكالسيوم واستعماله في حشو العظام. أوجد هذا العمل تقنية جديدة والتي قد تنتج بشكل مباشر فوسفات الكالسيوم كمواد لحشو العظام من خلال خطوة واحدة في درجة حرارة منخفضة. لقد استعمل كل من أكسيد الكالسيوم ، CaO ، و فوسفات الأمونيوم الهيدروجيني ،$NH_4H_2PO_4$، كأساس للفوسفور والكالسيوم في الماء المقطر في 80-100 درجة حرارة. وقد تفاوتت كمية أكسيد الكالسيوم من ٠ ، ١ ، ٢ ، ١٠ ، و ٢٠ ٪ مول فأكثر. وقد أظهرت ظروف عملية التركيب حالة التكوين آثارا مهمة و ملحوظة على حالة المادة و على فيزياء البلورات ، القوة الميكانيكية ، التفاعل مع البيئات الرطبة ، محاكاة السوائل او الموائع في الجسم (SBF) ، التصلب بعد الشيخوخة ، مقاومة تبييض الماء ،و تماسك الجزئيات مؤكدة عن طريق الانحناء ، القدرة على الحقن ، قدرات تشكيل خلية الأباتيت وقدرة خلايا فيرو على النمو. وقد اشتملت الدراسة على عدة تقنيات منها انحراف الأشعة السينية XRD ، طاقة تشتت الأشعة السينية EDX، التحليل الحراري بالقياس الوزني TGA ، التحليل الحراري التفاضلي DTA ، تحويل فوريير للأشعة تحت الحمراء FTIR ، المسح الضوئي المجهر الإلكتروني SEM وتحليل مساحة محددة BET . تم إنتاج حشوة العظام باستخدام استراتيجيتين هما الخلط بين المسحوق المترسب ، p ، والماء ، w ، (يسمى خليط) وفي حالة اختلاق نسبة p / w والتكوين المباشر (يسمى عجينة). تميزت نتائج الأباتيت غير البلورية أو ناقصة الكالسيوم هيدروكسيباتيت CDHA كمرحلة رئيسية من النتائج غير المتكلسة في جميع حالات زيادة أكسيد الكالسيوم المتفاوتة . قوة الضغط القصوى (ميغاباسكال) من الخلطات ٢.٠ بعد الشيخوخة في حالة الرطوبة و ٣.٤ في SBF ، بينما في حالة العجينة كانت قوة الضغط ٢.٣ في الرطوبة و ٢.٧ في SBF. كما أظهرت العجينة تحسناً في الأداء في كثير من جوانب القوة الميكانيكية، و المقاومة للماء، وأقل تذبذباً من شبكة البلورات بعد الشيخوخة وذلك بعد الحقن والانتظار لمدة 60 دقيقة . تنظم المصادر الأولية المنتجات لتكون مكونة من مرحلة واحدة أو عدة مراحل أو كمواد محتملة مضادة للجراثيم. تم إثبات تأثر قدرة الخلية على تشكيل الأباتيت بضغط الكريات. مادة الحشو كانت مناسبة لتطبيقات حشو العظم الإسفنجي أو غير الحاملة بينما كانت العجينة ممتازةً لمرونة الوقت اللازم لتحضيرها.

APPROVAL PAGE

The thesis of Asep Sofwan Faturohman Alqap has been approved by the following:

_____
Iis Sopyan
Supervisor

_____
Suryanto
Internal Examiner

_____
Souad A. Mohamad
Internal Examiner

_____
Mohd Hamdi Bin Abdul Shukor
External Examiner

_____
Amir Akramin Shafie
Chairperson

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Asep Sofwan Faturohman Alqap

Signature ……. Date: 29 September 2011

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Affirmed by Asep Sofwan Faturohman Alqap

