UNIVERSITI PUTRA MALAYSIA

PHYSICAL AND MECHANICAL PROPERTIES OF HYDROXYAPATITE REINFORCED WITH 45S5 BIOCOMPOSITE

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By

ZARIFAH BT HJ NADAKKAVIL ALASSAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

July 2016

DEDICATIONS

To my greatest heroes in the world
my late father, Hj Nadakkavil Alassan Kunju Ahmad
and
my lovely mother, Napisah Muhammaduni
for their steadfast love and support
This is for both of you

To my siblings and family
For their unconditional love
and helping me grow and bloom

To all my lecturers and teachers
For helping me a lot throughout my study

To all my very wonderful friends
For all your support and encouragement
and gives me great joy

To me
May Allah bless me always

Without whom none of my success would be possible

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman : Khamirul Amin Matori, PhD
Faculty : Science

The physical and chemical properties of bioglass have significance in both fundamental and practical applications such as to be used in bone replacements and dental implants which included excellent osteoconductivity and bioactivity, ability to deliver cells and controllable biodegradability. Hydroxyapatite (HA), which has a similar structure as natural bone is prominent due to its biocompatibility and structure. However, it's not suitable to be used in load bearing applications due to the low mechanical strength. The introduction of the bioglass in the HA can helps to increase the mechanical strength of the HA so that it's able to be used in load bearing application. Melt quenching technique is used to synthesis 45S5 bioglass because it's simple, low cost and applicable in large scale industry. Hence, in this study, the physical and mechanical properties of HA, reinforced with sample glass (SG) and treated glass (TG) at different sintering temperatures have been studied. SG has been prepared by the conventional melt quenching technique with 45S5 type of bioglass composition using 45% SiO_2, 24.5% $CaCO_3$, 24.5% Na_2CO_3 and 6% P_2O_5 as the starting raw materials. Two series of HA reinforced with 45S5 bioglass were produced. The HASG samples were produced by mixing HA and SG according to their weight ratios and followed by pressing them into a pellet form. While, the HATG samples were produced by mixing HA with TG. Whereas, TG is SG sintered at 800 °C. All samples were sintered at 800, 1000, and 1200 °C with a soaking time of 3 hours. All samples under study were tested for density, XRD, FTIR, FESEM and microhardness. The density of SG decreases from 2.26 to 0.44 gcm^{-3} while molar volume increases from 34.99 to 179.36 cm^3mol^{-1} as sintering temperature increased, which might be due to decomposition of carbonate group. Whereas, the density of HA increased from 1.99 to 3.11 gcm^{-3} with an increase in the sintering temperature and molar volume decreased from 252.03 to 162.30 cm^3mol^{-1} with the sintering temperature. The density of both HASG and HATG samples was found decrease with an increase in the SG and TG. The density also decreased with the sintering temperature. The molar volume decreased with increasing in the composition of SG and TG, which also increased with temperature. This might be attributed to the replacement of low density SG with

HA. The XRD results revealed amorphous phase of SG. After SG undergoes sintering process, the crystalline phase of sodium calcium silicate ($Na_2Ca_3Si_6O_{16}$), sodium, calcium phosphate ($NaCaPO_4$) and quartz (SiO_2) was observed. It is evident from the study of HASG and HATG samples that SG behaves more as a sintering aid and promotes the conversion of HA to as β–tetracalcium phosphate (β–TCP) and α–tetracalcium phosphate (α–TCP). The FTIR results revealed the presence of SiO_4, PO_4 vibrations in SG, HASG and HATG samples. In addition, the FESEM analysis revealed that by increasing the sintering temperature, the size of closed pores of SG samples increased, while the Ca/P ratio decreased. The FESEM morphology of the HASG and HATG samples showed irregular shapes of grains and closed pore formation. Smaller grain sizes and closed pores were observed in HATG samples. The incorporation of 45S5 bioglass in HA not only changes the crystal structure of HA but also introduced closed pores in the samples which caused the density and hardness reduced as well. This is due to decomposition of oxide material in the glass system. HA reinforced with 45S5 is suitable material for cancellous bone replacement, but the porosity of the sample not fulfilled the requirement for bone scaffold which is interconnected. Nearly, all the calculated Ca/P ratios were within a range for HA which is 1.3 to 2.0. Microvickers hardness of HASG and HATG increased with the sintering temperature and decreased as the composition of SG and TG is increased. This might be due to a coarser microstructure, crystal growth and porosity formation in the samples. Besides that, the hardness value in the range of 0.05–5.0 GPa shows that it's suitable used in cancellous bone applications. The compressive strength data of HATG were comparable to the cancellous bone which shows the compressive strength of 5–10 MPa.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

