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DEVELOPMENT OF POROUS MAGNESIUM-DOPED BIPHASIC CALCIUM PHOSPHATE (BCP) FOR BIOMEDICAL APPLICATIONS

BY

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ABSTRACT

Calcium phosphate is an interesting material for biomedical applications such as for artificial bone implant and cell culture. Their excellent bioactivity makes them suitable material for cells to grow. However, the application of porous calcium phosphate including biphasic calcium phosphate (BCP) in biomedical applications is limited to non-stressed loaded regions owning to the brittle nature and the low fracture toughness of the bioceramics. Incorporation of metal as sintering additive is a simple way to improve the densification, mechanical and biological performance of porous BCP. In this work, magnesium (Mg) was incorporated into the BCP as sintering additive to improve the performance of porous BCP. The work covered synthesis of calcium phosphate including magnesium doped-biphasic calcium phosphate through sol-gel method by varying the concentration of Mg. Porous calcium phosphate ceramics were prepared via polymeric sponge method using the synthesized powders. The biological performance of the pure BCP and Mg-doped BCP porous scaffold was tested using cell culture method. The crushed porous scaffolds, functioning as microcarrier were tested *in vitro* using spinner vessel cell culture for attachment and proliferation of Vero cells. Morphological evaluation by SEM measurement showed that the particles of Mg-BCP were tightly agglomerated, with primary particulates of 75-150 nm diameters. FESEM result also showed that doping of magnesium into BCP particles caused fusion of particles leading to more progressive densification of particles as shown by higher concentration of magnesium doped. Successful incorporation of Mg into BCP lattice structure was confirmed by higher crystallinity of Mg-BCP and by shifting of tricalcium phosphate (TCP) peaks in XRD patterns to higher 2θ angles as the Mg content increased. XRD and FTIR measurement showed that the increment of crystallinity was directly proportional to the amount of the dopant. Both analyses also revealed that TCP appeared only after calcination of 700ºC and above. The macroporous ceramics with different pore sizes ranged from 100 to 1000 μm have been successfully fabricated. The physical characterizations found that the density of the porous bodies varied from 1.90 g/cm^3 to 2.19 g/cm^3 with 31 –35 % porosities. Doping of 10 mol% magnesium has increased the compressive strength by over 5 times compared to pure BCP (0.395 MPa to 2.170 MPa). Cell culture studies revealed that porous pure BCP and Mg-doped BCP were suitable for attachment, spreading and proliferation of Vero cells. FESEM results showed that Mg substitution induced a spread-like and irregular morphology which was quite different from the cell grown onto the pure BCP where the cells just remain on the surface of the scaffold, or proliferated in a localized area within the porous ceramics.

