SYNTHESIS AND CHARACTERIZATION OF BIODEGRADABLE AND THERMORESPONSIVE POLY(1,8-OCTANEDIOL-GLYCEROL-1,12-DODECANEDIOATE) AND ITS COMPOSITES FOR TISSUE ENGINEERING APPLICATIONS

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DEDICATION

To my family for their love and support

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ABSTRACT

Biodegradable polyesters, poly(1,8-octanediol–glycerol–1,12-dodecanedioate)s (POGDA)s, were synthesized via polycondensation polymerization method without solvent and catalyst and with different monomer molar ratios. Formation of POGDA was confirmed with structural analysis by Fourier transform infrared spectroscopy. The effects of varying the monomer molar ratio on POGDA properties were illustrated in the gel content and swelling analysis, ultraviolet–visible spectroscopy, thermal gravimetric analysis, differential scanning calorimetry, x-ray diffraction, tensile test and *in vitro* degradation tests. *In vitro* degradation tests were performed in phosphate-buffered solution at 37 °C for 60 days. Thermoresponsive behavior was revealed by POGDA with 0.5 mole ratio of glycerol (Gly), and bending tests were performed to study the shape memory effect. *In vitro* cytotoxicity tests and cell proliferation tests suggested that these POGDA polymers have potential applications in biomedical fields such as tissue engineering. POGDA (0.5 Gly) loading with hydroxyapatite (HA) were prepared using similar polycondensation polymerization method. Addition of HA improved the mechanical properties and decreased the degradation rate of the composites. Scaffolds were fabricated from POGDA/HA composites using solvent-free salt leaching method. Pore structure in scaffold was visualized with field emission scanning electron microscope and porosities were measured using liquid displacement test. The porous scaffolds fabricated from POGDA/HA composites were found to exhibit thermoresponsive behavior. The cell proliferation tests suggested that the scaffolds have good biocompatibility with fibroblast cells and have potential to be used in tissue engineering.

ABSTRAK

Poliester terbiodegradasi, poli(1,8-oktanadiol-gliserol-1,12-dodekanadioat) (POGDA) telah disintesis dengan kaedah pempolimeran polikondensasi tanpa pelarut dan mangkin dan dengan nisbah molar monomer yang berbeza. Penghasilan POGDA telah disahkan melalui analisis struktur menggunakan spektroskopi transformasi infra merah Fourier. Kesan nisbah molar monomer yang berbeza terhadap sifat-sifat POGDA telah digambarkan dalam analisa kandungan gel dan pembengkakan, spektroskopi nampak-ultraungu, analisa gravimetrik terma, kalorimeter imbasan pembezaan, pembelauan sinar-x, ujian tegangan dan ujian degradasi *in vitro*. Ujian degradasi *in vitro* telah dijalankan dalam larutan penimbal fosfat selama 60 hari pada suhu 37 °C. Sifat responsif terma telah ditunjukkan oleh POGDA dengan 0.5 nisbah mol gliserol (Gly) dan ujian lenturan telah dijalankan untuk mengkaji kesan bentuk ingatan. Ujian kesitotoksikan *in vitro* dan ujian percambahan sel mencadangkan bahawa polimer POGDA mempunyai potensi aplikasi dalam bidang bioperubatan seperti kejuruteraan tisu. Muatan POGDA (0.5 Gly) dengan hidroksiapatit (HA) telah disediakan dengan kaedah pempolimeran polikondensasi yang sama. Penambahan HA telah meningkatkan sifat-sifat mekanik dan mengurangkan kadar degradasi komposit. Perancah telah diperbuat daripada komposit POGDA/HA menggunakan kaedah garam larut lesap tanpa pelarut. Struktur liang dalam perancah digambarkan dengan mikroskop elektron imbasan pancaran medan dan keliangan diukur dengan ujian sesaran cecair. Perancah berliang yang diperbuat daripada POGDA/HA komposit didapati mempamerkan sifat responsif terma. Ujian percambahan sel mencadangkan bahawa perancah mempunyai biokeserasian yang baik dengan sel fibroblas dan berpontensi untuk digunakan dalam kejuruteran tisu.