SIFAT FIZIKAL DAN MEKANIKAL BAGI HIDROKSIAPATIT DIPERKUKUH DENGAN BIOKOMPOSIT 45S5

Oleh

ZARIFAH BT HJ NADAKKAVIL ALASSAN

Julai 2016

Pengerusi : Khamirul Amin Matori, PhD
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Sifat fizikal dan kimia biokaca mempunyai kepentingan bagi kedua-dua aplikasi asas dan praktikal seperti digunakan dalam tulang gantian dan implan gigi yang merangkumi osteokonduktif dan bioaktiviti yang cemerlang, kebolehan menghantar sel dan biodegradasi terkawal. Hidroksiapatit (HA) yang mempunyai struktur yang sama dengan tulang semulajadi adalah penting oleh kerana keserasian biologi dan strukturnya. Walaubagaimanapun, ia tidak sesuai digunakan dalam aplikasi menahan beban kerana kekuatan mekanikal rendah. Dengan memperkenalkan biokaca ke dalam HA boleh meningkatkan kekuatan mekanikal HA supaya ia boleh digunakan di dalam aplikasi menahan beban. Teknik sepuh lindap digunakan untuk sintesis 45S5 biokaca kerana ia mudah, kos rendah dan dapat digunakan di dalam industri berskala besar. Oleh itu, dalam kajian ini, sifat fizikal dan mekanikal bagi HA yang diperkukuhkan dengan (kaca sampel) SG dan (kaca terawat) TG pada suhu persinteran berbeza telah dikaji. SG telah dihasilkan melalui teknik sepuh lindap konvensional dengan komposisi biokaca 45S5 menggunakan 45% SiO_2, 24.5% $CaCO_3$, 24.5% Na_2CO_3 dan 6% P_2O_5 sebagai bahan asas permulaan. Dua siri sampel hidroksiapatit diperkukuhkan dengan biokaca 45S5 dihasilkan. Sampel HASG dihasilkan dengan mencampurkan HA dengan SG mengikut nisbah beratnya dan diikuti dengan penekanan supaya membentuk pelet. Manakala, sampel HATG dihasilkan dengan mencampurkan HA dengan TG. Yang mana, TG adalah SG yang disinterkan pada 800 °C. Semua sampel disinter pada suhu 800, 1000, dan 1200 °C dengan masa rendaman 3 jam. Semua sampel di bawah kajian diuji untuk. ujian ketumpatan, XRD, FTIR, FESEM dan kekerasan mikro. Ketumpatan bagi SG berkurangan dari 2.26 ke 0.44 gcm^{-3} sementara isipadu molar bertambah dari 34.99 ke 179.36 cm^3mol^{-1} dengan penambahan suhu persinteran yang mana mungkin disebabkan penguraian kumpulan karbonat. Sementara, ketumpatan bagi HA meningkat dari 1.99 ke 3.11 gcm^{-3} dengan penambahan suhu persinteran manakala isipadu molar berkurangan dari 252.03 ke 162.30 cm^3mol^{-1} dengan suhu persinteran. Ketumpatan bagi kedua sampel HASG dan HATG didapati berkurangan dengan penambahan SG dan TG. Ketumpatan juga berkurangan dengan suhu persinteran. Isipadu molar berkurangan