ملخص البحث

إن مادة فوسفات الكالسيوم مادة جديرة بالاهتمام في التطبيقات الطبية، مثل صناعة العظام الاصطناعية. الطبيعة العالية الفعالية للمادة يجعلها ملائمة لنمو الخلايا. مع ذلك، فان التطبيقات لهذه المادة محدودة وليس عليها أي طلب بسبب الطبيعة الهشة والتكسر الحاصل في السيراميك المصنع منها. من الممكن إضافة مواد أخرى لتحسين المواصفات الميكانيكية والبيولوجية للمعدن المسامي (فوسفات الكالسيوم الثنائي). في هذا العمل، تم إدخال المغنيسيوم لتحسين المواصفات وإدخال نسب مختلفة من المغنيسيوم لغرض دراسة تأثير زيادة النسبة. تم تحضير سيراميك فوسفات الكالسيوم المسامي باستخدام الباودر الصناعي. تم فحص تأدية كل من فوسفات الكالسيوم الثنائي وفوسفات الكالسيوم مع إضافة المغنيسيوم.وضحت فحوصات (أس إي أم) إنه فوسفات الكالسيوم مع المغنيسيوم كانت متكتلّة بشدة وكان قطر الجزيئات بين 75 – 150 نانوميتير. أوضحت نتائج (أف إي أس إي أم) إنه باضافة المغنيسيوم تم تحسين الكثافة كلما زادت نسبة المغنيسيوم. تم التأكد من الاتحاد الناجح للمغنيسيوم مع فوسفات الكالسيوم الثنائية من البلورة العالية بينهما وزحف القراءات لفوسفات الكالسيوم الثلاثية في أشعة أكس الى زوايا أعلى من 2 ثيتا كلما زاد محتوى المغنيسيوم. أوضحت نتائج أشعة أكس و(أف تي آي آر) إن البلورة تزيد كلما زادت نسبة المغنيسيوم المضافة. أوضح كل من فحص أشعة أكس و(أف تي آي آر) إن فوسفات الكالسيوم الثلاثية تظهر في حالة الحرق بدرجات حرارة 700 درجة مئوية أو أعلىمنها. تم تصنيع السيراميك بحجوم بين 100 – 1000 مايكروميتر بنجاح. المواصفات الفيزياوية وهي الكثافة بين 1.9غم/سم^3 الى 2.19 غم/سم^3 مع مسامية 31 - 35% تم الحصول عليها باضافة 10 مول% من المغنيسيوم وتم زيادة قوة الضغط بخمسة أضعاف مقارنة بفوسفات الكالسيوم الثنائية النقي (من 0.395 الى 2.175 ميكا باسكال). أوضحت هذه الدراسة ان فوسفات الكالسيوم الثنائي والمعزز بالمغنيسيوم ملائم للاضافة أو لابدال العظام. أوضحت نتائج (أف إي أس إي أم) إن إضافة المغنيسيوم يتغلغل في التركيب ويجعله غير متجانس والذي يعتبر مختلف قليلا عن فوسفات الكالسيوم النقي الذي تكون الخلايا على السطح فقط أو تتكتل في أماكن معينة في تركيب السيراميك المسامي.

APPROVAL PAGE

I certify that I have supervised and read this study and 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 investigation, 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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ACRONYMS

HA	Hydroxyapatite
TCP	Tricalcium phosphate
β-TCP	β-tricalcium phosphate
BCP	Biphasic calcium phosphate
Mg	Magnesium
MgO	Magnesium oxide
BET	Brunauer-Emmet-Teller
SEM	Scanning Electron Microscope
XRD	X-ray diffraction
FESEM	Field emission scanning electron microscop
PSA	Particle size analyzer
TGA	Thermogravimetric analyzer
DTA	Differential thermal analyzer
FTIR	Furier transform infrared spectroscopy
EDS	Energy dispersive spectrometry
OH	Hydroxide
PO_4	Phosphate
CO_3	Carbonate
H_2O	Water
P_2O_7	Pyrophosphate
EDTA	Ethylene diamine tetra acetic acid

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Biomaterial by definition is an artificial non-drug substance suitable for inclusion in biological systems which augment, repair or replace the function of bodily tissues or organs (Heness, et al., 2004). Biomaterial also can be defined as a nonviable material used in a medical device, intended to interact with biological systems (Ratner, et al., 2004). Biomaterial improves the quality of life as it deals with the development of material used in medical field. Demand for development of biomaterial study arises due to improvement of average human lifespan, as well as higher expectation on the quality of life. The success of a biomaterial application critically depends on the achievement of a stable attachment to connective tissue. In producing a successful biomaterial which will survive in the body for a long period of time, the identified material needs to be developed specifically for clinical applications. The key factors in a biomaterial usage are its biocompatibility (Heness, et al., 2004; Agrawal, 1998), biofunctionality (Heness, et al., 2004), and availability to a lesser extent (Heness, et al., 2004). Moreover, it should be nonalergic, nonimflammatory, nontoxic, noncarcinogenic and owns sufficient physical and mechanical properties to serve as an augmentation or replacement for body tissues (Agrawal, 1998). From practicality point of view, a biomaterial should be amenable to be formed or machined into different shapes, has a relatively low cost, and be freely available.