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LIST OF ABBREVIATIONS

AF	-	Annulus fibrosus
APS	-	Poly(1,3-diamino-2-hydroxypropane-*co*-polyol sebacate)
ASTM	-	American Standard Testing Method
BPE	-	Poly(propylene sebacate)
CIP	-	Ciprofloxacin
CO_2	-	Carbon dioxide
DA	-	Dodecanedioic acid
DCF	-	Dichlorofluorescein
DCP	-	Dicumyl peroxide
DIA-DA	-	Poly(dimer acid-dodecanedioic acid)
DMEM	-	Dulbecco's modified eagle medium
DMSO	-	Dimethyl sulfoxide
DSC	-	Differential scanning calorimeter
DTG	-	Differential thermogravimetric
ECM	-	Extracellular matrix
EF	-	Equifunctional
FBS	-	Fetal bovine serum
FESEM	-	Field emission scanning electron microscope
FTIR	-	Fourier Transform infrared spectroscopy
Gly	-	Glycerol
HA	-	Hydroxyapatite
HAEC	-	Endothelial cells
HASMC	-	Human aortic smooth muscle cells
HD	-	Hydroxyl-dominant

HDPE/ UHMWPE	-	High-density polyethylene/ultrahigh molecular weight polyethylene
HSF	-	Human skin fibroblast
ISO	-	International Organization for Standardization
L 292	-	Mouse fibroblast cells
MSDS	-	Material safety data sheet
MTT	-	(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
MWCNT	-	Multi-walled carbon nanotube
min	-	Minute
NaOH	-	Sodium hydroxide
Oct	-	1, 8-Octanediol
PBS	-	Phosphate-buffered saline
PCL	-	Polycaprolactone
PCLF	-	Poly(3-caprolactone fumarate)
PDLA	-	Poly-D-lactide
PEO	-	Poly(ethylene oxide)
PEEK	-	Poly(ether-ether-ketone)
PenStrep	-	Penicillin streptomycin
PGA	-	Polyglycolide
PGD	-	Poly(glycerol dodecanoate)
PGS	-	Poly(glycerol sebacate)
PGSC	-	Poly(glycerol-sebacate-citrate)
PHBV	-	Poly(hydroxybutyrate-*co*-hydroxyvalerate)
PLA	-	Polylactide
PLGA	-	Poly(lactic-co-glycolic acid)
PLLA	-	Poly-L-lactide
PMMA	-	Poly(methyl methacrylate)
POC	-	Poly(1,8-octanediol-co-citric acid)
POCS	-	Polyoctanediol citrate/sebacate
POGDA	-	Poly(1,8-octandiol-glycerol-dodecanedioate)
POM	-	Poly(1,8-octanediol malate)
PPSC	-	Poly((1,2-propanediol-sebacate)-citrate)

PSCS	-	Poly(sorbitol citric sebacate)
PSTS	-	Poly(sorbitol tartaric sebacate)
PTFE	-	Polytetrafluoroethylene
PUUSD	-	Polyurethaneurea
PXS	-	Poly(xylitol sebacate)
ROP	-	Ring opening polymerization
SEM	-	Scanning electron microscope
SMP	-	Shape memory polymer
TG	-	Thermogravimetric
TGA	-	Thermogravimetric analysis
THF	-	Tetrahydrofuran
UV	-	Ultraviolet
wt	-	Weight
XRD	-	X-ray diffraction
3D	-	3 Dimensional

