EFFECT OF MAGNESIUM-SILICON SUBSTITUTION ON THE CHARACTERISTICS OF CARBONATED HYDROXYAPATITE FOR SYNTHETIC BONE

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by

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LIST OF ABREVIATIONS

BET	Brunauer, Emmett and Teller
BGS	Bone Graft Substitute
CHA	Carbonated Hydrosxyapatite
CHN	Carbon, Hydrogen and Nitrogen
CaP	Calcium Phosphate
CO_2	Carbon Dioxide
DP	Direct Pouring
DW	Dropwise
DTS	Diametral Tensile Strength
DMEM	Dulbecco's Modified Eagle's Medium
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
FWHM	Full Width at Half Maximum
HA	Hydroxyapatite
ICSD	International Centre of Standard Data
SBF	Simulated Body Fluid
TEM	Transmission Electron Microscopy
XRD	X-Ray Diffraction
XRF	X-Ray Fluorescence
RT	Room Temperature
i.e.	That is

LIST OF SYMBOLS

cm	Centimetre
°	Degree
°C	Degree Celsius
°C/min	Degree Celsius per minute
ρ	Density
g	Gram
h	Hour
L	Litre
<	Less than
m	Meter
ml	Millilitre
mm	Millimetre
min	Minute
>	More than
nm	Nanomater
%	Percentage
% D_s	Percentage of shrinkage in diameter
% t_s	Percentage of shrinkage in thickness
% T	Percentage of Transmittance
θ	Theta
wt %	Weight percent

KESAN PENGGANTIAN MAGNESIUM-SILIKON TERHADAP CIRI-CIRI HIDROKSIAPATIT KARBONAT TULANG SINTETIK

ABSTRAK

Serbuk magnesium-silikon hidroksiapatit terkarbonat bersaiz nano jenis B (Mg-Si CHA) di perolehi dengan kaedah pengemulsian nano melalui teknik tuangan langsung (DP) pada suhu ambien. Magnesium (Mg) dan silicon (Si) ditukar ganti ke dalam struktur kekisi hidroksiapatit karbonat supaya ia lebih menyamai komposisi kimia tulang semulajadi manusia. Partikel Mg-Si CHA menunjukkan bentuk sudut atau hampir bulat dengan dimensi 10-40 nm (panjang) dan 5-20 nm (lebar). Penggabungan Mg dan Si kurang dari 1% ke dalam kekisi CHA menunjukkan tiada kesan terus terhadap komposisi fasa, yang terbukti dari hasil keputusan Belauan sinar-X (XRD) dan Spektroskop infra merah jelmaan Fourier (FTIR). Persinteran kemudiannya dijalankan ke atas sampel Mg-Si CHA pada suhu 800°C dan dibiarkan selama 2 jam. Dalam peringkat penyejukkan, sampel yang telah disinter dikeluarkan dari relau bila suhu mencecah 200°C dan diletakkan dalam desikator. Gas CO_2 basah dipamkan ke dalam desikator untuk menggantikan karbonat yang terurai daripada sampel semasa persinteran. Kandungan karbonat Mg-Si CHA tersinter adalah 6.80%, iaitu jatuh dalam julat tipikal untuk tulang semulajadi. Kebioaktifan in vitro magnesium-silikon tertukar ganti hidroksiapatit karbonat (Mg-Si CHA) telah dinilai melalui rendaman sampel dalam larutan badan simulasi (SBF) sebagai medium dan ujian ketoksidan dalam erti kata tindakbalas sel kultur. Keputusan ujian menunjukkan Mg-Si CHA tersinter mempunyai kebolehan membentuk apatit dengan lebih cepat dan tidak toksik terhadap fibroblas manusia, seperti yang

didemonstrasikan oleh bilangan sel berdaya hidup yang tinggi. Kesimpulannya, Mg-Si CHA berkemungkinan boleh digunakan sebagai bahan pengganti tulang.