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Signature Date

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADP	Ammonium di-hydrogen phosphate
AcPho	Acid phosphatase
ALP	Alkaline phosphatase
BCP	Bicalcium phosphate
BET	Brunnauer – Emmet – Teller
BMD	Body mass density
BMSC	Bone marrow stromal cell
BSA	Bovine serum albumin
$CaAc_2$	Calcium acetate
CaO	Calcium Oxide
CC	Calcium carbonate
CDA	Calcium deficient apatite
CDHA	Calcium deficient hydroxyapatite
CDMS	Calcium deficient meta-stable material
CHA	Carbonated hydrpxyapatite
C-HA	Crystalline hydroxyapatite
Chit	Chitosan
CP	Calcium phosphate
CPP	Calcium pyrophosphate
CRMS	Calcium rich meta-stable material
CS	Compression strength
DAP	Diammonium hydrogen phosphate
DBM	Demineralized bone matrix
DCPA	Dicalcium phosphate anhydrous
DCPD	Dicalcium phosphate dihydrate
DMEM	Dubelco's modification of Eagle's medium
DTS	Diametrical tensile strength
DTA	Differential thermal analysis
EDX	Energy-dispersive x-ray
FA	Fluorapatite
FBS	Fetal bovine serum
FTIR	Fourier transmission infra-red
GIS	Glass ionomer cements
HA	Hydroxyapatite
HPMC	Hydroxyl propyl methyl cellulose
JCPDF	Joint committee for powder diffraction file
LPR	Liquid to powder ratio
MCPA	Monocalcium dihydrogen phosphate anhydrous
MCPM	Monocalcium dihydrogen phosphate monohydrate
MTT	Methyl-thiazolyl-tetrazolium
MWCNT	Multi-walled carbon nanotube
OCP	Octacalcium phosphate
PA	Phosphoric acid
PBS	Phosphate buffered saline

PEG	Poly (ethylene glycol)
PET	Poly (ethylene-terephthalate)
PLA	Poly (lactic acid)
PLLA	Poly (L-lactic acid)
PTFE	Poly tetra fluoro ethylene
RF	Radio frequency
SBF	Simulated body fluid
SCID	Severe combine immunodeficiency
SSA	Specific surface area
TCP	Tricalcium phosphate
TGA	Thermogravimetry analysis
TTCP	Tetracalcium phosphate
UHMWPE	Ultra high molecular weight poly-ethylene

LIST OF SYMBOLS

α	Alfa
β	Bheta
γ	Gamma
ν	Wave vibration mode of Infrared spectrum.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Bone is biologically smart material capable of self-regeneration. For instance, a bone fracture activates osteogenesis and, eventually, the fracture heals without any scar formation. Unfortunately, this ability for self-regeneration has limitations: if the size of large bone defects exceeds a certain threshold, the capacity of the body to repair these defects is insufficient. These defects, which are known as critical-sized defects, do not heal spontaneously without using bone replacing materials to restore the defect (Link, 2008). The bone replacing materials that clinically acceptable are calcium phosphate (CP) based ceramics.

Hydroxyapatite (HA), one of CP ceramics owing to similarity to main bone mineral, non toxicity and biocompatibility in the body have been the popular bone graft and filling materials. HA fillers are applied as bone grafts in rigid forms such as blocks and granules. The blocks are poor to fill bone defect hole. The granules are not stable to keep their dimension and place (Link, van den Dolder, Jurgens, Wolke & Jansen, 2006).

CP cement elements are solid ceramic compounds of calcium and phosphate. They harden when in contact with water or an aqueous solution, hence named as cement. There are two types of the CP cements: apatite transforming cements, that can be in the form of HA, carbonated HA or calcium deficient HA (CDHA), and brushite transforming cements in the form of dicalcium phosphate dihydrate (DCPD). The

apatite cements have better mechanical properties compared to brushite cements, while brushite cements are faster degradable than apatite cements (Bohner, 2007).