Biomaterials can be divided into four categories mainly governed by the tissue response. The categories are biotoxic, bioinert, bioactive and bioresorbable. The term

biotoxic refers to any material that will be rejected by living tissue once placed in human body and will result in the surrounding tissue to die. An example of this material is alloy containing cadmium. Bioinert material refers to material illicit no or minimal tissue response once placed in the human body. This material will maintain physical and mechanical properties while in the host. Generally, a fibrous tissue of various thicknesses might form around bioinert implants. Thus, its biofunctionality will rely on tissue integration with the implant. The examples of these materials are tantalum, titanium, aluminum, zirconia (PSZ), UHMW polyethylene and stainless steel. High density hydroxyapatite, glass-ceramics A-W, and certain bioglassses are examples of bioactive implant materials. These materials will encourage bonding of implant with surrounding tissue. Bioresorbable materials are materials that incorporate into the surrounding tissue and dissolve completely over a period of time. Common examples of bioresorbable materials are porous hydroxyapatite, tricalcium phosphate, polyurethane and polylactic-polyglycolic acid copolymer.

Additional factors shall be identified and thoroughly considered with respect to the basic categorization of biomaterial. By choosing the appropriate material, a desirable biological response such as good bonding between tissues and implants may be achieved. It is advantageous to have the ability to tailor the mechanical properties of the biomaterial to match those of the body component which it is replacing, that it is an analogue.

From a different aspect, biomaterial can be classified into four categories which are metals, polymers, ceramics, and natural materials (Agrawal, 1998.) This thesis focused on ceramics biomaterials. Bioceramic is within a class of advanced ceramics which are defined as ceramic products or components employed in medical and dental applications, mainly as implants and replacements (Paul, et al., 2005).

Ceramic examples are including but not limited to refractory, polycrystalline compounds, usually inorganic, including silicates, metallic oxides, carbides, and various refractory hydrides, sulfides and selenides (Praphulla, et al., 1995). Ceramics are materials that exhibit great strength and stiffness, having low density, and excellent resistance to corrosion and wear. Materials classified as bioceramics are alumina, zirconia, calcium phosphates, silica based glasses or glass ceramics, titania and pryolictic carbon (Paul, et al., 2005). Bioceramics have made significant contribution in modern health care industry by improving the quality of human life. Bioceramics can be used inside of human body without rejection due to their biocompatibility, low density, chemical stability, high wear resistance, and for calcium phosphates, mainly due to composition similarity with the mineral phase of bone (Kalita, et al., 2007).

Calcium phosphate bioceramics have widely been developed in biomedical in applications due to excellent biocompatibility, bioactivity and osteoconduction characteristics. Among various phases of calcium phosphate, hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] and bheta-tricalcium phosphate [$Ca_3(PO_4)_2$, β-TCP], with similar composition and crystal structure to natural bone (Hsu, 2003), are the two most commonly used calcium phosphate ceramics used for medical purposes. These materials are fabricated in porous, granular and dense forms (Liu, et al., 2008).

In biomedical applications, porous ceramics have been used for artificial bone substitutes, drug delivery and cell culture (Sopyan, et al., 2007). Porous ceramics exhibit strong bonding to the bone as the pores provide a mechanical interlock leading to a firm fixation of the material. Bone tissue grows well into the pores, which increases the strength of the porous ceramic implants. Highly porous scaffolds provide a framework for enhanced cell infiltration and migration throughout the scaffold

(Sunho, et al., 2006). Therefore, a porous structure will promote cell attachment, proliferation, and differentiation, provides pathways for transport of biofluids, nutrients and metabolic waste (Liu, et al., 2008). In addition to that, porous structures are light in weight, provide appropriate space for the ingrowth of the bone tissue, and accelerate the replacement of the material by bone tissue (He, et al., 2008).

