CALCIUM PHOSPHATE-MULTIWALLED CARBON NANOTUBES COMPOSITES FOR INJECTABLE BONE SUBSTITUTE

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CALCIUM PHOSPHATE-MULTIWALLED CARBON NANOTUBES COMPOSITES FOR INJECTABLE BONE SUBSTITUTE

By

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
aw-CPC	Anti-washout type calcium phosphate cement
CCD	Central composite design
CDHA	Calcium deficient hydroxyapatite
CHA	Carbonated hydroxyapatite
CI	Confident interval
CNTs	Carbon nanotubes
CPBC	Calcium phosphate bone cements
CPCs	Calcium phosphate cements
CS	Compressive strength
CVD	Chemical vapor deposition
DF	Degree of freedom
DOE	Design of experiments
EDS	Energy dispersive X-ray spectroscopy
F-value	Fisher test value
FTIR	Fourier transformed infrared spectroscopy
HA	Hydroxyapatite
IBS	Injectable bone substitute
i-CPCs	Injectable calcium phosphate cements
L/P ratio	Liquid to powder ratio
MWCNTs	Multiwalled carbon nanotubes
MWCNT-AP	As pristine multiwalled carbon nanotube
MWCNT-OH	Hydroxyl group functionalized multiwalled carbon nanotube
MWCNT-COOH	Carboxyl group functionalized multiwalled carbon nanotube
Prob	Probability
RSM	Response surface methodology
SBF	Stimulated body fluid
SEM	Scanning electron microscopy
SWCNTs	Single wall carbon nanotubes
TEM	Transmission electron microscopy
wt %	Weight percent
XRD	X-ray diffraction

LIST OF SYMBOLS

α	Rotatability of central composite design
α	Radiation for X-ray diffraction analysis
ε	Error
A	Coded term of wt % of MWCNTs
β	Beta
$\beta_{0,1,2\ldots,k}$	Regression coefficients in Equation (2.2)
B	Coded term of wt % of BSA
C	Coded term of type of MWCNTs
θ	Radiation angle for X-ray diffraction analysis
λ	Wavelength for X-ray diffraction analysis
$x_{0,\ 1,\ 2\ldots,\ k}$	Independent variables for regression in Equation (2.2)
Y	Dependent variables for regression in Equation (2.2)

KOMPOSIT KALSIUM FOSFAT- NANO TIUB KARBON BERBILANG SEBAGAI PENGGANTI TULANG MELALUI PENYUNTIKAN

ABSTRAK

Semen kalsium fosfat (CPCs) telah menunjukkan prestasi yang sangat baik sebagai bahan pengganti tulang tanpa kesan sampingan dalam kedua-dua *in vitro* dan *in vivo*. Namun, kelemahan CPCs mengakibatkan ia mengalami kekuatan mampatan yang rendah dan membataskan kebolehgunaannya dalam ortopedik. Oleh sebab itu, tujuan projek ini adalah untuk menghasilkan bahan pengganti tulang yang boleh suntik dan CPCs yang mengandungi pelbagai jenis nano tiub karbon berbilang (MWCNTs) dan "bovine serum albumin" (BSA) bagi membentukkan CPC/MWCNTs/BSA komposit dengan tujuan memperbaiki kekuatan mampatan bagi CPCs tulen. Kehadiran MWCNTs dan BSA mempunyai kesan yang signifikan dalam mempengaruhi morfologi hidroksiapatit (HA) kristal dalam matriks CPCs. BSA bertindak sebagai mangkin pertumbuhan HA pada permukaan CPCs. Dengan demikian, penambahan MWCNTs dan BSA menyebabkan peningkatan kekuatan mampatan dengan mengubahsuaikan ciri-ciri kristalit untuk komposit. Bagi proses pembelajaran yang sistematik, rekabentuk eksperimen (DOE) digabungkan dengan metodologi permukaan sambutan (RSM) dan rekabentuk ujikaji gabungan pusat (CCD) telah digunakan untuk mengkaji hubungkait antara kekuatan mampatan bagi komposit dengan parameter yang dikaji. Matlumat ini seterusnya digunakan untuk tujuan pengoptimuman kekuatan mampatan. Ujian kekuatan mampatan, pengimbasan mikroskop elektron (SEM), spektroskopi inframerah transformasi Fourier (FTIR) dan ujian kebolehsuntikan dijalankan untuk menilai sifat-sifat bagi komposit. Keputusan kajian menunjukkan bahawa CPCs komposit yang mengandungi MWCNT-OH dan

BSA menunjukkan kekuatan mampatan yang tertinggi (60 MPa) selepas 28 hari perendaman dalam simulasi bendalir tubuh (SBF).