dengan penambahan SG dan TG, yang mana turut meningkat dengan suhu persinteran. Ini mungkin disebabkan penggantian SG yang berketumpatan rendah dengan HA. Keputusan XRD mendedahkan fasa amorfus bagi SG. Setelah SG melalui proses persinteran, fasa hablur iaitu sodium kalsium silikat ($Na_2Ca_3Si_6O_{16}$), sodium kalsium fosfat ($NaCaPO_4$) dan kuarza (SiO_2) dilihat. Bukti kajian dalam sampel HASG dan HATG, menunjukkan SG bertindak sebagai pemangkin persinteran dan menggalakkan penukaran dari HA kepada β–kalsium fosfat (β–TCP) dan α–kalsium fosfat (α–TCP). Keputusan FTIR mendedahkan kehadiran getaran bagi SiO_4, PO_4 di dalam sampel SG, HASG dan HATG. Selain itu, analisis FESEM mendedahkan bahawa dengan peningkatan suhu persinteran, saiz liang tertutup bagi sampel SG meningkat manakala nisbah Ca/P berkurangan. Morfologi FESEM bagi HASG dan HATG sampel menunjukkan bentuk butiran tidak seragam dan pembentukan liang tertutup. Saiz butiran dan liang tertutup yang kecil dapat dilihat di dalam sampel HATG. Dengan penyertaan biokaca 45S5 dalam HA bukan sahaja mengubah struktur hablur HA tetapi juga memperkenalkan. liang tertutup dalam sampel yang menyebabkan ketumpatan dan kekerasan berkurangan juga. Ini adalah disebabkan oleh penguraian bahan oksida dalam sistem kaca. HA diperkukuhkan dengan 45S5 adalah bahan sesuai untuk penggantian tulang kancelus tetapi keliangan sampel tidak memenuhi kelayakan bagi rangka tulang iaitu bersambungan. Hampir kesemua nisbah Ca/P yang dikira berada pada julat bagi HA iaitu di antara 1.3 ke 2.0. Kekerasan vickers mikro bagi sampel HASG dan HATG meningkat dengan suhu persinteran dan berkurangan apabila komposisi SG dan TG meningkat. Ini mungkin kerana mikrostruktur yang kasar, pertumbuhan kristal dan pembentukan keliangan di dalam sampel. Selain itu, nilai kekerasan berada di dalam julat 0.05–5.0 GPa menunjukkan ia sesuai digunakan dalam aplikasi tulang kancelus. Data kekuatan mampatan bagi HATG berpadanan dengan tulang kancelus dengan menunjukkan kekuatan 5–10 MPa.

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I certify that a Thesis Examination Committee has met on 25 July 2016 to conduct the final examination of Zarifah bt Hj Nadakkavil Alassan on her thesis entitled "Physical and Mechanical Properties of Hydroxyapatite Reinforced with 45S5 Biocomposite" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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TABLE OF CONTENTS

			Page
ABSTRACT			i
ABSTRAK			iii
ACKNOWLEDGEMENTS			v
APPROVAL			vii
DECLARATION			ix
LIST OF FIGURES			xiv
LIST OF TABLES			xvii
LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERM			xviii
CHAPTER			
1.	**INTRODUCTION**		1
	1.1	Introduction	1
		1.1.1 Biomaterials/Bioceramic	1
		1.1.2 Bioglass	2
		1.1.3 Hydroxyapatite	3
	1.2	Problem Statements	3
	1.3	Objectives of the study	4
	1.4	Scopes of the study	5
		Outline of the thesis	5
2.	**LITERATURE REVIEWS**		6
	2.1	Biomaterials/Bioceramic	6
	2.2	Scaffold in tissue engineering	9
	2.3	Historical development of bone regeneration	10
	2.4	Bioglass	11
	2.4	HA	14
	2.6	HA with Bioglass	17
3.	**METHODOLOGY**		20
	3.1	Sample preparation	20
		3.1.1 Sample Glass (SG)	20
		3.1.2 Hydroxyapatite (HA)	22
		3.1.3 Hydroxyapatite reinforced with 45S5 bioglass (HASG)	22
		3.1.4 Hydroxyapatite reinforced with sintered 45S5 Bioglass (HATG)	24
	3.2	Sample characterization	26
		3.2.1 Density	26
		3.2.2 Molar volume	26
		3.2.3 X-ray diffraction Measurement (XRD)	26
		3.2.4 Fourier Transform Infrared Spectroscopy (FTIR)	27
		3.2.5 Field emission scanning electron microscope (FESEM) and Energy X-ray (EDX) analysis	27
		3.2.6 Micro Vickers Hardness (H_v)	28

