DEVELOPMENT OF CALCIUM PHOSPHATE/ POLY(ETHYLENE GLYCOL) COMPOSITE FOR INJECTABLE BONE CEMENT APPLICATION

BY

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ABSTRACT

The rising attention in micro-invasive bone grafting method in orthopaedics demanded for injectable bone filling materials. The injectable bone cement materials should have optimum setting time to provide sufficient time for implantation and prevent delay of operation, good injectability, mechanical strength similar to that of natural bone, and excellent biological response. However, the limitations of calcium phosphate cement (CPC) due to its low mechanical strength, poor injectability and weak cohesion. The objectives of this study are to develop calcium phosphate/poly(ethylene glycol) (CPC/PEG) composite bone cement and investigate the physical, mechanical and biological properties of the cement. In this method, hydroxyapatite (HA) powder was synthesized by using calcium hydroxide, $Ca(OH)_2$, and diammonium hydrogen phosphate, $(NH_4)_2HPO_4$. The mixture of calcium and phosphorus solution refluxed at 90°C. The production of CPC has been done by mixing the wet chemical precipitation derived HA powder with distilled water at certain powder-to-liquid (P/L) ratio, varied at 1.0, 1.3, 1.5 and 1.7. Bioactive ceramic matrix composite was produced by mixing the synthesized powder with liquid phase containing PEG at different PEG amount, varied at 1, 2, 3, 4 and 5 wt%. The powder characterizations involved X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). XRD and FTIR confirmed the formation of pure HA. Morphology analyses of FESEM and TEM illustrated the formation of agglomerated nanorod shape HA particles with size of 150-300 nm length and 10-30 nm width. Afterwards, the produced CPC was investigated for injectability, setting time, compression strength, porosity, anti-washout and cell proliferation capacity. The results of this study revealed that higher P/L ratio contributed to better setting time and compressive strength of CPC but worsen its injectability. The optimum condition achieved by CPC with the P/L ratio of 1.3, which shows 82.5% paste injectability, 88 min initial setting time, 228 min final setting time and 1.344 MPa compressive strength. The study on the effect of PEG on CPC properties has shown significant improvement in setting time, injectability and mechanical strength. The incorporation of 2% PEG into CPC with the P/L ratio 1.3 shows an optimum condition with 85.9% paste injectability, 60 min initial setting time, 209 min final setting time and 1.781 MPa compressive strength. All CPC compositions demonstrated an excellent performance since no cement dissolution or broken throughout 28 days soaking in Ringer's solution, except for the CPC/PEG with the P/L ratio of 1.0 with 3, 4, and 5 wt% PEG additions. The cell culture on CPC microcarriers has proven that the fabricated CPC shows no toxic reaction and cells grow well. This present study shows that the fabricated bioactive ceramic matrix composite is suitable for injectable bone cement applications.