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ABSTRACT

The nano-sized B-type magnesium-silicon carbonated hydroxyapatite (Mg-Si CHA) powders was successfully synthesized by nanoemulsion method through direct pouring (DP) technique at ambient temperature. Magnesium (Mg) and silicon (Si) were substituted into the carbonated hydroxyapatite (CHA) lattice structure in order to mimic the chemical composition of the human natural bone. The Mg-Si CHA particles exhibit angular or near spherical-like in shape with the dimension of 10-40 nm (length) and 5-20 nm (width). The incorporation of less than 1% of Mg and Si into the CHA lattice appears to have no direct effect on the phase composition, as evidenced by X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) results. Sintering was then performed on the Mg-Si CHA sample at 800°C and 2 hours soaking. During the cooling stage, the sintered sample was taken out from the furnace when the temperature reaches 200°C and placed in a desiccators. Wet carbon dioxide gas (CO_2) was pumped into the desiccators to compensate the carbonate loss during sintering. The carbonate content of sintered Mg-Si CHA was 6.80%, which is fall in the range typically reported for natural bone (3 to 8%). *In vitro* bioactivity of the Mg-Si CHA was evaluated through immersion sample in simulated body fluid (SBF) solution as a medium and cytotoxicity test in term of cell culture response. The results revealed that the Mg-Si CHA had good bioactivity by rapid apatite formation ability and found to be non-cytotoxic on the human fibroblast, as demonstrated by high cell viabilities. In conclusion, Mg-Si CHA may be used as a synthetic bone substitution material.

CHAPTER ONE
INTRODUCTION

1.1 Research Backgrounds

Surgical procedures are typically performed to replace, repair or regenerate bone defects, that are caused by numerous diseases, genetic abnormalities and traumatic injuries (Ana et al., 2010). These are commonly done in the field of orthopaedics, neurosurgery and dentistry. Conventionally, bone grafts adopted from the patient's own body (autograft) or from another person (allograft) or even from animal's tissue (xenograft) have been used to heal or to fill such bone defects. Nevertheless, the availability of autografts is limited, while harvest is expensive, painful and associated with donor-site morbidity due to infection and hematoma. On the other hand, allograft and xenograft procedures risked of transmitting disease from donor to patient or possible rejection by the patient's immune system (O'Brien, 2011). These shortcomings, including multiple operating procedures, have motivated and driven for synthetic bone grafts.

Bone is a complex living tissue with various levels of hierarchical structural units from macro to nano scales, to meet multiple functions (Murugan & Ramakrishna, 2005). It is a vascular dynamic tissue that has a unique capability of self-remodelling or self-regenerating throughout the lifetime of an individual. Typically, the bones assist in locomotion activity, while ensuring the skeleton has adequate load-bearing capacity, and acts as a protective casing for the delicate internal organs of the body as reported by Stevens (2008). Bones also serves a reservoir for the storage of essential minerals, particularly calcium and phosphate (Murugan & Ramakrishna, 2005). Although bone is considered the strongest tissue in the body, it is easy to fracture resulting from injury and degenerative diseases associated with ageing. The most frequent occurring fractures are hip, ankle, tibia and fibula fractures. The number of hip fractures are more than 280,000 every year in the United States and incidence is expected to double by 2050 (Isnoni et al., 2012). In the UK, hip fracture incidence will rise from 70,000 per year in 2006 to 101,000 in 2020 as reported by Departmental of Health Hospital (England). Moreover, Hernlund et al. (2013) had reported that the annual number of fractures in the European Union (EU) will rise from 3.5 million in 2010 to 4.5 million in 2025, corresponding to an increase of 28%. Many reports have shown an increase in the incidence of hip fractures in Asian population. There was 1.66 billion cases of hip fracture reported in 1990 and it is estimated to increase to 6.26 billion in 2050 due to the growth of the elderly population as reported by Aseel Hadi Abdulameer et al. (2017). As the longevity increased, older people (above 50 years old) are 10-15 times easily to fracture a hip than those younger. In Malaysia, there was 10 per 100,000 for the range of 50-54 years old, and rising to 510 per 100,000 in those over 75 years old (Yeap et al., 2016). According to ethnic background, Chinese and Malays have higher rates of hip fracture as compared to Indian (Aseel Hadi Abdulameer et al., 2017).