	3.2.7	Compressive Test	28
4	**RESULTS AND DISCUSSION**		29
4.1	Densities and Molar volume		29
	4.1.1	Sample Glass (SG)	29
	4.1.2	Hydroxyapatite (HA)	31
	4.1.3	Hydroxyapatite reinforced with 45S5 bioglass (HASG)	32
	4.1.4	Hydroxyapatite reinforced with sintered 45S5 Bioglass (HATG)	34
4.2	X-ray diffraction Measurement (XRD)		37
	4.2.1	Sample Glass (SG)	37
	4.2.2	Hydroxyapatite (HA)	40
	4.2.3	Hydroxyapatite reinforced with 45S5 bioglass (HASG)	42
	4.2.4	Hydroxyapatite reinforced with sintered 45S5 Bioglass (HATG)	53
4.3	Fourier Transform Infrared Spectroscopy (FTIR)		61
	4.3.1	Sample Glass (SG)	61
	4.3.2	Hydroxyapatite (HA)	65
	4.3.3	Hydroxyapatite reinforced with 45S5 bioglass (HASG)	67
	4.3.4	Hydroxyapatite reinforced with sintered 45S5 Bioglass (HATG)	74
4.4	Field emission scanning electron microscope (FESEM) and Energy X-ray (EDX) analysis		78
	4.4.1	Sample Glass (SG)	78
	4.4.2	Hydroxyapatite (HA)	81
	4.4.3	Hydroxyapatite reinforced with 45S5 bioglass (HASG)	83
	4.4.4	Hydroxyapatite reinforced with sintered 45S5 Bioglass (HATG)	90
4.5	Micro Vickers Hardness		96
	4.5.1	Hydroxyapatite (HA)	96
	4.5.2	Hydroxyapatite reinforced with 45S5 bioglass (HASG)	97
	4.5.2	Hydroxyapatite reinforced with sintered 45S5 Bioglass (HATG)	98
4.6	Compressive Strength		99
	4.6.1	Sample Glass (SG)	99
	4.6.2	Hydroxyapatite (HA)	100
	4.6.3	Hydroxyapatite reinforced with 45S5 bioglass (HASG)	101
	4.6.4	Hydroxyapatite reinforced with sintered 45S5 Bioglass (HATG)	102

5. CONCLUSION AND SUGGESTIONS FOR FUTURE WORKS 103
5.1 Conclusion 103
5.2 Suggestion for future work 106

REFERENCES 107
APPENDIX 117
BIODATA OF STUDENT 118
LIST OF PUBLICATIONS 119

LIST OF FIGURES

Figure		Page
2.1	Clinical applications of bioceramics.	8
2.2	Illustration of porous features of porous biomaterial.	9
2.3	The images of pores (a) closed pores (b) open pores.	14
3.1	Flowchart preparation of SG sample.	21
3.2	Flowchart preparation of HASG sample.	23
3.3	Flowchart preparation of HATG sample.	25
4.1	Density and molar volume of SG at different sintering temperatures.	30
4.2	Density and molar volume of HA at different sintering temperatures.	32
4.3	Densities of HASG with variations of SG composition (wt.%) at different sintering temperatures.	33
4.4	Molar volume of HASG with variations of SG composition (wt.%) at different sintering temperature.	34
4.5	Densities of HATG with variations of TG composition (wt.%) at different sintering temperatures.	36
4.6	Molar volume of HATG with variations of TG composition (wt.%) at different sintering temperatures.	36
4.7	XRD spectrum of SG at different sintering temperatures [(a) before sintering (b) 800 ℃ (c) 1000 ℃ and (d) 1200 ℃].	39
4.8	XRD spectrum of HA at different sintering temperature [(a) before sintering (b) 800 ℃ (c) 1000 ℃ and (d) 1200 ℃].	41
4.9	XRD spectra of HASG20 at different sintering temperatures [(a) before sintering (b) 800 ℃ (c) 1000 ℃ and (d) 1200 ℃].	43
4.10	XRD spectra of HASG40 at different sintering temperatures [(a) before sintering (b) 800 ℃ (c) 1000 ℃ and (d) 1200 ℃].	44
4.11	XRD spectra of HASG60 at different sintering temperatures [(a) before sintering (b) 800 ℃ (c) 1000 ℃ and (d) 1200 ℃].	45
4.12	XRD spectra of HASG80 at different sintering temperatures [(a) before sintering (b) 800 ℃ (c) 1000 ℃ and (d) 1200 ℃].	46
4.13	XRD spectra of HASG with different compositions of SG before sintering.	49
4.14	XRD spectra of HASG with different compositions of SG sintered at 800 ºC.	50
4.15	XRD spectra of HASG with different compositions of SG sintered at 1000 ºC.	51
4.16	XRD spectra of HASG with different compositions of SG sintered at 1200 ºC.	52
4.17	XRD spectra of HATG20 at different sintering temperatures [(a) before sintering (b) 800 ℃ (c) 1000 ℃ and (d) 1200° C].	54

