SYNTHESIZE AND CHARACTERIZATION OF BIOACTIVE GLASS POWDER BASED ON SiO_2-CaO-Na_2O-P_2O_5 SYSTEM

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by

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LIST OF ABBREVIATIONS

BG	Bioactive glass
CTE	Coefficient of thermal expansion
DMEM	Dulbecco's Modified Eagle Medium
DPSC	Dental pulp stem cell
DSC	Differential scanning calorimetry
EDX	Energy dispersive x-ray
FTIR	Fourier transform infrared spectroscopy
FESEM	Field emission scanning electron microscopy
HA	Hydroxylapatite
HCA	Hydroxylcarbonate apatite
ICP-OES	Inductively coupled plasma optical emission spectroscopy
PDF	Powder diffraction file
PSA	Particle size analysis
RMM	Relative molecular mass
rpm	Revolutions per minute
SBF	Simulated body fluid
TE	Tissue engineering
TTT	Time temperature transformation
XRD	X-ray diffraction
XRF	X-ray fluorescence
45S5	45SiO2-24.5CaO-24.5Na_2O-6P_2O_5 (wt. %)
50S8P	50SiO2-22CaO-20Na_2O-8P_2O_5 (wt. %)
54S4P	54SiO2-22CaO-20Na_2O-4P_2O_5 (wt. %)
46S0P	46SiO2-24CaO-30Na_2O-0P_2O_5 (wt. %)

LIST OF SYMBOLS

mol. %	Mole percentage
Nc	Network connectivity
T_c	Crystallization temperature
T_m	Melting temperature
T_g	Glass transition temperature
t	Time
%	Percentage
wt. %	Weight percentage
°	Degree
a.u	Arbitrary unit
θ	Incidence angle of X-ray beam

SINTESIS DAN PENCIRIAN SERBUK KACA BIOAKTIF BERASASKAN SISTEM SiO_2-CaO-Na_2O-P_2O_5

ABSTRAK

Serbuk kaca bioaktif (BG) telah digunakan sebagai pengisi dalam kecacatan tulang kerana keupayaan untuk berhubung dengan tisu tulang melalui pembentukan ikatan dengan lapisan apatit. Walau bagaimanapun, suhu lebur yang lebih tinggi (1450 °C-1570 °C) atau masa lebur yang lebih lama (3 jam) diperlukan untuk menghasilkan serbuk BG melalui kaedah lebur kaca konvensional. Dalam penyelidikan ini, komposisi baru (50S8P, Nc = 2.69), (54S4P, Nc = 2.60) dan (46S0P, Nc = 1.62) serbuk kaca bioaktif telah dibangunkan daripada sistem SiO_2-CaO-Na_2O-P_2O_5 untuk mendapatkan sifat-sifat pemprosesan dan biologi yang baik. Penghasilan BG termasuk pengelompokan, pencampuran, peleburan pada suhu yang berbeza, lindap kejut air, pengisaran dan pengayakkan. BG dengan komposisi 45S5 digunakan sebagai kawalan. Pembelaun sinar-X (XRD) memperlihatkan struktur kaca amorfus sepenuhnya diperolehi untuk semua komposisi BG dengan puncak lebar antara 30-35°. Kaca berasaskan rangkaian silika juga disahkan melalui transformasi Fourier spektroskopi inframerah (FTIR) dengan kumpulan berfungsi Si-O-Si (tetrahedral) dikenalpasti dalam spektrum. Analisis haba membuktikan bahawa semua komposisi BG boleh dileburkan pada suhu lebih rendah iaitu 45S5 pada 1377 °C, 50S8P dan 54S4P pada 1348 °C dengan 46S0P pada 1347 °C. Oleh itu, kesan suhu dan masa lebur yang berlainan (1.5, 1 dan 0.5 jam) juga dikaji. Berdasarkan XRD, struktur amorfus masih kekal walaupun serbuk BG dihasilkan pada suhu lebur yang lebih rendah pada 0.5 jam untuk semua komposisi BG. Bioaktiviti BG dinilai dengan pengeraman serbuk BG dengan larutan penimbal Tris (pH 8) selama 7, 14 dan 21 hari. Ujian *in vitro* mengesahkan pembentukan hidrosilapatit (HA) pada permukaan BG dengan kemunculan puncak berhablur dalam XRD dan ciri-ciri kumpulan berfungsi karbonat (C-O) dan fosfat (P-O) yang kelihatan dalam FTIR dengan keamatan puncak yang lebih tinggi telah diperhatikan pada 45S5 dan 50S8P BG berbanding dengan 54S4P dan 46S0P BG. Tindak balas biologi yang lebih baik diperhatikan pada BG yang dibuat pada suhu 1400 °C setelah pengeraman dan seterusnya diuji dengan *in vitro* simulasi cecair badan (SBF), pH 7.3 dan media sel. Namun, ciri-ciri pengamatan HA yang kurang telah diperhatikan pada XRD dan FTIR pada permukaan BG setelah direndam dalam SBF berbanding dengan larutan penimbal Tris. Kebolehserasian yang baik diperhatikan apabila sel stem pulpa gigi (DPSC) didedahkan kepada semua komposisi BG. Kesimpulannya, komposisi baru serbuk BG telah berjaya dibangunkan pada suhu dan masa lebur yang rendah dengan sifat biologi yang baik walaupun mempunyai sambungan rangkaian yang tinggi.