Porous HA and β-TCP have been extensively applied for artificial bone substitutes. The primary purpose of tissue engineering is for repair, regeneration, and reconstruction of lost, damaged or degenerative tissues (Sopyan, et al., 2008). Although bone tissue itself shows an excellent ability of bone regeneration, for big bony defect or for such situations that bone healing process is difficult, bone grafts are required. At this point, it is very crucial to match the osteoconductive properties of porous ceramic scaffolds in one side with the osteoinductive or osteogenic properties of living bone cells in the other side.

Great diversity of the biomedical usage has led to the development of various methods in preparation of porous ceramic materials. This has allowed the design and production of porous materials with controlled porosity, good pore interconnectivity, mechanical strength and surface properties.

Porous bioactive ceramics have been prepared by multiple methods, including introduction of porous structures using pore-creating volatile particles which burn away during sintering (Frieβ, et al., 2002), via direct conversion of marine coral skeleton and natural bone (Heness, et al., 2004), via ceramics foaming technique (Woyansky, et al., 1992) foam-gel technique (Tamai, et al., 2002) or hydrothermal hot pressing (Kusmant, et al, 2008). All the above-mentioned techniques have respective advantages and disadvantages. For example, gel casting of foams can be used in

producing ceramic scaffolds with high mechanical strength, but typically results in a structure with poorly interconnected pores and non-uniform pore size distribution.

Another approach for fabricating porous ceramics is via the replication of a polymeric sponge substrate to produce reticulated open-celled porous ceramics (Sopyan, et al., 2007). The polymeric sponge method, as the name suggest, is performed by impregnating porous polymeric substrates (sponges) with hydroxyapatite (HA) slurry. The method has been proven reliable in assuring a proper pore-size distribution, as osteoconduction requires, characterized by the existence of micro/meso/ macropores with a sufficient connection degree (Richart, et al., 2005). Therefore, one of the important aspects in the development of bone and organ substitute materials is the fabrication of supporting matrices or scaffolds with an appropriate micro- and macroscopic structural morphology including pore size, pore interconnectivity, mechanical strength and biodegradability.

1.2 PROBLEM STATEMENT

Theoretically, a degradagation rate of an implant similar to the rate of tissue formation is expected. However, porous HA has poor rate of biodegradability (Kalita, et al., 2006). In contrast, porous β-TCP is widely used as a biodegradable bone substitutes as it gives rise to extensive bone remodeling around the implant (Tas, et al., 1997). On the other hand, when used as biomaterial for alveolar ridge augmentation, the rate of biodegradation of β-TCP has been shown to be too fast compared to degradation of natural bone (Kivrak, et al., 1998). Moreover, β-TCP is difficult to sinter, exhibits poor mechanical strength and low resistance to crack-growth propagation (Kalita, et al., 2006). Thus, in order to achieve an optimized balance of the non-biodegrability of HA which is more stable phase and at the same time to slow the rate of biodegradation

of β-TCP, the interest of biphasic calcium phosphate (BCP) concept have been studied by multiple research groups.

Biphasic calcium phosphate (BCP) ceramics generally comprised of intimate mix of non-resorbable HA and resorbable β-TCP (Nilen, et al., 2008). Porous BCP is particularly suitable materials for synthetic bone substitute applications as to mimic the porous nature of cancellous bone (Nilen, et al., 2008) because the HA phase will provide a permanent scaffold for new bone formation via osteoconduction, and the resorption of the β-TCP oversaturates the local environment with Ca^{2+} and PO_4^{3-} ions to accelerate this new bone formation (Nilen, et al., 2008). The BCP allows its bioactivity and biodegradation to be controlled by varying the HA/ TCP ratio (Victor, et al., 2008).