CALCIUM PHOSPHATE-MULTIWALLED CARBON NANOTUBES COMPOSITES FOR INJECTABLE BONE SUBSTITUTE

ABSTRACT

Calcium phosphate cements (CPCs) has shown very good performance as bone substitute material without any side effects for both *in vitro* and *in vivo*. However, these CPCs suffer from a relatively low compressive strength, limiting its applicability in orthopedics. Therefore, the aim of this project is to develop the injectable bone substitute (IBS) consisting of CPCs with different types of multiwalled carbon nanotubes (MWCNTs) and bovine serum albumin (BSA) to create CPC/MWCNTs/BSA composites with the purpose of improving the mechanical properties of the pure CPCs. The presence of MWCNTs and BSA were found to have significant effects in influencing the morphology of hydroxyapatite (HA) crystals in CPCs matrix. BSA was found to act as promoter for HA growth when bounded to the surface of CPCs grains. Thus, the addition of MWCNTs and BSA could lead to an improvement of compressive strength by modifying the properties of the crystallites. In order to have a systematic process study, design of experiment (DOE) coupled with response surface methodology (RSM) and central composite design (CCD) was used to investigate the relationship between the compressive strength of the composites with the process parameters studied, which was then used for the optimization process. Compressive strength tests, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and injectability tests were used to evaluate the composites properties. Characterization results showed that CPCs composites containing hydroxyl group functionalized

MWCNT (MWCNT-OH) and BSA exhibited the highest compressive strength (60 MPa) after 28 days immersion in simulated body fluid (SBF).

CHAPTER ONE:

INTRODUCTION

This chapter provides detail introduction of this research project. Brief definition, current medical problems and benefits of using injectable bone substitute (IBS) are included in this chapter. It concludes with the problem statement, scope of study, objectives and thesis organization of this research project.

1.1 Why Injectable Bone Substitute (IBS)?

Calcium phosphate (CaP) ceramics are the main raw materials used in blocks or granules for bone substitutes (Suchanek and Yoshira, 1998). These forms are limited value when cavities are not easily accessible or when it would be preferable to perform micro-invasive percutaneous surgery. Contradict to the general practice in visceral surgery, percutaneous surgery is less frequently than open surgery in orthopedics. In the present time, improvements in specific instrumentation and the use of bioresorbable polymer implants for bone fracture healing have contributed to the development of this minimal invasive technique. Thus, there is a need to develop IBS (Weiss *et al.*, 1999; Daculsi *et al.*, 1999). IBS requires suitable properties to ensure bonding of the mineral phase in situ with good cell permeability. Contradict to the use of dense materials which do not have any inherent porosity, this approach provides rapid improvement in deep bone formation. IBS could be produced in a sterile stage ready to use. Its stable composition and mechanical properties are suitable for the reproducibility of the biological response. A list of desirable properties for an ideal IBS, as identified by different workers (Heini and Berleman, 2001; Phillips, 2003) is presented in Table 1.1. The most important properties are

easy injectability, high radiopacity, dough viscosity that does not change much between mixing and delivery into the vertebral body, a resorption rate that is neither too fast nor too slow and mechanical properties that are comparable to those of a healthy intact vertebral body (Heini and Berleman, 2001).

Table 1.1: Desirable properties of an ideal IBS (Heini and Berlemann, 2001; Phillips, 2003).

- Very high radiopacity
- Ease of preparation and handling
- Very easy injectability into the collapsed vertebral body
- No toxicity
- Low cost
- Low curing temperature
- Working time of about 6-10 min
- Setting time of about 15 min
- Resorption rate that is neither too high nor too low
- Excellent osteoconductivity
- Excellent osteoinductivity
- Excellent biocompatibility
- Excellent bioactivity
- Adequate mechanical properties that would allow for immediate reinforcement of the vertebral body and ensure early ambulation of the patient; for example, values of modulus of elasticity and strength should be comparable to those of a healthy vertebral body
- Appropriate cohesion; that is, dough sets in a fluid without disintegration (this is achieved by keeping a high viscosity for the dough)
- A curing dough whose initial viscosity is low (but not low enough to have the potential for extravasation) and a change in that viscosity that is practically invariant with setting time
- Microporosity (mean pore diameter < 10 μm), to allow circulation of body fluid
- Macroporosity (mean pore diameter >100 μm), to provide a scaffold for blood-cell colonization