خلاصة البحث

أثارت تقنية التطعيم العظمي الدقيق الانتباه في مجال جراحة العظام مما أدى إلى زيادة الطلب على المواد اللازمة لحقنها بداخل العظم. تتطلَّب المواد الأسمنتيّة القابلة للحقن داخل العظم تحقيق وقت مثالي لكي يتوفر الوقت الكافي لعملية الزرع ويُمنَع أي تأخير في العمليّة. يجب كذلك أن تتمتع بجودة عالية للحقن، وقوَّة ميكانيكية مُطابقة للعظم الطبيعي، وآخيرًا إستجابة بيولوجيّة ممتازة. ولكن، يُعاني فوسفات الكالسيوم الأسمنتي(CPC) من ضعف قوَّته الميكانيكيَّة وضعف حقنه وكذلك ضعف تماسُكه، لذلك، فالهدف من هذه الدراسة هو تطوير مُركَّب سيراميك العظام المُتكوّن من فوسفات الكالسيوم الأسمنتي (CPC) و غليكول بولي إيثيلين (PEG) ، وكذلك التحقق من الخصائص الفيزيائية والميكانيكية والبيولوجية للأسمنت.في هذه التجربة، تم تركيب مسحوق الهيدروكسيل أباتيت (HA) باستخدام هيدروكسيد الكالسيوم $Ca(OH)_2$ وثُنائي فوسفات الأمونيوم $(NH_4)_2HPO_4$ وتم تكثيفهم على درجة 90 مئوية. تم إنتاج فوسفات الكالسيوم الأسمنتي (CPC) بخلط المترسِّبات الكيميائية الرَّطبة المُستمَدَّة من مسحوق الهيدروكسيل أباتايت HA مع ماء مُقطَّر بنِسب مُعيَّنة من المسحوق والسائل تتنوّع بين 1.0 و 1.3 و 1.5 و 1.7.تم تكوين مصفوفة السيراميك النشطة بيولوجيا عن طريق خلط المسحوق المُركب مع السائل الذي يحتوي على غليكول بولي إيثيلين (PEG) بنِسَب مئوية مُختلفة تتنوَّع بين 1 و 2 و 3 و 4 و 5 نسبة مائوية. أما تمييز المسحوق تطلَّب تقنيات عديدة وهي تشتت الأشعة السينيّة (XRD) و تصوير طيفي بالأشعة تحت الحمراء(FTIR) والمجهر الإلكتروني الماسح ذو الإصدار الحقليّ (FESEM) والمجهَر الإلكتروني النافذ (TEM). أكدت كل من الأشعة السينيَّة وتحت الحمراء تكوُّن هيدروكسيل أباتيت (HA) نقيّ. أظهرت التحليلات المُورْفُولُوجيَّة المُعتدمة على كل من المجهر الإلكتروني الماسح ذو الإصدار الحقليّ FESEM والمجهر الإلكتروني النافذ TEMتشكيل كُتل من جزيئات صغيرة الحجم لهيدروكسيل الأباتيت (HA) يتراوح طولها بين 300-150 نانو متر وعرضها بين 10-30 نانو متر. بعد ذلك، تم فحص فوسفات الكالسيوم الأسمنتي (CPC) المُنتَج فيما يخُصُّ قوة حقنه، وضبطه للوقت، وقوة الضغط، وقدرته المسامية ، قوته المُضادة للانجراف و قدرة خلاياه على التكاثُر.أظهرت نتائج هذه الدراسة أن زيادة نسبة المسحوق للسائل تُحسِّن من نسبة ضبط الوقت وقوَّة ضغط فوسفات الكالسيوم الأسمنتي(CPC)، لكن لها تأثيرًا سلبيًّا على جودة الحقن. وتُعَد النسبة الأمثل للمسحوق والسائل هي 1.3 والتي تُشير إلى 82.5% لجودة الحَقن، 88 دقيقة لضبط الوقت المبدئي و 228 لضبط الوقت النهائي، و1.344 ميجا باسكال لقوة الضغط. دراسة تأثير غليكول بولي إيثيلين (PEG)على خصائص فوسفات الكالسيوم الأسمنتي (CPC) كانت لها نتائج مُعتبرة فيما يخص ضبط الوقت، وجودة الحَقن والقوة الميكانيكية. أظهر دمج 2% من غليكول بولي إيثيلين (PEG) إلى فوسفات الكالسيوم الأسمنتي (CPC) بنسبة مسحوق للسائل 1.3

نسبة 85.9% لجودة الحَقن، 60 دقيقة لضبط الوقت المبدئي و 209 لضبط الوقت النهائي، و 1.781 ميجا باسكال لقوة الضغط. وأظهرت جميع تركيبات فوسفات الكالسيوم الأسمنتي (CPC) أداءًا متميّزًا والسبب هو عدم وجود أي ذوبان أو انكسار للاسمنت أثناء فترة تركها في محلول الرينجر لمدة 28 يوم ماعدا فوسفات الكالسيوم الأسمنتي (CPC) ذو نسبة مسحوق للسائل 1 ونسب إضافية لغليكول بولي إيثيلين (PEG) تتنوّع بين 3 و 4 و 5 بالمائة. وقد أثبتت الزراعة الخَلَوِيَّة على الحاملات الدقيقة لسي.بي.سي(CPC) أن فوسفات الكالسيوم الأسمنتي (CPC) المُرَكّب لم يُظهر أي رد فعل سام كما أن الخلايا تنمو بشكلٍ جيد للغاية. ولهذا، فإن هذه الدراسة أثبتت أن مركَّب مصفوفة السيراميك النشطة بيولوجيا مُناسب لاستعمال المواد الأسمنتيّة القابلة للحقن داخل العظام.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Materials Engineering).

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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.

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Alhamdulillah, with permission and mercy from Allah, I am able to successfully write this thesis. My efforts and struggles throughout two years study period in Master of Science (Materials Engineering) are finally rewarding through the completion of this thesis.

