DEVELOPMENT AND CHARACTERIZATION OF *PENNISETUM PURPUREUM*/PLA BIOCOMPOSITE SCAFFOLD

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UNIVERSITI MALAYSIA PERLIS

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DEVELOPMENT AND CHARACTERIZATION OF *PENNISETUM PURPUREUM*/PLA BIOCOMPOSITE SCAFFOLD

by

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LIST OF ABBREVIATIONS

3D	3-Dimension
ASTM	American Standard Testing Material
DTG	Derivative Thermogravimetric
ECM	Extracellular matrix
FDA	Food and Drug Administration
FDT	Final Degradation Temperature
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
FRP	Fiber Reinforced Polymers
KBr	Potassium Bromide
MC	Moisture Content
NFRC	Natural Fibre Reinforced Composite
PAs	Polyamides
PBS	Phosphate-Bufferes Saline
PCL	Polycaprolactone
PDLA	Poly-D-lactide
PDLLA	Poly-DL-lactide
PEG	Polyethylene Glycol
PGA	Polyglycolide
PLA	Polylactic acid
PLA_C	Polylactic acid as controlled scaffold
PLA/PP_{10}	Polylactic acid reinforced with 10 wt% *P. purpureum* fibre

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PLA/PP_{20}	Polylactic acid reinforced with 20 wt% *P. purpureum* fibre
PLA/PP_{30}	Polylactic acid reinforced with 30 wt% *P. purpureum* fibre
PLGA	Poly Lactic-co-Glycolic acid
PLLA	Poly-L-lactide acid
PP	*Pennisetum purpureum*
PUs	Polyurethanes
SCPL	Solvent Casting and Particulate Leaching
TGA	Thermogravimetric Analysis
XRD	X-Ray Diffraction
MC	Moisture Content
NaCl	Sodium Chloride

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LIST OF SYMBOLS

°C	Temperature
A_o	Cross-sectional area of the specimen
E_c	Compressive modulus
F	Applied load
µm	Micrometer
g/cm^3	Gram per centimeter cube
MPa	Megapascal
kV	Kilovolt
l	Decrease in length of the compressed specimen
l_o	Original length of the specimen
mA	Miliampere
mL/min	Millimeter per minute flow rate
w_o	Weight after immersed in distilled water
w_{od}	Weight of the scaffold after drying
w_t	Weight before immersed in distilled water
T_c	Crystallization temperature
T_d	Degradation temperature
T_g	Glass transition temperature
T_m	Melting temperature
θ	Theta
ε_c	Compressive strain
σ_c	Compressive stress

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Pembentukan dan Pencirian daripada *Pennisetum Purpureum*/PLA Biokomposit untuk Kejuruteraan Tisu

ABSTRAK

Penyelidikan tentang ciri ciri mekanikal, haba, morfologi dan degrasi 'in vitro' berkaitan liang 3D *Pennisetum purpureum* (PP)/polylactic acid (PLA) berdasarkan perancah telah dijalankan. Dalam penyelidikan ini, perancah yang mengandungi *P. purpureum* and PLA dihasilkan daripada kaedah pemutus pelarut dan kaedah larut lesap zarahan. Komposit PLA yang mengandungi pelbagai *P. purpureum* kandungan (10 wt%, 20 wt%, and 30 wt%) disediakan dan dan kemudiannya dikelaskan. Ciri-ciri morfologi, struktur dan keadaan haba 'scaffold' dikelaskan mengguna alatan seperti 'field-emission' mikroskop elektron pengimbas (FESEM), 'Fourier transform infrared spectroscopy' (FTIR), 'X-ray diffraction' (XRD) dan 'thermogravimetric analysis' (TGA). Ciri-ciri morfologi dikaji menggunakan 'field-emission' mikroskop elektron pengimbas (FESEM) dimana perancah mengandungi liang yang saiznya antara 70-200 μm dan mempunyai faktor keliangan berjumlah 99% yang mana merupakan antara faktor keliangan yang paling besar dengan tahap saling hubung yang tinggi. Ciri-ciri mekanikal dan degrasi '*in-vitro'* daripada liang perancah yang dikembangkan seterusnya dikaji dengan lebih mendalam. Ujian mampatan dilakukan untuk menilai kekuatan mampatan dan modulus perancah mengikut ASTM F451-95. Kekuatan perancah dilihat meningkat daripada 1.94 ke 9.32 MPa, manakala kekuatan modulus meningkat daripada 1.73 ke 5.25 MPa seiring dengan peningkatan kandungan pengisi daripada 0 wt% ke 30 wt%. Sintesis komposit perancah telah direndam dalam larutan PBS pada suhu 37 °C selama 40 hari. Hasilnya menunjukkan kadar degrasi menurun untuk PLA/PP_{20} perancah walaupun kadar penurunananya rendah, tetapi ini boleh menyumbang kepada penambah baikan ciri-ciri mekanikal dan menguatkan antara muka gentian matriks. Perubahan mikrostruktur dikaji mengunakan FESEM. Hasil kajian daripada FESEM menunjukkan antara muka gentian matrik yang kuat terjalin dibentuk PLA/PP_{20} perancah, dimana ia mencerminkan penambahan *P. purpureum* dalam PLA menurunkan kadar degrasi jika dibandingkan dengan PLA perancah yang asli. Dengan keputusan ini, boleh disimpulkan bahawa ciri-ciri liang yang tinggi dalam *P. purpureum*/PLA perancah yang terhasil boleh dikawal dan dioptimumkan. Ini boleh digunakan untuk memudahkan pembentukan implant tulang rawan dalam tisu kejuruteraan.