Furthermore, there has been considerable work on the development of IBS materials to improve the currently surgical implant method (Grimandi *et al.*, 1998; Knaack *et al.*, 1998; Gauthier *et al.*, 1999; Tamada *et al.*, 1999). IBS formulations are attractive since they can be used for the purpose of minimally invasive surgical procedures and can be molded to exactly fill irregular bone defects (Peter *et al.*, 1998). Grimandi *et al.* (1998) and Gauthier *et al.* (1999) worked on an IBS composed of a methylhydroxypropylcellulose (MHPC) matrix incorporating bicalcium

phosphate (BCP) granules. Both groups found that the IBS provides an excellent bioactive matrix for bone ingrowth promoting cell colonization, but the material is lacked suitable mechanical properties. The latter group also distinguished that smaller BCP granules obtained greater inflammatory response, with macrophages being recruited to the implant site. This in turn tended to increase the degradation rate of the bone substitute, apparently through signaling and recruitment of other bone remodeling cells (Daculsi *et al.*, 1995; Grimandi *et al.*, 1998; Gauthier *et al.*, 1999). To improve the mechanical properties of an IBS and to stabilize it at the implant site, many researchers investigated alternatives to IBS formulations that set in situ, primarily through cross linking reactions. Polyalkenoate cements, used as dental cements and fillers, consist of a basic metal oxide like zinc oxide and a polyacid, such as poly(acrylic acid) (PAA). The acid reacts with the base to form a cross linked metal-polyacrylate salt. In order to improve the bioactivity of these cements, Kenny *et al.* (2000) mixed apatite into the formulations and varied the molecular weight of the polyacid. They obtained cements that set at body temperature, with the potential to chemically bond to bone, exhibit no shrinkage and possess mechanical properties comparable to acrylic cements. The mechanical performance of these systems improved with increasing polyacid molecular weight. Watson *et al.* (1999) reacted tetracalcium phosphate (TTCP) with PAA, forming a cross linked hydroxyapatite (HA)-calcium polyacrylate composite. In aqueous solution, TTCP hydrolyzes to HA and the calcium cation neutralize the PAA, forming the cross linked network. The reaction between TTCP and PAA was very fast and controlled by prehydration of the TTCP to form a slower reacting HA surface layer. In contrast, Reed *et al.* (1996) synthesized a dicarboxy polyphosphazene that can be cross linked by di- or trivalent cation. The cations are

calcium ions from TTCP and dicalcium phosphate dehydrate (DCPD). They obtained cements with a compressive strength of the order of 10 MPa and 65 % porosity. Mimicking the setting of polymethylmethacrylate (PMMA) cements, Peter *et al.* (1999) used a vinyl monomer to crosslink poly(propylene-co-fumarate) (PPF). They were able to make quick setting, degradable cements, with low heat output and compressive strengths in the range of 1-12 MPa by varying the PPF molecular weight, as well as monomer, initiator and porogens content like sodium chloride (NaCl). Li *et al.* (2000) prepared an acrylic cement with a strontium-containing HA (Sr-HA) that cured at temperatures lower than PMMA-based cements, but with comparable mechanical properties, with the intention that the Sr-HA would improve the ability to bond the bone. Finally, a novel IBS was described by Turczyn *et al.* (2000), where a silanized hydroxyethylcellulose carrier was mixed with BCP. The suspension was in liquid form at pH 10-12, but gels quickly at pH < 9.

Self-setting calcium phosphate cements (CPCs) have been first expanded and many of them are commercially available (Frankenburg *et al.*, 1998; Larsson and Bauer, 2002; Cassidy *et al.*, 2003). IBS are being developed based on the suspension of CaP granules in a carrier phase consisting of polymeric water solution of non-ionic cellulose ether especially for the application in invasive surgery and drug delivery system. The resulting composite provides a ready to use, sterile, injectable material (Daculsi *et al.*, 1995; Grimandi *et al.*, 1998). Such IBS have shown ability to support more extensive and early bone substitution than macroporous implants or CaP bone cement but have not been yet studied for biomechanical competence after implantation (Gauthier *et al.*, 1999; Gauthier *et al.*, 2003).

Two different types of IBS compounds have been developed for example CPCs that harden in situ and exhibit interesting mechanical properties and IBS associating with polymer and CaP mineral phase, which possess osteoconduction qualities similar to those of macroporous ceramics (Iijima, 1991; Munting *et al.*, 1993; Miyamoto *et al.*, 1997; Bo *et al.*, 1999; Grimandi *et al.*, 1998; Gauthier, Boix *et al.*, 1999). New injectable biomaterials consisting of bioactive CaP ceramics fillers and hydrophilic polymer matrix were studied by Weiss *et al.* (1999) for bone and dental surgery.

In summary, IBS represent an ideal material for many bone repair applications provided that the osteoconductive properties and ease of use can be associated with adequate mechanical properties. It is also clear that any effort to develop IBS should include a fundamental examination of all items listed in Table 1.2 (Kenny and Buggy, 2003). Furthermore, IBS materials should exhibit good workability, have a suitable set time with low heat output, good mechanical properties, non-toxic, biocompatible, osteoconductive and they should integrate with bone over the time scale of bone ingrowth and remodeling (Rafal, 2001).

Table 1.2: List of criteria for the design of new IBS materials (Kenny and Buggy, 2003).