Injuries due to road mishaps also contribute to high demands of bone grafting procedure. Malaysia had a high number of road accidents, with the total number of accident reported at 489,606 cases in 2015, which was an increase from 476,196 in 2014, corresponding to an increase of 2.8% (Malaysia Transport Statistics 2015). This reflects orthopaedic injuries was high among Malaysian road users. As a result, bone grafting procedures become one of the important surgeries performed at hospitals. Therefore, there is a great need to develop synthetic alternative biomaterials for bone replacement, repair and augmentation (LeGeros, 2008).

Various kinds of biomaterials have been developed and used as bone graft substitutes (BGS) such as ceramics, metals, polymers and their composites. Among the ceramic materials, hydroxyapatite (HA) has been used widely as bone graft substitute due to its biocompatibility, bioactivity, osteoconductivity, non-toxicity and non-inflammatory properties (Malina et al., 2013). Moreover, the chemical and phase composition of HA also mimics the mineral phase of natural bone (Cacciotti, 2016). Although synthetic HA has the ability to allow new bone to form along its surface, the usage of synthetic HA in medical applications is limited, because of its slow rate of osseointegration, remodelling in the body over a long period of time, as well as a relatively low fracture strength and toughness (Gibson et al., 2002). Synthetic HA also does not degrade significantly but rather remains as a nearly permanent fixture susceptible to long term failure (Ishikawa et al., 2003).

One way to enhance the bioactive behaviour of HA is to develop substituted apatites, resembling the chemical composition and structure of the mineral phases in human bones. These ionic substitution can modify the surface structure and electrical charge of HA, leading to major changes in biological performance upon implantation (Supova, 2015).Carbonate ion is the most abundant substitutions and has been reported to be about 3-8 wt%, depending on the age of the individual (Barinov et al., 2006). In this sense, the biological apatite is regarded as carbonated hydroxyapatite (CHA), with a variety of other ions substituted in its lattice such as sodium, magnesium and strontium. Recently, Sr, Mg, Zn, Mn, Li, Cu, F and Si ions have attracted much interest because of the positive effects on bone repair and regeneration (Xia, 2013). Sr ion has been shown to stimulate osteoblast activity, bone strength and bone growth. Li ion could enhance the proliferation and cementogenic differentiation of human periodontal ligament-derived cells (Han et al., 2012). Similarly, Zn ion inhibitsbone resorption, supports bone growth, enhances antimicrobial resistance, as well as increases the activity of osteoblasts, thus resulting in increased bone formation (Gopi et al., 2015). Si substitution has been proven to enhance the apatite solubility, thus increase the bioactivity (Tampieri et al., 2016), whereas Mg plays an important role in bone metabolism stimulating osteoblast cell proliferation (Lala et al., 2016).

1.2 Problem Statement

Substituted hydroxyapatites have significant advantages in terms of biodegradability and bioactivity compared to the stoichiometric hydroxyapatite, as reported by Supova (2015). Currently, CHA is accepted as a prospective material in medical applications in order to mimic the composition of natural bone (Roveri & Iafisco, 2010). The co-substitution of carbonate with silicate, strontium or magnesium has been shown to ensure controlled release and improved biological response *in vitro* (Landi et al., 2010; Sprio et al., 2008). Co-substitutions have also been carried out in order to counteract the structural changes that occur during substitution and prevent destabilization of the structure and subsequent decomposition during heat treatment. These include the co-substitution of carbonate with magnesium (Abdelkader et al., 2014) or yttrium with silicon (Stephen et al., 2007). However, there is lacking of research on the co-substitution of multiple (more than two) ions in HA crystal structure which would accurately mirror the chemistry of the natural bone, in order to produce the most effective bone substitute materials. As such, this research embarks on the study of co-substitution of HA with carbonate, Si and Mg. The limited thermal stability of the CHA is also a challenge, as poor