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LIST OF ABBREVIATIONS

BG	Bioactive glass
Ca/P	Calcium-to-phosphorus
CaP	Calcium phosphate
CDHA	Calcium deficient hydroxyapatite
CPC	Calcium phosphate cement
CSC	Calcium sulfate cement
CSiC	Calcium silicate cement
DCPA	Dicalcium phosphate anhydrous
DCPD	Dicalcium phosphate dihydrate
DMEM	Dulbecco's Modification of Eagle's Medium
DSHP	Disodium hydrogen phosphate
FBS	Fetal Bovine Serum
HA	Hydroxyapatite
MCPM	Monocalcium phosphate monohydrate
MPC	Magnesium phosphate cement
P/L	Powder-to-liquid
PBS	Phosphate buffered solution
PEG	Poly(ethylene glycol)
PLGA	Poly(lacti-*co*-glycolic acid)
PLR	P/L ratio
PMMA	Polymethylmethacrylate
PVA	Poly(vinyl alcohol)
SBF	Simulated body fluid
TTCP	Tetracalcium phosphate
α-TCP	α-tricalcium phosphate
β-TCP	β-tricalcium phosphate

LIST OF SYMBOLS

α	Alpha
β	Beta
γ	Gamma
υ	Wave vibration mode of Infrared spectrum

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Present year shows the increasing demand for bone graft causes by trauma, diseases and degeneration. In USA, people suffer from osteoporosis is about 10 million. The occurrence of osteoporotic vertebral fracture reported every year is more than 1.5 million, which incur health care cost of USD 12-18 billion per year (Gao et al., 2015). Moreover, the Organ Procurement and Transplantation Network reported that more than 120000 patients are in waiting list of organ transplantation in the USA as of 15 November 2015 (Abbott & Kaplan, 2016).

The increasing demand for biomaterials has raised the global biomaterials market, which is estimated to reach USD 139 billion with 11.8% Compound Annual Growth Rate (CAGR) by 2022 (Tatkare, 2016). Furthermore, North America becomes the leading region of biomaterials market forecasted until 2022, followed by Europe and Asia Pacific. It is expected that Asia Pacific region to have the fastest growing rate with CAGR of 15.8% from 2016 to 2022 (Tatkare, 2016). In the Asia Pacific region, China is expected to have rapid biomaterials market growth with CAGR of 18.5% from 2016 to 2022 (Allied Market Research, 2016). In terms of types of materials, global market revenue of metallic biomaterials has been forecasted to be the highest until 2022. However, polymeric biomaterials is predicted to have rapid grow with 13.0% CAGR from 2016 to 2022 (Allied Market Research, 2016; Tatkare, 2016).

Conventional bone grafting which are autografts, allografts and xenografts have been implemented to treat bone related ailments (Wang et al., 2016). Autograft

provided the best results for integration and regeneration of bone, which indicate its osteoinductivity, osteoconductivity and nonimmunogenic properties (Nandi et al., 2010). However, availability of autograft is limited and additional surgeries is required which incurs an additional cost as well as more pain for patients (Wang et al., 2016; Zhang et al., 2014). Moreover, precise duplication of mechanical strength and shape of the replaced bone cannot be achieved. On the other hand, allograft availability is vast and able to duplicate the strength and shape of the replaced bone (Shepherd & Best, 2011; Zhang et al., 2014). However, it shows immunogenic reaction, low osteoinductivity, and high risk of disease transmission such as hepatitis and HIV. In addition, the high cost of surgery and storage is of significant concern (Nandi et al., 2010; Shepherd & Best, 2011; Zhang et al., 2014).

In this recent period, the development of synthetic bone filling materials becomes more significant and has prompted various researches in the field of biomaterials. Synthetic biomaterials can be classified into metals, polymers and ceramics (Bohner, 2010). These synthetic biomaterials show biocompatibility and bioactivity, with low infection rate, rejection rate as well as inflammatory reactions. In addition, bioresorbability of the synthetic biomaterials allows its removal which filling the defect site entirely with new bone. The resorption rate of synthetic biomaterials should be similar to the bone regeneration rate. Among the synthetic biomaterials available in the market, calcium phosphate materials exhibit this bioactivity and are viable bone graft materials (Shepherd & Best, 2011).

Recent development in surgical techniques has introduced the minimally invasive technique. This new emerging technique includes micro-invasive bone grafting. The micro-invasive bone grafting involves the delivery of bone graft materials via a small incision or local percutaneous puncture or injection to the defect

site. This surgical technique is simple, prevents bone harvesting and offers little injury. Moreover, it shows fast healing process without immunological reactions and is favorable for bone regeneration (Mostafa & Zaki, 2015; Weitao et al., 2007).