Criterion	Purpose
Mechanical properties to match those of bone	Enhance the elimination of the phenomenon known as stress shielding
Quick setting (5-15 minutes)	To assists in clinical use and after care of patient
In situ setting at body temperature	Elimination of necrosis of the adjacent tissue
Bonding to bone and medical grade alloys	Elimination of fibrous capsule and thus loosening of the implant
Bioactive bone in-growth	Enhancing both stress transfer and chemical attachment of the implant
Radio opaque	Subsequent monitoring of implant

The advantages of injectable CPC include easy placement in surgery, able to be used in difficult surgical sites that are not freely accessible by open surgery, capable of filling narrow defects and facilitating minimally invasive techniques. Furthermore, to improve the mechanical properties of CPCs, many researchers have blended polymers, organic or inorganic additives, bioglass and carbon nanotubes (CNTs) with the cements (Low *et al.*, 2010).

1.2 Problem Statement

Due to increasing of population ageing, osteoporosis is becoming progressively a more common medical problem leading to higher rate of bone fractures (Bohner *et al.*, 2003). For example vertebral fractures, may cause persistent, often excruciating pain, which impairs mobility and reduces the patient's quality of life. Management of vertebral bone fractures includes analgesics, bed rest and external bracing. Even with these types of treatment, progressive kyphosis, prolonged pain and disability still may occur. A major clinical problem with a high risk of severe kyphosis can develop such as multiple contiguous compression fractures in the thoracic and thoracolumbar spine. Therefore, there is a need for treatment that could decrease the occurrence of these fractures and improve management options once these fractures occur. It has been demonstrated that using minimally invasive bone cement injection for stabilizing osteoporosis or treating vertebral bone fracture has significant clinical benefits. At present, there is an increase in the usage of cement augmentation techniques such as vertebroplasty and kyphoplasty for treating persistent painful vertebral compression fractures (Bo *et al.*, 1999). One extensive category of potential intervention involves the fortification or augmentation of the vertebral bones. In addition to prophylactically stabilizing

osteoporotic bones at risk for fracture, augmentation of vertebral bones that already have fractured may prove useful by reducing pain, improving function and preventing further collapse and deformation (Bostrom *et al.,* 1997).

Therefore, there are several limitations and problems faced for IBS. Hence, this research is carried out focusing on the development of CPC composites and it application as IBS. The study will also focus on how to improve the CPC mechanical properties by using multiwalled CNTs (MWCNTs) and overcome their drawback which limits its clinical use such as, low mechanical strength and poor injectability. Besides, the bovine serum albumin (BSA) was also incorporated in the CPC composites in order to improve the mechanical properties by promoting the HA crystal growth.

1.3 Scope of Study

CPCs are bone substitute material which has shown very good performance without outside effects, both *in vitro* and *in vivo* (Knaack *et al.*, 1998; Lee *et al.*, 1999). However, the final cement suffers from a relatively low compressive strength, limiting its applicability in orthopedics. The aim of this project is to develop IBS consisting of CPC with different types and weight percent (wt %) of MWCNTs and different wt % of BSA to create CPC/MWCNTs/BSA composites with the purpose of improving the mechanical properties of the CPC. Drawing on the results from the literature, MWCNTs have been chosen as a result of their impressive list of superlatives including mechanical strength, stiffness and their applications in biological and biomedical systems. BSA acts as promoters of CaP crystal growth when bound to a surface and hence has also included in this study. Thus, the addition

of MWCNTs and BSA could lead to an improvement of mechanical properties by modifying the properties of the crystallites, thereby possibly resulting in a composite of higher density and improved mechanical properties. The composites formulated will be expected to exhibit compressive strengths higher than the pure IBS material. In each case, characterization techniques will be considered to evaluate the final product properties. Moreover, the interfacial bonding between the phases, CPC, MWCNTs and BSA which is an important consideration for composite materials will also considered. Finally, the biocompatibility of selected samples will be carried out by *in-vitro* for biological evaluation.

The compressive strength of the CPC/MWCNTs/BSA composites will also be studied and performed using design of experiment (DOE) occupying response surface methodology (RSM) coupled with central composite design (CCD) with the assistant of Design Expert 6.0.6 software.

1.4 Objectives

The aim of this project is:

i. To develop IBS consisting of CPC with different types of MWCNTs (e.g. as-pristine MWCNT (MWCNT-AP), hydroxyl group functionalized MWCNT (MWCNT-OH) and carboxyl group functionalized MWCNT (MWCNT-COOH)), different wt % of respective MWCNTs and BSA to create CPC/MWCNTs/BSA composites with the purpose of improving the compressive strength and injectability of the CPC.

ii. To optimize the compressive strength of the CPC/MWCNTs/BSA composites produced using RSM couple with CCD.

iii. To study the *in vitro* biological test on the optimum CPC/MWCNTs/BSA composites.