LIST OF SYMBOLS

cm^2	-	Centimeter square
°C	-	Degree Celsius
ΔH_c	-	Enthalpy of crystallization
ΔH_m	-	Enthalpy of melting
GPa	-	Gigapascal
g/mol	-	Gram per mole
kV	-	Kilovoltage
MPa	-	Megapascal
µl	-	Microliter
µm	-	Micrometer
ml	-	Millimeter
Mw	-	Molecular weight
nm	-	Nanometer
n	-	Number of specimen for each formulation
T_b	-	Boiling temperature
T_c	-	Crystallization temperature
T_g	-	Glass transition temperature
T_m	-	Melting temperature
T_{trans}	-	Transition temperature
%	-	Percentage
ε	-	Elongation at break
σ	-	Tensile strength
E	-	Young's modulus

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Treatment of damaged organs or tissues using traditional therapy such as organ transplants often encounter problems associated with donor site shortage, complex surgery, immunogenicity, enormous expenses and post-operative care have limit the use of this method (Cohen *et al.*, 1993). Therefore, a new therapeutic solution termed regenerative medicine has been explored to accelerate the proliferation and differentiation of cells for regeneration of tissues and organs (Tabata, 2005). Hence, a local in vivo environment of artificial extracellular matrix (ECM) which suitable for cell to proliferate and differentiate is necessary. Tissue engineering is designed to build up such environment for cell proliferation and differentiation to regenerate natural tissues or to create biological substitutes for defective of lost organs (Tabata, 2005;Hosseinkhani and Hosseinkhani, 2009). In tissue engineering, biomaterial is used to prepare an artificial scaffold as ECM for cell proliferation and differentiation. Most tissue engineering strategies based on the principal that cells seeded into three dimensional (3D) scaffolds will be re-assemble into functional structures which resembling native tissues (Shi *et al.*, 2010).

Biomaterial is defined by the National Institutes of Health Consensus Development Conference of November 1982 as “any substances (other than drug) or combination of substances, synthetic or natural in origin, which can be used to for any period of time, as a whole or as a part of system which treat, augments, or replace any tissue, organ, or function of the body” (Boretos and Eden, 1984). Common synthetic biomaterials for biomedical application included metals and alloy, polymers, and ceramic. Polymer-based biomaterials have been developed since 1920s (Moukwa, 1997). Market research reports by MarketsAndMarkets has indicated that the biomaterials market will be worth US$149.17 billion by 2021. Polymeric biomaterials are the fastest growing field in biomaterials. Biodegradable and biocompatible polymers have been considered as replacements for permanent prosthetic devices used for temporary treatments (Nair and Laurencin, 2007;Erbel *et al.*). The development of a new generation of synthetic biodegradable polymers is underway and being driven by the latest biomedical technologies, such as tissue engineering and controlled drug delivery (Griffith, 2000;Furth *et al.*, 2007;Garg *et al.*, 2012).

Polymeric biomaterials market contains only a small fraction of degradable polymers, although the consumption of polymeric biomaterials is more than 8000 kilotons annually (Lendlein *et al.*, 2011;Yin and Luan, 2016). Public concerns about the environment have created an interest in biodegradable polymeric biomaterials synthesized from renewable resources. Attention has been paid to biodegradable polyesters that are easily produced from renewable resources such as polylactic acid and poly(glycerol sebacate) (Liu and Ma, 2004;Sundback *et al.*, 2005;Shah Mohammadi *et al.*, 2014). Polyesters are hydrolytically degradable and offer a wide range of properties that can be achieved through the manipulation of monomers and synthesis conditions (Bruggeman *et al.*, 2008b;Lei *et al.*, 2007b).

Biodegradable polymers with smart functions besides biodegradable, such as thermoresponsivity, have been reported in the area of petroleum-based and bio-based polymers including poly(ε-caprolactone) (PCL), polyurethanes, propylene sebacate, etc. (Ajili *et al.*, 2009;Guo *et al.*, 2011;Yang *et al.*, 2013b;Alvarado-Tenorio *et al.*, 2015;Li *et al.*, 2015a). However, only few thermoresponsive polyesters synthesized

from renewable resources have been reported, including poly(glycerol-sebacate) (PGS) (Cai and Liu, 2008), poly(glycerol dodecanoate) (PGD) (Migneco *et al.*, 2009) and polydiolcitrate (Serrano *et al.*, 2011). For thermally responsive or shape memory polymers (SMP), two shapes are formed in the shape memory process, one permanent shape and another temporary shape. Hard segments determine the permanent shape of a polymer, whereas soft segments determine the temporary shape. Shapes can be switched at the transition temperature (T_{trans}). Deformation occurs above T_{trans} and cooling below T_{trans} allows a SMP to obtain a temporary shape. The permanent shape of a SMP can be recovered through heating to above T_{trans}. The glass-transition temperature (T_g) or melting temperature (T_m) can be a T_{trans}, depends on the type of polymer.