Micro-invasive bone graft demanded for injectable materials. The graft materials used in this technique may be in the form of paste-like solution of fine particulate materials (Mostafa & Zaki, 2015; Weitao et al., 2007). The paste is formed from the mixture of powder and liquid phases. Injectable bone cement is the material in liquid or paste forms that can be injected or molded to take the shape of defect bone upon solidification. This materials should aid the regeneration of bone and provide strong mechanical support (No et al., 2014). The clinically available injectable bone cements are polymethylmethacrylate (PMMA), calcium phosphate cement (CPC) and calcium sulfate cement (CSC) (Gao et al., 2015; He et al., 2015; No et al., 2014). Other cements that are available including calcium silicate cement (CSiC) (Gao et al., 2015; No et al., 2014) and magnesium phosphate cement (MPC) (He et al., 2015).

Calcium phosphate cement (CPC) is one of biomaterial that shows great potential as injectable bone cement materials. CPC fabrication involves the mixing of powder phase of any calcium phosphate (CaP) compounds with liquid phase of distilled water or calcium/phosphate aqueous solution. CPC shows injectability, *in vivo* setting ability and microporosity. CPC also offers excellent biological response, which are bioactive, biocompatible and biodegradable. However, CPC has poor injectability, weak cohesion and low mechanical strength without addition of additives (Zhang et al., 2014).

In the development of CPC, it is important to fabricate CPC that has sufficient setting time for surgeon to finish the implantation procedure without delay (Zhang et al., 2014). Moreover, the strength and biological response of the implant should be

similar to the natural bone. This can be achieved by adding polymeric materials into CPC. Polymeric additives that have been used in CPC fabrication including chitosan, alginate, cellulose, collagen, poly(lactic-*co*-glycolic acid) (PLGA), poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG) (Perez et al., 2012). .

1.2 PROBLEM STATEMENT AND ITS SIGNIFICANCE

A major concern in developing injectable bone cement is to produce paste that could provide controllable setting time with good injectability. Generally, cement paste with good injectability has long setting time which will delay the operation and can cause adverse effect. Meanwhile, cement paste with poor injectability has short setting time which will become unworkable before the surgeon finishes the operation. Therefore, it is critical to prepare a cement paste with long enough setting time to complete implantation procedure and short enough setting time to prevent delays of operation. Moreover, the materials should have high mechanical strength and excellent biological response. However, CPC without addition of additives has poor injectability and low mechanical strength which limited their applications in non-load bearing bone defects only. Furthermore, CPC shows weak cohesiveness and disintegration tends to happen upon injection into the physiological body solutions.

The significance of this research is developing a novel synthesis method of CPC. The modified wet chemical precipitation method used in this study shortened the synthesis time as compared with the previous synthesis route by Angelescu et al. (2011) and Monmaturapoj & Yatongchai (2010). This research also fabricating a new injectable CPC by incorporating PEG into CPC to produce CPC/PEG composite with desired physical properties and high bioactivity. The new injectable CPC is expected to be used in injectable bone cement applications.

1.3 RESEARCH OBJECTIVES

The objectives of this research are:

1. To synthesize calcium phosphate cement via wet chemical precipitation method.
2. To prepare the wet chemical precipitation derived calcium phosphate/poly(ethylene glycol) composite.
3. To characterize the physical and mechanical properties of calcium phosphate cement and calcium phosphate/poly(ethylene glycol) composite.
4. To investigate the biological activity of the fabricated calcium phosphate cement and calcium phosphate/poly(ethylene glycol) composite using Vero cell culture.

1.4 RESEARCH METHODOLOGY

Research methodology is summarized in Figure 1.1.

1. Synthesis is the fabrication of CPC via wet chemical precipitation method.
2. Powder characterization is works involving morphological and phase analyses of the synthesized powder using field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy.
3. Preparation of CPC is the mixing of powder and liquid phase to form cement paste.

4. Characterization and properties evaluation of the fabricated CPC are the series of testing and methods to evaluate the injectability, setting time, mechanical, anti-washout and cytotoxicity properties of CPC.