1.5 Organization of The Thesis

This thesis consists of five chapters. Chapter one provides an outline of the overall research project including introduction on IBS. Problem statement was written after reviewing the current scenario of the CPC. The problem statement reveals the problems faced by the surgical implant and the importance of this research project. The original objectives of this research project were then carefully formulated with the intention to address the problems encountered by the bone fracture patients. Lastly, the organization of the thesis highlights the content of each chapter.

Chapter two gives an overall review of various research works reported in the literature in this area of study which includes properties of CPC, MWCNTs and BSA, also the current status of using MWCNTs in the CPC and its application are reported in the review.

Experimental materials and methodology were discussed in chapter three. This chapter describes details information on the overall flow of this research works and some experimental methods in conducting this research project. In addition, material, chemicals and equipments used in this study were also reported. This chapter also includes the information required for the calculation of injectability and compressive strength. The characterization process and data analysis also will be including in this chapter.

Chapter four perhaps is the heart of the thesis since it includes detail discussion on the results obtained in the present research work. This chapter consists of four sections which have been divided according to the stages of this research work. First section described the formation of CPC/MWCNTs/BSA composites in details. Section two of this chapter presents the investigated the effect of different types of MWCNTs, different wt % of MWCNTs and BSA in the compressive strength of the CPC composites by using the DOE approach before further experimental works were carried out. At the end of this section, characterization of CPC/MWCNTs/BSA composites produced under optimum conditions was reported. This section also reports the characterization result of CPC/MWCNTs/BSA composites for the effect of using different types of MWCNTs. Process study on the compressive strength of CPC/MWCNTs/BSA composites was being discussed in this section. Section three will discuss on the *in vitro* biological evaluation result for the CPC/MWCNTs/BSA composites.

Chapter five, the last chapter of this thesis, provides a summary on the results obtained in this research project. This chapter concludes the overall research project and gives some recommendations for future studies related to this research works.

CHAPTER TWO:

LITERATURE REVIEW

2.1 Bone Substitute Material: Calcium Phosphate Cements (CPCs)

2.1.1 Properties of Calcium Phosphate Cements (CPCs)

The possibility to obtain monolithic calcium orthophosphate ceramics at ambient or body temperature via a cementation reaction was put forward by LeGeros *et al.* (1982) and Brown and Chow (Brown and Chow, 1983; Brown and Chow, 1985; Brown and Chow, 1986) in the early 1980s. Currently, this type of material is known as CPC and due to their suitability for repair, augmentation and regeneration of bones, they might be named as calcium phosphate bone cements (CPBC) (Driessens *et al.*, 1995). CPC is a synthetic bone graft material that was invented in 1986 by Brown and Chow, scientists at the American Dental Association (Brown and Chow, 1985). The cement is a white powder consisting of equimolar amounts of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) or dicalcium phosphate dehydrate (DCPD) or β-tricalcium phosphate (β-TCP), which are mixed with water in a liquid-to-powder (L/P) ratio of 1:4 to form a paste that can be conventional to osseous defects with complex shapes and set *in vivo* to form HA with excellent osteoconductivity without any acidic or basic byproduct (Brown and Chow, 1985; Bo *et al.*, 1999). CaP is used by our body to build bone tissue and is being applied to produce biomaterials for bone repair. The CaP biomaterials are discussed in relation to bone repair because CaP, in particular carbonated HA (Bo *et al.*, 1999) is the major inorganic component of bone. The CPCs are hydraulic cements. This setting is attained by an acid-base or hydrolysis reaction between a powder and liquid. After mixing, a CaP of intermediate alkalinity precipitates. A

cement material consists of solid powder phase which initially forms a plastic paste by mixing with liquid phase which can be shaped during surgery to fit the contours of a wound. This viscous paste will convert into a stiff paste during setting, increasing its mechanical strength up to saturation or hardening. CPCs generally consist of an aqueous solution and powder that typically contains several CaP compounds. Upon mixing, the powder dissolves in the aqueous solution and new forms of crystals precipitate, the reaction proceeds until all reactive CaP compounds react. Cement hardening occurs with the entanglement of CaP crystals, hence leading to a highly porous structure. The final product has porosity close to 40 to 60 %, with pores ranging typically from 0.1 to 10 μm. It is important to note that CPCs are mechanically much stronger in compression than in tension or shear, because entangled crystals are not well bonded. Compressive strength values are typically 5 to 10 times larger than the tensile strength (Bohner and Baroud, 2008).