In this research, biodegradable polyester, poly(1,8-octanediol-glycerol-1,12-dodecanedioate) (POGDA) with thermoresponsive behaviour were successfully synthesized from 1,8-octanediol (Oct), glycerol (Gly) and 1,12-dodecanedioic acid (DA) via solvent- and catalyst-free polycondensation polymerization method. The degree of crosslinking and crystallinity was manipulated through variation of the monomer molar ratios. Based on the material properties analysis, POGDA (0.5 Gly) was selected to load with hydroxyapatite (HA) particles to produce POGDA/HA composites. The effects of HA concentration on the properties of the composites were investigated through a series of tests. Fabrication of scaffolds was carried out using salt-leaching method. Conventional salt-leaching method involves usage of solvent to dissolve polymer and mix with sieved salt. The use of solvent in salt-leaching method was eliminated in this research to reduce the toxicity of the scaffolds.

1.2 Problem Statement

Biomedical application demands materials with specific physical, chemical, mechanical, biodegradability and biocompatibility properties for efficient therapy. Thus, the important of developing diverse biodegradable polymers is increasing in the advancement of biomedical materials since the market has contains only a small fraction of biodegradable polymer (Yin and Luan, 2016). Public concern and critical discussion about the preservation of natural resources and environment have led to the rise of interest on biomaterials with the focus on renewable raw materials. Consequently, polyester which can be synthesized by monomers from renewable resources has attracted huge attention in the biomaterials field. To increase the biocompatibility of the polyester with the host, monomers with low toxicity and can be cleared from body after degraded are favourable to synthesize a new polyester.

Besides biodegradable and biocompatible, an ideal implant with extra functionality to meet specific demands is desired. A polymer with thermoresponsive behaviour will serves as an ideal biomaterial for implantation. Thermoresponsive polymers show shape memory effect by their ability to change shape in different temperature. During implantation, thermoresponsive polymers able to pass through a small incision and regain its original shape at desired conditions. In current works, most of the thermoresponsive polymers are petroleum-based polymers and bio-based polymers. Only few polyester synthesized from renewable resources thermoresponsive behaviours are reported. Unfortunately, these polyesters including PGS, PGD and polydiolcitrate have T_{trans} below 30 °C which is far below body temperature and early shape recovery might occurs before implantation.

Therefore, a thermoresponsive biodegradable polyester synthesized from monomers that can be produced from renewable resources and low in toxicity is needed in current market. The monomers used in the present research were chosen to meet these criteria. Gly is mainly produced from renewable resources as a byproduct during the transesterification of vegetable oils and animal fat in the production of

biodiesel (Solomon *et al.*, 1995;Demirbaş, 2003). DA can also be produced from renewable resources, such as vernonia galamensis oil (Ayorinde *et al.*, 1989). Introduction of Oct into the polymer network was decided based on previous studies on PGD and polydiolcitrate. PGD showed T_{trans} below 30 °C. In study of polydiolcitrate, only polydiolcitrate synthesized from monomers with longer backbone chains exhibited thermoresponsive behaviour. Therefore, Oct was chosen to react with Gly and DA to increase the length of backbone and increase the T_{trans} higher than 30 °C for shape memory effect. To improve mechanical properties and biocompatibility, HA fillers were incorporated into the polyester.