4.18	XRD spectra of HATG40 at different sintering temperatures [(a) before sintering (b) 800 ℃ (c) 1000 ℃ and (d) 1200 °C].	55
4.19	XRD spectra of HATG60 at different sintering temperatures [(a) before sintering (b) 800 ℃ (c) 1000 ℃ and (d) 1200 °C].	56
4.20	XRD spectra of HATG with different TG composition sintered at 800 ºC.	58
4.21	XRD spectra of HATG with different TG composition sintered at 1000 ºC.	59
4.22	XRD spectra of HATG with different TG composition sintered at 1200 ºC.	60
4.23	FTIR spectra of SG at different sintering temperatures [(a) before sintering (b) 800 °C (c) 1000 °C and (d) 1200 °C].	64
4.24	FTIR spectra of HA at different sintering temperatures [(a) before sintering (b) 800 °C (c) 1000 °C and (d) 1200 °C].	66
4.25	FTIR spectra of HASG with different SG composition before sintering.	70
4.26	FTIR spectra of HASG with different SG composition sintered at 800 °C.	71
4.27	FTIR spectra of HASG with different SG composition sintered at 1000 °C.	72
4.28	FTIR spectra of HASG with different SG composition sintered at 1200 ºC.	73
4.29	FTIR spectra of HATG with different TG composition sintered at 800 °C.	75
4.30	FTIR spectra of HATG with different TG composition sintered at 1000 °C.	76
4.31	FTIR spectra of HATG with different TG composition sintered at 1200 °C.	77
4.32	FESEM micrographs and EDX spectra with calculated Ca/P ratio of SG at different sintering temperatures [(a) 800 ℃ (b) 1000 ℃ (c) 1200 °C].	80
4.33	FESEM micrographs and EDX spectra with calculated Ca/P ratio of HA at different sintering temperatures [(a) 800 ℃ (b) 1000 ℃ (c) 1200 °C].	82
4.34	FESEM micrographs and EDX of HASG20 after sintering at 800-1200 °C for 3 hours.	85
4.35	FESEM micrographs and EDX of HASG40 after sintering at 800-1200 °C for 3 hours.	86
4.36	FESEM micrographs and EDX of HASG60 after sintering at 800-1200 °C for 3 hours.	87
4.37	FESEM micrographs and EDX of HASG80 after sintering at 800-1200 °C for 3 hours.	88
4.38	Images of air traps (closed pores) observed in HASG samples at 100x magnification.	89

4.39 FESEM micrographs and EDX spectra with calculated Ca/P ratio of HATG20 at different sintering temperatures [(a) 800 °C (b) 1000 °C (c) 1200 °C]. 92
4.40 FESEM micrographs and EDX spectra with calculated Ca/P ratio of HATG40 at different sintering temperatures [(a) 800 °C (b) 1000 °C (c) 1200 °C]. 93
4.41 FESEM micrographs and EDX spectra with calculated Ca/P ratio of HATG60 at different sintering temperatures [(a) 800 °C (b) 1000 °C (c) 1200 °C]. 94
4.42 Images of closed pores observed in HATG samples at low magnification. 95

LIST OF TABLES

Table		Page
1.1	Scaffold design parameters for bone tissue engineering.	2
2.1	Ceramic processing methods.	7
2.2	Class of material used in body and parameter to be used in tissue engineering.	7
2.3	Types and composition of bioglass.	13
3.1	HASG samples nominal composition and abbreviation.	22
3.2	HATG samples nominal composition and abbreviation.	24
4.1	Crystalline phase of SG at different sintering temperature.	38
4.2	Crystalline phases of HASG at different composition and temperature.	48
4.3	Crystalline phases of HATG at different composition and temperature.	61
4.4	Vibration modes of SG at different IR wave numbers.	63
4.5	Vibration modes of HA at different IR wave numbers.	65
4.6	Vibration modes of HASG at different IR wave numbers.	69
4.7	Vibration modes of HATG at different IR wave numbers.	74
4.8	Ca/P ratio value of HASG at different composition and sintering temperature.	90
4.9	Ca/P ratio value of HATG at different composition and sintering temperature.	95
4.1	The Hv value of HA at different sintering temperature.	96
4.11	Hv of HASG at different sintering temperature at variation SG composition.	98
4.12	Hv of HATG at different sintering temperature and variation TG composition.	99
4.13	Compressive strength of SG at different sintering temperature.	100
4.14	Compressive strength of HA at different sintering temperature.	100
4.15	Compressive strength of HASG at different sintering temperature and SG composition.	101
4.16	Compressive strength of HATG at different sintering temperature and TG composition.	102