Two categories of CaP exist including apatite CPC and brushite CPC. The main difference between apatite and brushite CPCs lies in their solubility and hence resorption rate with brushite being much more soluble than apatite, so brushite CPCs in principle resorb faster than apatite CPCs (Bohner, 2000). For the majority of apatite cements, water is not a reactant in the setting reaction. Therefore, the quantity of water actually needed for setting of apatite cements is very small (Lacout *et al.*, 1996; Weiss *et al.*, 2003). However, for brushite cements, water always participates in the chemical transformations because it is necessary for DCPD formation. Due to this reason, brushite cements are always hydraulic, while usually this term is not associated with apatite cements (Sergey, 2008). In contrast to apatite cements, brushite cements can be initially liquid and still set within a short period of time

(Bohner, 2000; Bohner, 2001). Brushite cements are remarkable biocompatible and bioresorbable. Due to both a better solubility of DCPD if compared to that of calcium-deficient hydroxyapatite (CDHA) and metastability of DCPD under physiological conditions (Vereecke and Lema îre, 1990), brushite cements are faster degradable than apatite cements (Klein *et al.*, 1985; Apelt *et al.*, 2004). They are quickly resorbed *in vivo* and suffered from a rapid decrease in strength (although the mechanical properties of the healing bone increase as bone ingrowth occurs (Ikenaga *et al.*, 1998)). However, short setting times, low mechanical strength and limited injectability prevent brushite cements from a broader clinical application (Sergey, 2008). In addition, brushite cements has such a fast setting reaction (several times faster than apatite cements), that high liquid to powder (L/P) ratios have to be used for brushite cements to keep the cement paste workable or injectable over an adequate time. The increase in liquid content resulted in a highly porous thus weaken the material (Barraleta *et al.*, 2004).

Furthermore, synthetic apatite CaP bone substitutes, fillers and cements have excellent biocompatibility and have been the focus of considerable applications in the dental and orthopedic fields since early 1980s (Jarcho, 1981; Brown and Chow, 1985; Hollinger and Battistone, 1986; Hollinger *et al.*, 1996; Driessens *et al.*, 1995). Previously, CaP bone substitutes have a tendency to be developed either by HA or β-TCP. Although HA and β-TCP were found to be useful as bone fillers, they have specific weakness. Crystalline forms of HA undergo osseointegration but resorption rates have been relatively slow compared to rates of new bone formation (Klein *et al.*, 1983; Whittaker *et al.*, 1989; Frayssinet *et al.*, 1993). The β-TCP tends to be more resorbable than HA, but its resorption rates are still unsatisfactory and somewhat

unpredictable (Mors and Kaminski, 1975; Metsger *et al.*, 1982; Hollinger and Battistone, 1986). Besides that, the most common CPCs yield a poorly crystalline, precipitated HA similar in structure to natural bone mineral. There is a number of CPCs currently commercially available. However, due to limited compressive strength, they are limited to non-stress-bearing applications, including maxillofacial surgery, repair of cranial defects and dental fillings (Rafal *et al.*, 2002). Table 2.1 and Table 2.2 give common types of CaPs and the main features of most common CPCs for bone substitute materials respectively. Table 2.3 lists the properties of 3 common formulations of CPCs. In short, the advantages of CPCs include being injectable, moldable, bone defects, to exhibit excellent biocompatibility and to be osteoconductors.

Table 2.1: Characteristic of common type of CaPs (Evalina and Vikas, 2008).

Calcium Phosphates (CaPs)	Characteristic
Dicalcium phosphate	Present in renal calculi and fracture callus, a synthetic source of calcium and phosphate
Dicalcium phosphate dehydrate (DCPD)	Contains brushite, hydrated CaP, a common and widely used animal supplement
Octocalcium phosphate	Precursor for HA in teeth, bioactive and biodegradable grafting material
Amorphous calcium phosphate (ACP)	TCP, substance used as a dental treatment
Precipitated HA	TCP, a naturally occurring mineral form of calcium apatite, commonly used as a filler to replace amputated bone or as a coating to promote bone ingrowth into prosthetic implants

Table 2.2: Main features of most common CPCs for bone substitute materials (Becker and Ogon, 2008; Gladius, 2005).

Features	Calcium phosphate cements (CPCs)
Hydrophilicity	Hydrophilic
Injectability	Critical
Setting time	< 20 minutes
Setting rate	Slow
Temperature change	Negligible
Tensile strength	< 15 MPa
Compressive strength	< 100 MPa
Porosity	40 - 60 %
Pore diameter	0.1 – 10 μm
Resorption	Little to great
Bone-cement contact	Excellent
Radiopacity	Very high
Toxicity	No
Resorption rate	Neither too high nor too low
Osteoconductivity	Excellent
Osteoinductivity	Excellent
Biocompatibility	Excellent
Bioactivity	Excellent
Cost	Low

Table 2.3: Properties of CPCs (Schmit, 1999).