SYNTHESIZE AND CHARACTERIZATION OF BIOACTIVE GLASS POWDER BASED ON SiO_2-CaO-Na_2O-P_2O_5 SYSTEM

ABSTRACT

Bioactive glass (BG) powder have been used as a filler in bone defects due to the ability to connect with bone tissue through bonding formation with apatite layer. However, higher melting temperature (1450 °C-1570 °C) or longer soaking time (3 hours) is required to produce BG powder *via* conventional glass melting route. In this research work, new composition (50S8P, Nc =2.69), (54S4P, Nc =2.60) and (46S0P, Nc =1.62) of bioactive glass powder was developed from SiO_2-CaO-Na_2O-P_2O_5 system to obtain good processing and biological properties. The BG preparations included batching, mixing, melting at different temperature, water quench, milling and sieving. BG with 45S5 composition was used as a control. X-ray diffraction (XRD) revealed that fully amorphous glass structure was obtained for all BG composition with broad peaks between 30-35°. Silica network based glass was also confirmed through Fourier transform infrared spectroscopy (FTIR) with Si-O-Si (tetrahedral) functional group was observed in the spectrum. Thermal analysis proved that all BG composition can be melted at lower temperature where 45S5 at 1377 °C, 50S8P and 54S4P at 1348 °C with 46S0P at 1347 °C. Hence, the effect of different melting temperature and time (1.5, 1 and 0.5 hour) were also studied. Amorphous structure was still retained based on XRD although BG powder was synthesized with lower melting temperature at shorter melting time, 0.5 hour for all BG composition. The BG bioactivity was evaluated by incubating the BG powder with Tris buffer solution (pH 8) for 7, 14 and 21 days. *In vitro* test confirmed on the hydroxylapatite (HA) formation on the BG surface with emerging of crystalline peaks in XRD. Characteristic of carbonate (C-O) and phosphate (P-O) functional group noticed in FTIR with more intense peaks was observed on 45S5 and 50S8P BG compared to 54S4P and 46S0P BG. Better biological responds was observed on BG synthesized at 1400 °C upon incubation and was further evaluated by *in vitro* test in simulated body fluid (SBF), pH 7.3 and cell culture. However, less intense HA characteristic was observed in XRD and FTIR on the BG surface upon immersion in SBF compared to Tris buffer solution. Good compatibility was observed when dental pulp stem cell (DPSC) was exposed to all BG composition. In conclusion, new composition of BG powder was successfully developed at lower melting temperature and soaking time with good biological properties although possess high network connectivity.