LIST OF ABBREVIATIONS AND SYMBOLS

EDX	Energy dispersive x-ray spectrometer
FESEM	Field emission scanning electron microscope
FTIR	Fourier transform infrared spectroscopy
HA	Hydroxyapatite
HASG	Hydroxyapatite reinforced with 45S5 bioglass
HATG	Hydroxyapatite reinforced with treated glass
H_v	Microvickers hardness
ICDD	International Center Diffraction Data
IR	Infrared
JCPDS	Joint Committee on Powder Diffraction Standards
mol%	Mol percentage
SG	Sample glass
TCP	Tricalcium phosphate
TG	Treated glass
UATR	Universal Attenuated Total Internal Reflection
wt. %	Weight percentage
XRD	X-ray diffraction
α–TCP	α-tricalcium phosphate
β–TCP	β-tricalcium phosphate

CHAPTER 1

INTRODUCTION

1.1 Introduction

1.1.1 Biomaterials/ Bioceramic

An inorganic compound that consists of metallic and non–metallic materials, which harden at high temperatures, is defined as ceramics. They can be single crystal, polycrystalline, glass, ceramic or composites. It is a widely known fact that glasses and ceramics have been widely used outside the body for various applications. They are commonly used in large industrial applications such as housewares, automotive industries, building's construction, chemical wares and health care industries. Nowadays, ceramic can also be employed in the body as implants and dental applications. Ceramic is widely used as a restorative material such as gold, porcelain crowns, glass-filled ionomer cements and dentures.

Bioceramics are ceramic materials that are used in medical and dental applications such as, repair and reconstruction of diseased or damaged parts of the body (Rukiye, 2000). Bioceramic is a type of biomaterial that is produced in a variety of forms and phases and serves in different applications within a human body. A biocompatible ceramic is composed of calcium and phosphate such as hydroxyapatite (HA) or tricalcium phosphate (TCP). It is either intended for a permanent replacement, such as, coating gliding surfaces to reduce wear in prosthetic joints, or as a temporary structure, as in the case of bioresorbable pins, plates and screws.

Various studies have been conducted for the design and construction of engineering scaffolds for the regeneration of different tissues with natural materials and artificial, or a combination of them. Any material that is prone to the purpose for repair and reconstruction of lost, damaged or deceased tissue can be referred as biomaterials (Seeram *et al.*, 2004). According to William, "biomaterial is a material that is used in implants or medical devices, designed to interact with the biological systems" (Hench, 2013).

In order to be used for medical application, the material must possess lots of specific characteristics whose fundamental requirements are related to a biocompatibility. The compatible materials are considered as biomaterials due to their biocompatibility, which is a descriptive term and indicates an ability of a material to perform the appropriate host response, in a particular application (Seeram *et al.*, 2004).

In order to form an ideal scaffold that can be used in bone tissue engineering, it requires certain criteria as follows; an ability to deliver cells, excellent osteoconductivity, good biodegradability, appropriate mechanical properties, which include an extremely porous structure with porosity > 90%. Moreover, it must also possess an ability of irregular shape fabrication, and a commercialization potential (Chen *et al.*, 2006). Table 1.1 summarized important scaffold design parameters.

Table 1.1: Scaffold design parameters for bone tissue engineering (Chu and Liu, 2008).

Parameters	Requirements
Porosity	Maximum possible without comprising mechanical properties
Pore size	200-400 μm
Pore structure	Interconnected
Mechanical properties of the cancellous bone	
Tension and compression	Strength: 5-10 MPa
Hardness	0.05-5 GPa
Mechanical properties of the cortical bone	
Tension	Strength: 80-150 MPa
Compression	Strength: 130-220 MPa
	Fracture toughness: 6-8 $MPam^{1/2}$
Hardness	7-30 GPa

1.1.2 Bioglass

The basic component of bioglass is composed of SiO_2, Na_2O, CaO and P_2O_5. The 45S5 bioglass is a type of bioglass which consists of 45% SiO_2, 24.5% $CaCO_3$, 24.5% Na_2CO_3 and 6% P_2O_5. The glass is known as bioactive based on its definition by Hench, "a bioactive material is one that elicits a specific biological response at an interface of a material that results in a formation of a bond between the tissues and material" (Hench, 1993).