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Development and Characterization of *Pennisetum Purpureum*/PLA Biocomposite for Tissue Engineering

ABSTRACT

The mechanical, thermal, morphological properties and *in vitro* degradation study of a 3D porous *Pennisetum purpureum* (PP)/polylactic acid (PLA) based scaffold were investigated. In this study, a novel scaffold containing *P. purpureum* and PLA was produced using of the solvent casting and particulate leaching method. PLA composite with various *P. purpureum* contents (10 wt%, 20 wt%, and 30 wt%) were prepared and subsequently characterised. The morphologies, structures and thermal behaviours of the prepared composite scaffolds were characterised using field-emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and thermogravimetric analysis (TGA). The morphology was studied using FESEM; the scaffold possessed 70-200 µm-sized pores and had a greater porosity factor (99%) with a high level of interconnectivity. The mechanical properties and *in vitro* degradation of the developed porous scaffolds were further characterized. Compression tests were conducted to evaluate the compressive strength and modulus of the scaffolds, according to ASTM F451-95. The compression strength of the scaffolds was found to increase from 1.94 to 9.32 MPa, while the compressive modulus increased from 1.73 to 5.25 MPa as the fillers' content increased from 0 wt% to 30 wt%. In this study, the synthesized composite scaffolds were immersed in a PBS solution at 37 °C for 40 days. Interestingly, the degradation rate was reduced for the PLA/PP_{20} scaffold, though insignificantly, this could be attributed to the improved mechanical properties and stronger fibre-matrix interface. Microstructure changes after degradation were observed using FESEM. The FESEM results indicated that a strong fibre-matrix interface was formed in the PLA/PP_{20} scaffold, which reflected the addition of *P. purpureum* into PLA decreasing the degradation rate compared to in pure PLA scaffolds. From the results, it can be concluded that the properties of the highly porous *P. purpureum*/PLA scaffold developed in this study can be controlled and optimized. This can be used to facilitate the construction of implantable tissue-engineered cartilage.

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CHAPTER 1

INTRODUCTION

1.1 Background

There are three types of cartilage in the body: fibrocartilage, hyaline cartilage and elastic cartilage (Rotter *et al.*, 1998). Hyaline cartilage is the most concentrated type in recent researches, due to its role as the surface covering for all the diarthrodial joints in the human body from abrasion and wear. Hyaline cartilage is also frequently called as articular cartilage. Articular cartilage is a heterogeneous and mechanically anisotropic tissue. Since cartilage does not contain nerve, cartilage has a very limited capacity to repair itself because of the absence of blood vessels and low cellular content (Naseri, Lenart, & Kristiina, 2016). Developing a new artificial cartilage to repair and restore defected cartilage is of great interest.

The availability of biomedical composites as a scaffold for tissue repair and degeneration, or cells preceding implantations and for the deposition of engineered tissue has significant market potential together with continuous financial investment. The concept of long-term sustainability and the emerging "green" economy and ecological awareness have motivated the search for green materials compatible with the living human cells. Unlike synthetic polymers, natural fibre composites (NFCs) gained more popularity among researchers due to their availability, as they are carbon neutral, low density, renewable and biodegradable and they can be used without damaging the environment (Cheung, Ho, Lau, Cardona, & Hui, 2009). Moreover, the intrinsic properties of natural fibre such as low cost,

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low weight, and high mechanical strength made them attractive to the tissue engineering industry (Reddy & Yang, 2009).