The main advantage of CP cements over CP ceramics is the handling properties (Bohner, 2000). As a mixture of CP ceramic powder and liquid, known as CP paste, the CP cement becomes injectable, and hence can be shaped perfectly *in situ* according to the defect dimension. The CP cement can be injected directly into a bone defect, easy to keep stable in place, and shaped before complete setting. The injectability of *in situ* setting cement results in an optimal filling of the cement with the defect dimensions. However, the CP cement must be applied before the initial setting time and the wound should be closed after the final setting time. Otherwise, CP cements could disintegrate upon early contact with aqueous solutions, or body fluids such as blood.

Although giving better mechanical strength properties, the HA based cement shows several drawbacks: difficult storage due to reactivity to open air (Gbureck, Barralet, Hofmann & Thull, 2004), cumbersome precursor development, consuming high energy and cost, fast phase transformation leading to disintegration, loosening and leaking (Seo & Lee, 2007), very short setting time which makes difficult handling (Driessens, Boltong, de Maeyer, Wenz, Nies & Planell, 2002) and relatively no resorbability (Verron, Khairoun, Guicheux & Bouler, 2010).

The strength of material is affected by two aspects: the inherent strength of material and application technology. The strength of material is results of inter-atomic bonding system, crystal density, degree of lattice imperfection and impurities, design and developing process. The application technology also affects strength. A material ready for use with good mechanical strength, process and design will possess better strength or even inversely worse after application. HA which is famous for its

best strength among CP ceramics and non degradable becomes degradable very shortly because of implant misplacement when isolated from load transmission (Dubok, 2000). Tricalcium phosphate (TCP), the famous degradable material does not degrade even after 6 months and a thin fibrous layer surrounds the non loaded ceramic at all times (Korkusuz & Korkusuz, 2004). In opposite, free flow individual powders when placed or molded with high pressurized jet injection or compaction will have strong consolidation (Dunne, Orr & Beverland, 2004; Surin, 2007) and withstand under high loading. Paste strength inducing force by jet injection as addition of the material strength is effective to material construction properties as the result of strengthening by application technology. Besides, successfully remodeled bone graft can create the strong interface even stronger than the graft and the host bone (Dubok, 2000). Weak and resorbable powder will be replaced by new bone due to remodeling by host bone and suitably strengthened *in situ* as required.

Different phase of CP ceramics is different in degradation rate when in contact with water. Dicalcium phosphate dihydrate (DCPD) or brushite is very much fast in degradation rate, and β-tricalcium phosphate (β-TCP) is faster than HA. This degradation rate should be considered in application. Where loading on site is high while fluid is flowing, the least degradable CP is applied like HA and β-TCP. In opposite, when the loading is very low and less fluid flow, β-TCP and DCPD are favourable. Degradation of CP is necessary for bone remodeling. Less or un-degradability property of HA makes it late to be replaced by bone, although it bonds the host stronger. Therefore, porosity in HA is necessary for the possibility of cells to penetrate, grow and bond particles. Fortunately, less crystalline HA and β-TCP are degradable faster than crystalline HA. It is predictable that cells can penetrate easily to the materials when the degradability is faster (Kasten, Luginbühl, Vogel, Niemeyer,

Weiss, van Griensven, Krettek, Bohner, Bosch & Tonak, 2004), therefore less crystalline HA, CDHA and β-TCP are promising CP ceramics for bone filling applications.

CP ceramics are osseoinductive only, not osseogenic. To be osseogenic, CP ceramics are necessarily mixed with bone cells and proteins in order to enable cell differentiation and faster bone remodeling. It is known that bones strengthen by aging from infant to mature, and HA minerals enrich and then the bones become harder but then become brittle and prone to osteoporosis. In the bone, there are osteoblast, *i.e.* bone mineralization promoter cell, and osteoclast, *i.e.* bone demineralization promoter cell. Their activity can be known from alkaline phosphatase (ALP). ALP is evidence marker of both two cells (Fong, Cassir, Le Nihouannen & Komarova, 2008). In denser bone or more crystalline HA, ALP is dominant. Conversely in less crystalline HA, ALP concentration is low. ALP activity can be associated with bone embrittlement due to osteoporosis (Ross & Knowlton, 1998; Talwar & Aloia, n.d.; Klatt, n.d.). It suggests that the osteoclasts works well in crystalline HA but not in less crystalline HA while the osteoblast works well in less crystalline HA and not in crystalline HA. Less crystalline HA, CDHA and β-TCP are promising materials as mixture with bone cells and proteins.