CHAPTER ONE

INTRODUCTION

1.1 Biomaterials

A material that is able to interact with biological system by providing treatment, tissue or organ replacement and function of body is known as biomaterial. Initially, the first generation of biomaterial was focused on the mechanical performances of implant material and the material selection was limited to those that exhibit inert characteristics (Crovace *et al*., 2016). Inert material such as steels, carbon materials, silicones, and poly (methyl methacrylate) were examples of biomaterials. These materials exhibit biocompatibility characteristic yet suffer on non-degradation properties which limit their usage in clinical applications (Wang, 2016). These provided the basis for the invention of second and third generation of biomaterials (Crovace *et al*., 2016).

Development of biomaterials for clinical applications require some additional excellent important characteristic such as the ability of the biomaterial to be harmonized with micro-environment of defective tissue, ability to support the mechanical stability of defective tissue during tissue repair and possess the adaptable biodegradability characteristic which matches the new tissue formation (Wang, 2016). The second generation of biomaterials demonstrate such characteristic as the ability to induce reaction in the physiological environment. Meanwhile, the growth of third generation of biomaterials received great attention due to the capability of the biomaterials to stimulate specific cellular responses at the molecular stage and able to activate genes responsible for living tissue regeneration. Bioactive glass and glass ceramic are examples of third generation of biomaterials (Crovace *et al*., 2016).

Biomaterials is widely used in clinical applications such as in repairing bone defects. Several reasons that induced bone defects are infection, trauma, tumor and congenital deformity. The autogenous bone transplantation is optimum alternative for the treatment. However this procedure has drawbacks such as limited source, might induce damage at the site of transplantation. The other option is to introduce biomaterials that have potential restorative effects in bone defects. Biomaterial such as bioactive glass (BG) is widely used to repair bone defect due to the ability to bond and integrate with bone in living body through rapid formation of apatite layer on the material surface upon exposure to biological environment (Mosbahi *et al*., 2016). Biomaterials also being implemented in dental treatment applications. The use of bioactive glass in dental treatment enables the induction of remineralization and assist against local irritation. In addition, the possibility to use BG in periodontal disease treatment is also recognized. It was reported that the combination of BG and clodronate enhanced ion exchange resulting in apatite formation in dental application to treat periodontitis during maintenance phase (Rosenqvist *et al*., 2014). Tooth sensitivity can also be treated using biomaterial such bioactive glass where the BG is added in tooth paste for the treatment (Fernando *et al*., 2017). The use of biomaterial in wound healing treatment (Lv *et al*., 2017) and bone fracture (Arcos *et al*., 2014) due to osteoporosis also has been widely explored and studied.

1.1.1 Bioactive glass

A glass is a material which is obtained by heating a solid mixture material until it reaches a viscous state and quickly cooled to prevent the formation of crystalline structure. Upon quenching, the atom remains in the disordered state characteristic of liquids (Salinas, 2014). The glass structure exhibit random array of atoms and are linked by directional bonding and glass network which contains no

regular pattern to the spacing among neighbour, and thus are often called 'amorphous' or without form (Kelly and Benetti, 2011; Wright, 2014). The open structure of amorphous glass facilitates the inclusion of network modifiers inducing the discontinuity of the glass network. The disordered structure leads to the high reactivity of glass in aqueous environments. The high surface reactivity of glass is the prime advantages of their application in bone repair and replacement (El-Kheshen *et al*., 2008).

The use of bioactive glass (BG) has received a great attention for bone and dental treatment since its first invention by Hench in the 1970s. The primary characteristic of BG which includes the ability to integrate with living tissue has induced rapid development of BG in biomedical applications (Rahaman *et al*., 2011). Formerly, implant material exhibit only bioinert character and tends to evoke undesirable fibrous encapsulation around the material upon implantation. However, BG demonstrates a positive response upon implantation by developing a stable bond and interface with living tissue without formation of contact between fibrous tissue and the living tissue. The bond between BG and tissue is formed through apatite layer formation (Miguez-Pacheco *et al*., 2015).