Fibres can be classified into 2 main categories: natural and synthetic. Natural fibres are obtained from natural sources such as animals and plants, while those which are not obtained from natural sources are called synthetic fibres (Thomas, Paul, Pothan, & Deepa, 2011). Since natural fibres are in opposition to synthetic fibers, they can be directly used without chemical processing, in contrast to regenerative fibers (Avella, Buzarovska, Errico, Gentile, & Grozdanov, 2009). However, natural fibres have disadvantages like moisture absorption, poor matrix-fibre adhesion, and low durability which prevent them from industrial usage (Avella *et al.*, 2009; Babu, O'Connor, & Seeram, 2013). In order to improve the composite's interfacial bonding and durability, chemical treatments such as surface modification technique, alkylation, acetylation and etc. is being applied to increase the fibre's quality (Reddy, 2009). Over the last 55 years, the use of synthetic polymer has shown a dramatic increase, due to its benefits of low cost and manufactured easily in bulk together by modifying the physical properties (Shankar, Seyam, & Hudson, 2013). On the other hand, the production of synthetic polymers is then become a major contributor to carbon emission and waste which lead to harmfulness towards the environments (Fowler, Hughes, & Elias, 2007). Therefore, United Nations had announced 2009 as International Year of Natural Fibres; the year brought global awareness about natural fibers with particular focus on expanding market interest to guarantee the long-term sustainability for agriculturists who fully depend on their production (Maria & Bicalho, 2009). Polymer matrix composites are also known as fiber reinforced polymers (FRP), in these composites, a polymer-based resin is the matrix and the reinforcement is produced by various of fibers

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such as carbon, aramid and glass (Fowler, Hughes, & Elias, 2006). In contrast with isotropic materials such as steel and aluminum, the physical properties of the resulting composite material are mostly anisotropic as the composite is dependent on directional orientation of the applied force (Ramakrishna, Mayer, Wintermantel, & Leong, 2001).

Biocomposites or "Green" composites can be defined as the combination of bio-fibers with biopolymer matrices from both renewable and non-renewable resource, used to replace the function of living tissue or organs (H. Y. Cheung, Lau, Tao, & Hui, 2008). The fibre reinforcement with polymer matrix is proven to increase the mechanical strength of the composite and improve other crucial parameters such as degradation rate, cell proliferation and cell viability, structural properties and etc. (Chirayil, Mathew, & Thomas, 2014). Moreover, the reinforcement of fibres reduces the quantity of polymer matrix used. The composite's strength and stiffness can be enhanced by reinforcing bio-fillers with weaker material which in this case is the polymer matrix where the structural load of the composite is supported by reinforcement (Adeosun, Lawal, Balogun, & Akpan, 2012). The polymer matrix, normally known as bio-binder, maintains the reinforcement in the desired position and orientation (Chandramohan & Marimuthu, 2011). The polymer matrix prevents the fibre reinforcement in a composite from chemical and environmental attack, and it bonds the reinforcement so that applied stresses can be effectively transferred into the fibres (Thomas *et al.*, 2011). Besides, these natural fibres reinforced polymer composites have an extended lifetime which improves the quality of life for people with an increasing demand. The scope of their application varies and more suitable for orthopedic application and tissue engineering. Finally, the development of new natural fibre-based composites will enable various applications in biomedical field is to be the focus of future research.

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Among various synthetic polymers used in the fabrication of highly porous polymeric scaffold, polylactic acid (PLA) has currently received significant consideration among researchers. Currently, polylactic acid is the most chosen biodegradable polymer for this purpose and has conclusively illustrated the proof of idea as biodegradable materials application in polymeric scaffold (H. Y. Cheung *et al*., 2008). This is due to their mechanical strength and biological feature such as biocompatibility and the ability to control biodegradability. Basically reinforcement of PLA with natural polymers likes collagen, chitosan, gelatin and polyethylene glycol (PEG) improved the scaffold's mechanical strength with controllable rate of degradation. Particularly, PLA has been mainly considered for the advancement of scaffold in tissue engineering applications such as ligament, cartilage, bone, skin, vascular tissue and as controlled delivery system for drugs, DNA, and proteins in concerning marketing use and in research (Lasprilla, Martinez, Lunelli, Jardini, & Filho, 2012).

To take benefit of the outstanding properties of natural fibre and its derivatives, we have designed a highly porous *Pennisetum purpureum*/PLA scaffolds through solvent-casting and particulate leaching technique. This technique was chosen based on its ability to control the porosity and pore size of a scaffold. Furthermore, this technique requires less energy and time consumption while it is also low-cost, more customers friendly. The resulting biocomposite's physical, morphological, thermal, mechanical, and degradation properties of the *Pennisetum purpureum*/PLA scaffolds with various compositions are included and discussed.