Glass is an inorganic substance that is produced by melting several minerals together at high temperature and cooling the molten to its solid state through its glass transition temperature without crystallizing them. Bioglass is different from glass ceramic due to its possibilities to control a range of chemical properties and rate of bonding with the tissues.

Bioglass, which consists of basic components such as SiO_2, Na_2O, CaO and P_2O_5 is known to have the most stimulatory effect on bone cell function (El-Ghannam, 2004). Moreover, the fabrication techniques for bioglass include both traditional melting methods and sol gel techniques. At first, the bioglass was used in a form of substitute for small solid bone that was used in a middle of ear surgery. Not long after that, bioglass was also used in other applications such as in periodontology, endodontology or as coatings on metallic orthopedic implants. Recently, bioglass has been considered as one of the potential material in tissue engineering and regenerative medicine (Boccaccini *et al.*, 2010).

Bioglass has gained attention of many researchers due to its unique characteristics such as: relatively low softening temperature that can be used as a sintering aid which is required during sintering to bond ceramic particles and fill the micropores. Besides that, bioglass also has the ease of compositional design based on properties unique to a particular clinical applications. Its also have a wide range controllability of chemical properties and rate of bonding with tissues and a rapid rate of surface reaction that leads to their direct attachment to bone via a chemical bond (Balamurugan *et al.*, 2007).

1.1.3 Hydroxyapatite

The chemical formula of HA is $Ca_5(PO_4)_3(OH)$, which is a form of calcium phosphate. It is also written as $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell is comprised of two entities. This material has a similar structure as natural bone mineral that's why it has been used as a bone substitute because of its biocompatibility and structural properties (El-Ghannam, 2004). Almost, 70% of the biological apatite is found in bones by weight.

HA has been classified as one of the best biocompatible and bioactive material, which has many biological applications such as, bone repair scaffolds. Besides that, it also possesses several advantages such as, it is found to be osteoconductive, which enhances the growth of bone cells (Maryam and Fathi, 2012). Furthermore, when implanted in vivo, the presence of HA can also induce osteogenesis because of its osteoinductive and bone bonding ability (Deplaine *et al.*, 2010). A bone graft material that is osteoconductive and osteoinductive does not only serve as a scaffold for currently existing osteoblasts, but it also triggers the formation of new osteoblasts, theoretically promoting the faster integration of a graft.

HA is entirely compatible with a body because when exposed to body fluids, HA bonds to bones by forming indistinguishable unions. This bond begins with a formation of carbonate apatite crystals in bone, where it promotes the adhesion of matrix–producing cells and organic molecules due to a surface chemistry and surface charges (Racquel and John, 1993). However, HA is unsuitable for load bearing applications. This is due to low tensile strength and fracture toughness compared to natural bone which gives drawback to HA derived implants.

In this study, reinforcement of HA with an incorporation of 45S5 system perhaps is a suitable choice for improving its mechanical properties so that's its able to be used as bone generation scaffolding. This research is focused on the improving the physical and mechanical properties so that's its able to be used as bone scaffold.

1.2 Problem Statements

According to the World Health Organization (WHO), an estimated 20 to 50 million people sustain an injury and most of them suffer permanent injury level due to road accidents ("World report on road traffic injury prevention," 2015). Most of the injuries in vehicle accidents involve broken bones and fractures. These broken bones sustained in any vehicle accident can be more severe than in a fall or sports accident. People who

suffer fractures in car accidents often require surgery and the victim may require reconstructive surgery involving hardware to secure the bones. With a recent advancement in the field of biomaterials can be used as a bone replacement.

HA has been classified as one of the best biomaterials. HA possesses a similar structure as a natural bone mineral that is why, it has been used as a bone substitute (El-Ghannam, 2004). Due to this particular property, they can be used as implant materials in the human body to replace and/or repair diseased or damaged bone. However, the drawback of hydroxyapatite in scaffold engineering is not suitable to be used in load bearing applications due to their properties which is brittleness and low mechanical strength compared to bone. The low mechanical strength is due to porosity, grain size and amorphous phase (Valeri and Aleksandra, 2012). This can be improve by reinforced with several filler such as polymers (collagen), metals and inorganic materials (carbon nanotubes) (Valeri and Aleksandra, 2012). Even so, combining HA with polymer may mask the osteoinductive properties of HA itself. Nevertheless, it only can be attempt for dense type of materials only. Availability of HA in porous form encourages the extensive use of these biomaterials to serve as tissue engineering scaffolds for cells (El-Ghannam, 2004; Maria *et al.*, 2000). Porous HA can be develop by salt leaching, gas foaming, phase separation, freeze-drying and sintering. Unfortunately, the fabrication only focusing the open porosity without taking account the closed pores and it's also decreased the mechanical strength of HA.