However, the application of porous calcium phosphate including BCP in clinical orthopaedic and dental applications is limited to non-stressed loaded regions owning to the brittle nature and the low fracture toughness of the bioceramic (Tan, et al., 2008). In addition to that, a three dimensional (3-D) interconnected porous structure is necessary to allow cell attachment, proliferation, and differentiation, and to provide pathways for biofluids (Ramay, et al., 2003). In fact, it is generally known that the mechanical strength of porous ceramic usually decreases as the porosity increases. Thus, optimizing balance between the biological requirements and mechanical properties of porous scaffold of BCP is very desirable. Multiple researches and development effort have been carried out in enhancing the mechanical properties with respect to the biological compatibility of porous BCP including varying the powder processing technique (Tan, et al., 2008), manipulation of processing parameters such as particle size and shape, distribution and morphology of the starting

powders, control of sintering temperatures and incorporation of metal as sintering additive into the BCP.

Incorporation of metal as sintering additive (Bhatt, et al., 2007, Kalita, et al., 2006; Itatani, et al., 2002) is a simple and economical way to improve the densification, mechanical and biological performance of porous BCP. In this work, magnesium was incorporated into the BCP as sintering additive to improve the performance of porous BCP.

Various research groups have attempted to dope calcium phosphate materials with magnesium (Zyman, et al., 2008; Kalita, et al., 2007; Landi, et al., 2006; Kannan, et al., 2005; Gibson, et al., 2002; Fadeev, et al., 2003) for better performance bone implant materials. Doping of magnesium ions into BCP will results in biological improvement as the ion will cause the acceleration of nucleation kinetics of bone minerals (Landi, et al., 2006). Magnesium depletion adversely affects all stages of skeletal metabolism, leading to decrease in osteoblastic activities and bone fragility. Addition of magnesium might as well improve thermal stability of TCP which prevents phase transformation of β-TCP to α-TCP at high temperature (Xue, et al., 2008). Thus, this will results in a better mechanical properties of porous BCP.

Addition of magnesium into porous BCP has encouraged the spreading and improves the adhesiveness of cells onto bioceramic matrices (Paul, et al., 2007). Landi et al. (2006) has revealed that doping of magnesium into the apatite has improved the behaviors of cultured cells in term of adhesion, proliferation and metabolic activation, compared to stoichiometric.

1.3 SCOPE OF RESEARCH

Here, BCP powder doped by magnesium was synthesized via a sol-gel method by utilizing non-alkoxide compounds as the raw materials. Several advantages gained by producing ceramic powder through the sol-gel method are good homogeneity of powder (Bezzi, et al., 2003; Gibson, et al., 2001), nanosize dimensional of the primary particles, and high reactivity (Bezzi, et al., 2003) compared to conventional methods such as solid-state reaction (Suchanek, et al., 1998), hydrothermal (Suchanek, et al., 1998), and wet chemical precipitation (Suchanek, et al., 1998). Moreover, the sol-gel method employed in this work is economically attractive, using raw materials which are easily obtained, compared to conventional sol-gel method which usually uses expensive alkoxide compounds (Xiu, et al., 2005).

The advantage of using chemical methods like sol-gel method is that magnesium will replace calcium in molecular level to join the lattice of BCP. This is better approach if comparison is made with a physical method such as milling method in mixing MgO and BCP (Tan, et al., 2008) which may produce residual MgO that cannot be accepted as bone implant.

In order to produce porous ceramic implants of BCP and Mg-doped BCP to mimic scaffolds for spongy bone application, water based suspensions from the synthesized powders were prepared using Duramax D3005 as the dispersant agent. Each slurry was homogenized by stirring. Commercial cellulosic sponges were shaped and impregnated in the slurry and left to dry off. The porous samples were later heat treated to remove organic matrix and densify the porous phase. Physical, chemical and mechanical properties of the prepared porous ceramics were characterized by using XRD, FESEM, TG/ DTA, densitometer (Archimedes principle), FT-IR and mechanical testing machine.