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1.2 Problem Statement

Metallic implants containing titanium alloys are extensively applied for internal fixation of bone fractures in human body. These implants are believed as the gold standard for the most of the bone fracture fixation treatments owing to their superior mechanical properties. However, metallic implants have some critical weakness such as the effect of stress shielding which causes to implant loosening, bone resorption and, indirectly leads to the need for a second surgery to take off the implants particularly in pediatrics. The need for second surgery has certain possibilities such as infections, removal difficulty of jammed implants, migration of implants, and combined with additional health care costs (O'Brien, 2011). Resultantly, in order to overcome stress shielding it has been widely studied for many years to utilize a material that will gradually degrade and lose its strength identical to the rate of bone recovering (Mehboob & Chang, 2014).

Natural fibers gained researchers attention due to excellent beneficial traits such as inexpensive, high mechanical strength to weight ratio, light in weight, non-corrosive character, and sufficient specific strength, together with their renewable source and degradable properties. Natural fibers are available large in number as nature resource, and these could be utilized as reinforcement/bio-fillers in producing of polymer composite. For the past few years, requirement for natural fibers has proved a rapid increase for inventing new kinds of environmentally–friendly composites.

Polylactic acid (PLA) is biodegradable, and it has extensive applications in biomedical fields, including suture, bone fixation material, drug delivery microsphere, and tissue engineering. In this work, the blend of PLA with *Pennisetum purpureum* fibres is

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investigated in order to invent an ideal scaffold with sufficient mechanical properties and desired shape. Thus, rational design of PLA based scaffolds with *Pennisetum purpureum* fibre is integrated together for enhancing each limitation and advantages of materials to improve tissue regeneration and fulfills the requirements of tissue engineering applications.

1.3 Research Objectives

The main objective of this research is to characterise the development of polylactic acid (PLA) reinforced with *Pennisetum purpureum* fibre as filler. As developed the incorporating of *Pennisetum purpureum* fibre in the biodegradable polylactic acid (PLA) could provide stiffness and strength to the polymer composite. Following are the objectives of this research:

a) To fabricate composite scaffolds with higher porosity and interconnectivity by controlling the variables in fabrication technique.

b) To study the influence of *Pennisetum purpureum* fibre's content on the morphology and thermal properties of the polymer composite.

c) To investigate the mechanical properties of the biodegradable polymer composite with *Pennisetum purpureum* fibre as filler.

d) To study the influence of *Pennisetum purpureum* fibre on the *in vitro* degradation of polymer composite.

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1.4 Research Scope

The scope of this project is to establish the background study about the development and characterization of *Purpureum pennisetum*/PLA biocomposite as a tissue engineering scaffold. In this investigation, fabrications of the PLA composite scaffold with various *P. purpureum* contents will be prepared using solvent casting-particulate leaching technique. The composite scaffolds will be characterized using field emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), and thermogravimetric analysis (TGA). The compression testing will then be carried out by following ASTM F451-95; the compressed scaffold undergoes FESEM to study the impact on its microstructure. Further investigation on the *in vitro* degradation of composite scaffolds will be evaluated in this study. The prepared scaffolds will be immersed in phosphate buffered saline (PBS) medium for 40 days to determine the weight loss, pH changes, and water uptake and conduct the FESEM test on the degraded scaffolds.

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1.5 Structure of the Thesis

This thesis consists of five chapters. Chapter 1 of this thesis describes the introductory chapter to this project. The main objective of this chapter is to give a brief introduction to the readers. It will help readers to understand the direction of the thesis and what they can expect in this investigation at the end. Also this chapter will cover the problem statement, objective, and scope of this research.

Chapter 2 covers the relevant literature review in support of the remainder of the thesis. This includes a broad understanding of biocomposite materials, characteristics of

PLA, composition of natural fibres, and an overview of the biocomposite fabrication techniques. In addition, this chapter consists of brief review of recent works on the natural fibre reinforced PLA biocomposites.

Chapter 3 provides a brief description on materials, methodology of fibre extraction and fabrication of composite. Also included is the configuration of specimens, instrumentation, characterization techniques and testing of composites.

In Chapter 4, we present the results and discussion obtained from this investigation. Moreover, in this chapter there will be discussion mainly focusing on the characterization of *Pennisetum purpureum*/PLA composite scaffold. This chapter also presents the thermal, mechanical and *in vitro* degradation analysis of composite scaffolds with varying fibre contents.

Chapter 5, we summarize the overall findings obtained from this project as well as discusses on directions for future work.

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