1.2 PROBLEM STATEMENTS AND ITS SIGNIFICANCE

Synthesis process to produce hydroxyapatite (HA) is limited because it needs to lengthy precipitation time in low temperature operation or to pressure control and high temperature to ensure HA achievable thereof. Moreover the results are crystalline HA that is not resorbable or degradable even up to years. *In situ* porous creating process by cells penetration is less possible in this HA. However less crystalline HA, calcium

deficient HA (CDHA) and β-tricalcium phosphate (β-TCP) are favourable to designate for this significant point.

CP cements are made of different calcium and phosphate base compounds, such as mixing of tetracalcium phosphate (TTCP) or α-tricalcium phosphate (α-TCP) with dicalcium phosphate dihydrate (DCPD) or dicalcium phosphate anhydrous (DCPA). TTCP and α-TCP are produced from high temperature process higher than 1200 °C while DCPD is produced from β-TCP decalcification along with hydroxylation. DCPA comes from the heating process of DCPD. Other method to produce DCPA / DCPD is a long process in acid environment. These processes are highly risks in terms of safety work, high energy and high cost. In addition to that, the drawbacks of the products are poor injectability and fast setting time. The setting time determines injection time. Set cement is hard to be moldable and injectable through canula. Faster setting time and shorter injectability cause material wasting and trouble surgeon tasks.

Study on low temperature synthesized CP filler materials with high Ca/P ratio is rather scarce. Although research showed that high calcium positively improved the natural bone, however many researchers on CP filler materials worked in the range of Ca/P ratio $\leq$ 1.67. Researchers who concern with HA never come to high Ca/P ratio because the synthesis with higher Ca/P ratio produces non-single HA based on isothermal solubility diagram. This work is devoted to study on the effect of excess calcium of the product synthesized at low temperature in water as media, on phases after calcinations, on treating as single pot straightforward process from its synthesis to hardening of aging in media, on filling character and biochemical responses, to state its position as solution to the above problems.

1.3 RESEARCH PHILOSOPHY

Knowing that the living bone is great smart material, densification, strengthening or hardening of CP powders before application or implantation is beyond the scope of this work. Densification of CP powders can occur after the material is implanted when chemically reacts with body fluid and biologically regulated by bone cells. It is an advantage if there are the accumulation of properties of hardenable by aging and resorbable to be new bone. When HA is immersed in water or an aqueous solution the strength increases with time (Fu, Zhou, Huang, Wang, Zhang & Li, 2005), meaning hydration process affects the strength. While when resorbable material is soaked in biological environment it resorbs and is totally substituted to be a new bone (Okuda, Ioku, Yonezawa, Minagi, Gonda, Kawachi, Kamitakahara, Shibata, Murayama, Kurosawa and Ikeda, 2008). Addition to that, biological system can even create a joining system to the implant which is stronger than the implant and host bone (Dubok, 2000).

Solid phase like clay is flowing when contains water but lower in strength. As that nature explains, a series of wheels that performs concurrent motion action depends on which way they are being in order and on media. When they are being in continuous order and homogenous smooth media, they move continuously. Once one of the wheels slips or blocks by the media they move no more concurrently and the blocked wheel becomes an obstacle for the overall wheels moving system. Continuity and homogeneity are prerequisite for the series of the wheels move concurrently travelling long distance. This also is a natural teaching of dislocation in materials science and engineering to explain why a material is being strained longer and strengthened higher.