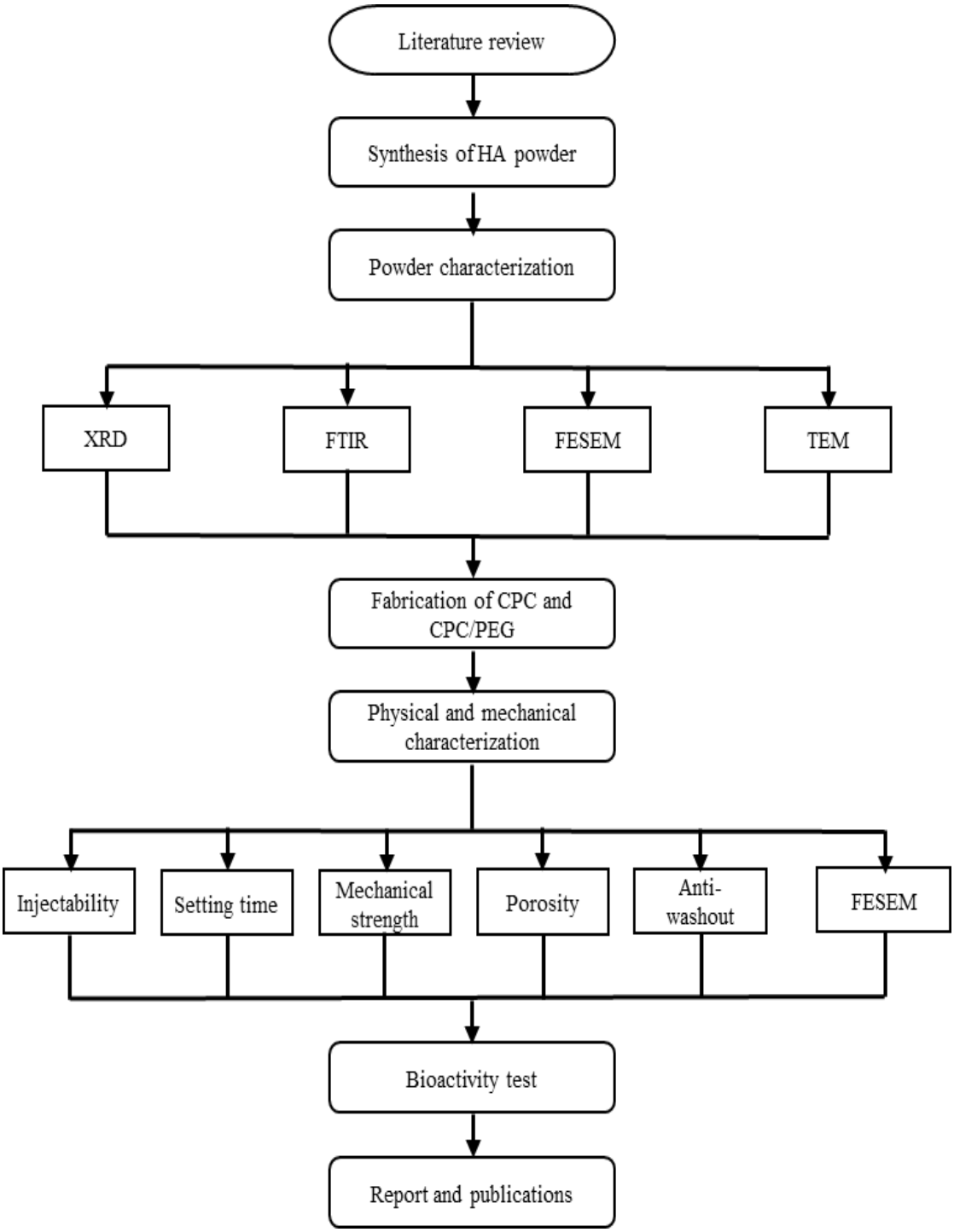

Figure 1.1 Flowchart of research methodology

5. Injectability is the ability of CPC to be extruded out from syringe under extrusion force by compression machine.
6. Setting time is the time taken for cement paste to solidify and measured using Gillmore needle method.
7. Mechanical strength of the fabricated CPC was determined through compression strength test using universal testing machine (UTM). Compression strength is the longitudinal material integrity, in which the ability to withstand under axial-loading through cross section.
8. Anti-washout properties is the ability of materials to retain its shape without disintegration when in contact with liquid. The anti-washout properties is determined via visual observation of CPC after soaking in Ringer's solution for certain period of time and strength evaluation.
9. Bioactivity and biocompatibility is evaluated from the ability of CPC to achieve direct bonding with living bone without destructive effect on living cells. The fabricated CPC is tested using Vero cells in the solution of Dulbecco's modification of eagle's medium (DMEM) with fetal bovine serum (FBS).

1.5 SCOPE OF RESEARCH

This present study comprised of three parts of researches.

The first part is about synthesis of CPC via wet chemical precipitation method and its properties evaluation. The synthesized powder was characterized by using XRD, FTIR, FESEM and TEM. Afterwards, CPC was fabricated by mixing the wet chemical derived powder with distilled water at certain powder-to-liquid (P/L) ratio. In this study, the P/L ratios varied at 1.0, 1.3, 1.5 and 1.7. The fabricated CPC