POGDA polyesters and POGDA/HA composites were synthesized using the polycondensation method demonstrated by previous study (Bruggeman *et al.*, 2008a) but with some modification. This research was carried out to synthesis POGDA and POGDA/HA, to characterize the polyesters and composites, to study the biodegradation rate and to investigate the biocompatibility of the polyesters and composites. For application in tissue engineering, scaffolds were fabricated using salt-leaching method without solvent.

List of research questions:

(a) What are the effect of molar ratio of monomers on thermal properties, mechanical properties, thermoresponsive behaviour, biodegradation rate and biocompatibility of the POGDA polyesters?
(b) What are the effect of HA particles with different weight percentage on thermal properties, mechanical properties, biodegradation rate and biocompatibility of the POGDA/HA composites?
(c) What are the pore size, porosity and biocompatibility of scaffolds fabricated using solvent-free salt leaching method?

1.3 Objectives

The main objective of this research was to develop biodegradable POGDA polyester with thermoresponsive behaviour from Oct, Gly and DA through solvent- and catalyst-free polycondensation method. The mechanical and biocompatibility of POGDA was to be improved by incorporation of HA particles. Last part of this research was to fabricate scaffolds using solvent-free salt leaching method suitable for tissue engineering applications.

The sub-objectives set to achieve the goal of this research:

(a) To determine the effects of molar ratio of monomers on the structural, thermal properties, crystallinity, mechanical properties, thermoresponsive behaviour, biodegradation rate and biocompatibility of the POGDA polyesters.

(b) To investigate the effect of HA particles with different weight percentage on the thermal properties, crystallinity, mechanical properties, biodegradation rate and biocompatibility of the POGDA/HA composites.

(c) To identify the mixing ratio between sieved salt and pre-polymer composite solution for porous scaffold fabrication and to determine the pore size, porosity and biocompatibility of scaffold prepared from solvent-free salt leaching method.

1.4 Scopes

The scopes of this research are identified and divided into few parts.

a) POGDA polyesters were synthesized using polycondensation polymerization route with different molar ratio of monomers. The effects of molar ratio of monomers on the structural, thermal properties, mechanical properties, thermoresponsive behaviour, biodegradation rate and biocompatibility of the POGDA polyesters were examined via Fourier Transform infra-red (FTIR) spectroscopy, UV/vis spectroscopy, swelling tests, differential scanning calorimeter (DSC), thermogravimetric analysis (TGA), X-ray diffraction (XRD), tensile tests, bending tests, *in vitro* biodegradation test, *in vitro* cytotoxicity and *in vitro* cell proliferation tests.

b) Incorporation of HA with different weight percentage into POGDA using same polycondensation method. The effects of HA particles with different weight percentage on the structural, thermal properties, mechanical properties, biodegradation rate and biocompatibility of the POGDA/HA composites were investigated via FTIR, DSC, XRD, tensile tests, field emission scanning electron microscope (FESEM), *in vitro* biodegradation test and *in vitro* cytotoxicity.

c) Scaffolds were fabricated from POGDA/HA composites using solvent-free salt leaching method. The morphology, porosity and biocompatibility of scaffold were determined via TGA, FESEM, liquid displacement test and *in vitro* cell proliferation tests.

1.5 Significant of Study

Oct, Gly and DA were used together for the first time to synthesise biodegradable polyesters with thermoresponsive behaviours. The material properties of the newly develop polyesters, POGDAs, were studied in detail. Different composition of monomers was used to create different crosslinking density in the polyesters. The effect of monomers' composition of the polyesters on material properties were determined for better understanding in the relationship between crosslinking density and material properties. The synthesized polyesters in present study was shown to have T_{tans} higher than PGS, PGD and polydiolcitrates. POGDA with selected monomer composition showed the ability to recover to its original shape at body temperature which is benefit for biomedical application. POGDA/HA composites were prepared with different loading percentage of HA particles. The effects of HA concentration on the composites properties were disclosed. In additional, the minimum mixing ratio of pre-polymer composite solution and sieved salt was determined to fabricate a fully porous scaffold. The results are important for further study of scaffold.

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