The important characteristics of BG such as high bioactivity which has the ability to develop hydroxyapatite layer on the glass surface, osteoconduction and osteostimulation make them suitable to be used for bone and tooth repair regeneration (Miguez-Pacheco *et al*., 2015). The bioactivity depends on the ability to develop a bone-like mineral on the glass surface when in contact with physiological fluids (Orgaz *et al*., 2016). BG also shows excellent osteogenic characteristic due to ion released during glass dissolution which has the ability to stimulate expression of numerous genes that promote osteoblastic cell proliferation

and differentiation (Xynos *et al*., 2001; Hench, 2009). The classic application of BG includes bone filling materials, bioactive coating on orthopedic implant, dental applications and small bone implant (Jones, 2015).

1.1.2 45S5 Bioglass

The first bioactive glass was synthesized based on SiO_2-CaO-Na_2O-P_2O_5 system. The classical bioactive glass is generally referred as 45S5 Bioglass® and originally developed by Hench. The glass is characterized in nominal composition of 45% silicon dioxide (SiO_2), 24.5% calcium oxide (CaO), 24.5% sodium oxide (Na_2O) and 6% phosphorus pentoxide (P_2O_5) (in weight percent) (Desogus *et al*., 2015). The unique glass composition was reported as 45S5 to indicate the weight percentage of silica (SiO_2) used as the network former and a 5-fold ratio of Ca/P (Hench, 2013). The lower content of network former silicon dioxide (SiO_2) and higher content of glass network modifier, sodium oxide (Na_2O) and calcium oxide (CaO) is the key feature that contributes in the bioactivity of 45S5 glass (Rahaman *et al*., 2011). P_2O_5 was added in the glass composition in order to stimulate the Ca/P component of hydroxyapatite (HA), the inorganic mineral of bone (Hench, 2013).

The oxide composition of 45S5 allows it to bond with both hard and soft tissues (Faure *et al*., 2015). *In vitro* experiment using 45S5 glass, heterogeneous nucleation of the HA layers on the glass surface was observed indicating the process of mimicking bone mineralization during bone repair. This finding indicated the possibility to develop HA layer on the implant glass which later will provide interfacial bonding with living bone. During, *in vivo* experiment in mid shaft femur of rats, evidence of development of polycrystalline HA layer on the implant 45S5 glass was observed to form bonding between collagen fiber (Hench, 2016). 45S5 bioactive glass has been widely used since mid 1980s in clinical treatments in

powder form as regenerative bone filler with product names such as Perioglas® and Novabone® (Novabone Corporation, Alachua Florida) (Jones *et al*., 2007). It has been use clinically in medical and dental application since then (Hench *et al*., 2014).

45S5 bioactive glass powder was first synthesized *via* conventional melt derived route. Melt derived route is a simple method and able to produce BG in massive production (Ma *et al*., 2010). Bioactive glass produced *via* melt derived is fully amorphous without existence of other crystalline phase. The amorphous phase is obtained due to the sudden cooling effect after quenched (Aguilar-Reyes *et al*., 2017). The bioactive glass produced *via* melt derived also exists in more disordered three-dimensional glass structure. The fully amorphous glass structure induced higher ability in formation of apatite layer compared to crystalline phase due to lower solubility that might be due to strong bonding between atoms in crystal lattice (Dziadek *et al*., 2016). This route requires mixing stoichiometric amounts of oxides which is high purity silica (SiO_2), calcium carbonate ($CaCO_3$), sodium carbonate (Na_2CO_3) and phosphorus pentoxide (P_2O_5). The mixture will then be melted at high temperature 1450 °C (Hench *et al*., 1971). Available literature also indicate range of melting temperature to synthesize 45S5 bioactive glass from above oxides, melted at 1400 °C for four hours (Lefebvre *et al*., 2008; Yang *et al*., 2013) and 1380 °C for two hours (Zarifah *et al*., 2015). The molten glass was then subsequently quenched in graphite (Yang *et al*., 2013) or stainless steel (Aguilar-Reyes *et al*., 2017) mold to obtain glass block or in water to obtain glass frit (Zarifah *et al*., 2015). In order to obtain glass powder, the glass frit or even glass block was crush and ground *via* mortar or milling process (Yang *et al*., 2013; Araújo *et al*., 2015). Synthesize of BG powder through melt derived is an alternative method to obtain glass without destructing the amorphous network structure. The mixture of reactants will be