Formulation	Bone Source	A-BSM Embarc	Norian SRS/CRS
Components	Tetracalcium phosphate (TTCP), $Ca_4(PO_4)_2O$ and dicalcium phosphate dihydrate (DCPD), $Ca(HPO_4).H_2O$	Decarbonated ACP and either DCPD, calcium metaphosphate, calcium heptaphosphate, calcium pyrophosphate, or TCP	Monocalcium phosphate, β-TCP, calcium carbonate
Compressive strength	36 MPa (for first 24 hours)	Unknown	55 MPa
Resorbable	Minimally	Yes	Completely
Commercially available	Yes	Yes	Yes
Pore diameter	2-5 nm	Unknown	300 Å
Initial setting time	10-15 min	15-20 min	10 min
Final setting time	4 hours	1 hour	12 hours
Osteoconductive	Yes	Yes	Yes
Sets in presence of fluid	No (must be kept dry)	Yes	Yes

The most generally used injectable bone cement is PMMA, but it suffers from the fact that it does not degrade and its high curing temperatures can cause necrosis of the surrounding tissue (Peter *et al.*, 1997). Therefore, further development of alternative injectable materials is necessary. CPC is an attractive candidate because of it self-hardens to form HA, excellent osteoconductivity and bone-replacement ability (Brown and Chow, 1986; Chohayeb *et al.*, 1987; Chow and Takagi, 1989; Fukase *et al.*, 1990; Chow, 1991; Costantino, 1991; Friedman *et al.*, 1991; Sugawara *et al.*, 1992; Ishikawa *et al.*, 1994; Ishikawa and Asoaka, 1995; Ginebra *et al.*, 1997; Constantz *et al.*, 1998; Miyamoto *et al.*, 1999) and have promising for use in craniofacial and orthopedic repair (Driessens *et al.*, 1993; Peter *et al.*, 1997; Chow *et al.*, 1998; Becker and Ogon, 2008; Evalina and Vikas, 2008).

The development of i-CPC formulations has good prospects for minimally invasive surgical techniques developed in recent years. Since then, CPCs have attracted much attention and different formulations have been proposed (LeGeros *et al.*, 1982; Driessens *et al.*, 1994; Constantz *et al.*, 1995; Ginebra *et al.*, 1997; Freche *et al.*, 1999; Tofighi *et al.*, 2000). The development of self-setting CPCs has extended the application of CaP to IBS that can be shaped and molded to fit irregular defects, with osseointegrative properties comparable to or better than bulk CaP (Brown and Chow, 1985). Compared with non-setting IBS, CPCs showed excellent mechanical properties and do not involve potentially toxic reagents or strongly exothermic setting reactions (Brown and Chow, 1985; Fernandez *et al.*, 1996; Liu *et al.*, 1997; Fernandez *et al.*, 1998; Boudeville *et al.*, 1999; De Maeyer *et al.*, 2000; Yuasa *et al.*, 2001). CPCs are a rising class of bone substitute materials that can be identified as excellent alloplastic materials for osseous augmentation due to the

reason of the unique combination of osteoconductivity, biocompatibility and mouldability. CPCs can be molded and shaped to fill intricate bony cavities or narrow dental defect sites. In this context, CPCs are more attractive than HA ceramic granules. In addition, CPCs are more suitable than PMMA bone cements due to the non-toxic, non-carcinogenic, non-exothermic setting and negligible shrinkage properties of CPCs. They show excellent *in vivo* resorption (Manoj and Varma, 2003).

In contrast to PMMA-based cement (Leeson and Lippett, 1993), the setting reaction of CPC occurs with minimal exothermic at a physiological pH value without the release of monomer. Several apatite cements are accepted for repair of cranial defects in humans (Friedman *et al.*, 1998; Sarkar *et al.*, 2001; Kopylov *et al.*, 2002). However, the use of apatite cements is limited to non-load-bearing or low-load-bearing applications due to their poor mechanical performance relative to bone (Reilly and Burstein, 1975). Kingery (1950) was the first to report the formation of CPCs as part of a larger study of metal ion phosphate cements. He proposed that acid-base systems produce the strongest cements when the rate of reaction is slow. This rule is commonly used to modify CPCs, especially cements forming brushite that proceed through a fairly vigorous reaction between a basic CaP and phosphoric acid. In addition to adjust the rate of reaction, some additives are used to modify the rheology of the paste or to act as porogens. CPC has been broadly considered because of its attractive characters as self-setting, good biocompatibility and being readily molding. Basically, i-CPC has been developed for non invasive surgery (Manoj and Varma, 2003). The most important character of i-CPC is plasticity. Moreover by changing the associated parameters for example the injectable time and

porosity, the strength and degradation speed can be adjusted for the different clinical applications. Also, it can be an excellent carrier for the growth factor, medicine or gene through the simple physical mixture (Li *et al.*, 2007).