Bioglass is a silica based glass that binds to bone more efficiently. It is a synthetic amorphous material with high biocompatibility (Mistry *et al.*, 2011). Due to this particular property, it can be used as an implant material in a human body to replace and/or repair diseased or damaged bone in orthopedic, cranio–maxillao facial and periodontal surgeries as well as a filling material for human teeth (Mistry *et al.*, 2011).

The use of HA in load bearing parts can be explored by provided the strength and toughness of HA by reinforcement with 45S5 bioglass. Despite the fact bioglass is brittle, the brittleness of the glass can be improved by sintering process. High sintered density and ultra fine particles will ensure leading to improve mechanical properties of the composites via dispersion strengthening. The solid state method is chosen as the method of synthesis of HA reinforced with bioglass due to their simplicity and low cost production. Besides that, its offer large scale production, which saves energy and time.

Therefore, this research has focused on the fabrication of bioceramic composite materials via solid state method using HA and 45S5 bioglass to be used as tissue engineering scaffolds. In this study, the reinforcement of HA with the incorporation of glasses within the SiO_2–$CaCO_3$–Na_2CO_3–P_2O_5 glass system is a suitable choice for improving physical, mechanical and microstructure properties.

1.3 Objectives of the study

The major part of this research deals with a characterization of SG, HA, HA reinforced with SG and HA reinforced with TG. The main objectives of this research are summarized as follows:

1. To synthesize a sample glass (SG) based on 45S5 composition: SiO_2–$CaCO_3$–Na_2CO_3–P_2O_5 through melting and water quenching technique.
2. To determine the effect of sintering on the physical, structural, and mechanical properties of SG.
3. To investigate the impact of the SG and TG on the physical, structural, and mechanical properties of HA.
4. To examine the effect of sintering on the physical, structural, and mechanical properties of HA reinforced with SG and TG.

1.4 Scopes of the study

The melt quenching and thermal treatment technique is used in this study. The research has been focused on the physical and mechanical properties of HA reinforced with 45S5 bioglass prepared using melt quenching technique. The research is done in order to achieve optimum physical and mechanical properties of the sample by excluding the bioactivity study such as invivo and invitro test. The SG samples was prepared based on 45S5 compositions: 45% SiO_2, 24.5% $CaCO_3$, 24.5% Na_2CO_3 and 6% P_2O_5 using conventional solid state method through water quenching and followed by sintering at 800, 1000 and 1200 °C for 3 hours. The HASG sample was prepared by mixing SG with HA at 20, 40, 60 and 80 wt.%, which was followed by sintering at 800, 1000 and 1200 °C for 3 hours. TG was prepared by sintering SG at 800 °C for 3 hours. The HATG sample was prepared by mixing TG with HA at 20, 40, 60 and 80 wt.% and followed by sintering at 800, 1000 and 1200 °C for 3 hours. The density of the samples was measured by density meter, with ethanol as immersion–liquid while; molar volume is calculated based on density and molecular weight of the samples. The structure of the samples was measured using x-ray diffraction technique to study the phase and crystal structure of the samples. In order to evaluate the bonding structure of the samples, FTIR spectroscopy was used in this study. The surface morphology and microstructure of samples were analyzed using Field emission scanning electron microscopy (FESEM) while the chemical composition was detected by energy dispersive x–ray spectrometer (EDX) and Ca/P ratio value of the samples was determined by Ca and P ratio. The micro Vickers hardness test was used to determine the hardness of samples.

1.5 Outline of thesis

This thesis is structured as follows: Chapter 1 gives an introduction of biomaterials, bioglass and HA. The previous works, including the past and current literature of bioglass and HA with bioglass, is covered in Chapter 2. In Chapter 3, the methodologies employed for the preparations and characterization of the SG, HA, HASG and HATG are discussed. The results concerning the effect of SG and TG on physical, structural, mechanical properties of HA are analyzed and discussed in Chapter 4. The conclusion and suggestions for future works are given in Chapter 5.

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