Different studies on CPCs have shown that they are highly biocompatible and osteoconductive materials, with potential use in tissue regeneration (Jansen *et al.*, 1995; Kurashina *et al.*, 1997; Friedman *et al.*, 1998; Yuan *et al.*, 2000; Torner, 2001; Larsson and Bauer, 2002; Ooms *et al.*, 2003). One of the most important characteristics of CPCs is that they are supposed to be osteotransductive. Thus, they could be resorbed slowly and transformed simultaneously into new bone tissue, when placed in a bony environment (Yuan *et al.*, 2000). The CPC paste can thoroughly adapt to neighboring bone even with irregular shaped cavities and then harden in situ to form HA. Since the HA from CPC is formed in an aqueous environment at 37℃, it is more similar to biological apatite than sintered HA formed at higher temperatures. Additionally, the benefit of low temperature formation could allow organic molecules and even living cells to be incorporated into the cement (Yuan *et al.*, 2000). Thus, CPC are highly promising for large clinical uses due to their self-setting ability, highly bioactivity, bioresorbability, excellent osteoconductivity and capability to be replaced by new bone (Costantino *et al.*, 1992; Friedman *et al.*, 1998; Chow, 2000). Besides, CPC was approved in 1996 by the Food and Drug Administration for repairing craniofacial defects in humans (Friedman *et al.*, 1998). On the other hand, the low strength and susceptibility to catastrophic fracture have limited CPC to only non load-bearing repairs (Friedman *et al.*, 1998; Costantino *et al.*, 1992; Chow, 2000).

To facilitate bony ingrowth and implant fixation, macropores are needed (Hing *et al.*, 1999; Pilliar *et al.*, 2001; Livingston *et al.*, 2002; Gan and Pilliar, 2004). Macroporous CPC can be produced in situ by using a foaming agent such as hydrogen peroxide solution (Almirall *et al.*, 2004), a hydrophobic liquid or oil (Bohner, 2001), calcium sulfate (Fernandez *et al.*, 2005) and degradable polymer microparticles (Link *et al.*, 2006). This represents an advantage of CPC over sintered HA but macroporous severely degrade the CPC strength (Xu *et al.*, 2001). A study showed that the strength only increases once new bone starts to grow into the macroporous (Martin *et al.*, 1989; Shors and Holmes, 1993).

In order to achieve a controlled combination of degradation behavior and bioactivity in order to promote bone formation, β-TCP and DCPA were used as the main components of the CPC material (Kitamura *et al.*, 2004). As a bone graft material, β-TCP showed excellent performance in bone formation (Epstein, 2006). Mixtures of β-TCP and DCPA for bone substitution have been used for many years (Weitao *et al.*, 2007). The more slowly resorbing granules were surrounded by newly grown bone, thus providing an inverse scaffold for bone regeneration (Gisep *et al.*, 2003). Thus, by adding of β-TCP granules, the overall resorption rate of the cement can be tailored to specific needs and the bone formation rate can be controlled. Cements containing β-TCP and DCPA have been found to degrade completely *in vivo* after 16 weeks (Weitao *et al.*, 2007).

2.1.2 Injectability of Calcium Phosphate Cements (CPCs)

One of the drawbacks of CPCs is their poor ability to be injected through a thin long cannula attached to a syringe, such as in minimally invasive clinical

applications (Leroux *et al.*, 1999; Khairoun *et al.*, 1998; Bohner and Baroud, 2005). In most research works, injectability has been related to the viscosity of the CPC (Leroux *et al.*, 1999; Khairoun *et al.*, 1998; Ratier *et al.*, 2004; Bohner and Baroud, 2005; Wang *et al.*, 2006). Full injectability could not be reached even when no cannula was used. This implies that filter pressing occurred even for very small forces. Consequently, a solution might be obtained by reducing the ability of the mixing liquid to pass through the powder. Thus, injectability of cement could be improved by increasing the viscosity of the mixing liquid or reducing the permeability of the powder (Otsuka *et al.*, 1995; Leroux *et al.*, 1999; Khairoun *et al.*, 1998; Ratier *et al.*, 2004; Bohner and Baroud, 2005; Wang *et al.*, 2006; Habib *et al.*, 2008).

Several approaches can be used to improve and manipulate the CPC injectability such as changes of particle size (Bohner and Baroud, 2005), particle shape (Ishikawa, 2003), mixing liquid viscosity (Khairoun *et al.*, 1998; Bohner and Baroud, 2005), L/P ratio (Livingston *et al.*, 2002), paste rheology and additives (Gbureck *et al.*, 2004). Any change in L/P ratio can influence the basic properties of CPC such as setting time, injectability, porosity, compressive strength and resorbability. For example, a low L/P ratio decreases the porosity and increases compressive strength (Hesaraki *et al.*, 2007) while high L/P ratios improve the injectability of the cement paste, but this may cause a prolonged setting time and decrease the mechanical property of the cement because a high L/P ratio can produce a fluid with low viscosity paste through dilution (Bohner and Baroud, 2005; Hugo *et al.*, 2008; Qi *et al.*, 2008). Besides that, the experimental and theoretical approach predicted that the injectability of the cement paste could be improved by increasing