SYNTHESIS AND MECHANICAL PROPERTIES OF CONDUCTIVE COMPOSITE POLYLACTIC ACID/POLYANILINE SCAFFOLD FOR POTENTIAL TISSUE ENGINEERING

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ABSTRACT

This thesis reports a new composite scaffold material that is conductive and porous made from degradable polylactic acid (PLA) and conducting polyaniline (PANI) which has the potential for use in promoting tissue regeneration. The conductive scaffold was successfully prepared using a simple yet effective method known as freeze extraction method. The doped PANI was synthesised using conventional method of oxidative chemical polymerization. The electrical percolation state was successfully obtained at 3 wt% of PANI inclusion and reached at useable conductivity level for tissue engineering application at 4 wt% PANI, 2.91 x 10-3 Scm-1. 4 wt% inclusion of PANI was justified as the best PLA/PANI composite scaffold because it met the criterion as an electro-responsive material where the conductivity achieved was higher than 10-3 Scm-1. It is also much suitable material in the regeneration of skin tissue (fibroblast) because the mean pore size achieved was at 35.82 μm and optimum tensile strength at 3.08 MPa. The UV-spectrum of the conductive scaffold displayed transition peaks of PANI indicating the PANI was still in its conducting doped state inside the scaffold. Incubation for 24 weeks for in-vitro degradation revealed that the PANI component delayed the degradation of PLA. Preliminary bioactivity test results indicated that the doping agent able to form chelate at the scaffold surface and this could assist in the formation of in-vitro apatite during the biomimetic immersion.

ABSTRAK

Tesis ini melaporkan bahan komposit perancah terbaharu berkonduktif dan berliang yang diperbuat daripada asid polilaktik (PLA) berdegradasi dan polianilina (PANI) berkonduktif di mana berpotensi untuk menggalakkan pertumbuhan semula tisu. Perancah berkonduktif ini berjaya dihasilkan dengan menggunakan kaedah yang mudah tetapi berkesan yang dikenali sebagai pengekstrakan beku. PANI terdop telah disintesis dengan cara yang konvensional iaitu pempolimeran kimia secara oksidatif. Tahap perkolasi elektrik berjaya diperoleh pada 3% kemasukan PANI dan mencapai tahap konduktiviti yang berguna untuk kejuruteraan tisu pada 4% PANI iaitu 2.91 x 10-3 Scm-1. Kemasukan 4% PANI telah dibuktikan sebagai PLA/PANI perancah komposit yang terbaik kerana ianya memenuhi kriteria sebagai bahan yang elektro-responsif di mana pencapaian konduktiviti adalah lebih tinggi daripada 10-3 Scm-1. Ianya juga bahan yang sangat sesuai dalam pertumbuhan semula tisu kulit (fibroblas) kerana purata saiz liang yang dicapai pada 35.82 μm dan kekuatan tegangan yang optimum pada 3.08 MPa. Spektra UV perancah berkonduktif ini menunjukkan kewujudan peralihan spektra PANI dan ini menunjukkan bahawa PANI masih berkeadaan terdop di dalam perancah tersebut. Tempoh pengeraman selama 24 minggu untuk degradasi secara in-vitro menunjukkan komponen PANI telah melambatkan degradasi PLA. Keputusan awal ujian bioaktiviti menunjukkan agen dop mampu membentuk sebagai kelat pada permukaan perancah dan ini dapat membantu pembentukan in-vitro apatit ketika rendaman cecair biomimetik.

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LIST OF ABBREVIATIONS

1D	-	One dimensional
3D	-	Three dimensional
CA-*cit*	-	Calcium-citric acid
$CHCl^3$	-	trichloromethane
DC	-	Direct Current
FC	-	Fast cooling rate
FDA	-	Food and Drug Administration
HA	-	hydroxyl apatite
m-ABA	-	m-Aminobenzoic acid
mmol	-	Milimole
MWCNT	-	Multiwall Carbon Nanotubes
PANI	-	Polyaniline
PANI-CSA		PANI-camphor sulfonic acid
PEDOT	-	Poly(3,4-ethylene dioxythiophene)
PLA	-	Polylactic Acid
PLLA	-	Poly-L-Lactic Acid
PGA	-	Polyglycolic Acid
PPY	-	Polypyrrole
PPV	-	Poly(p-phenylene-vinylene)
PYG	-	Polypyrrole/graphene
SBF	-	Simulated body fluid
SC	-	Slow cooling rate
SEM	-	Scanning Electron Microscopy
UV-vis	-	Ultraviolet-Visible Spectroscopy

LIST OF SYMBOLS

α - Alpha
A - Area
E - Electric field intensity
I - Current
J - Current density
σ - Conductivity of the material
M_s - Mass of scaffold after immersion in water
M_d - Mass of dry scaffold
ρ - Resistivity
π - Pi bonding orbital
π^* - Antibonding Pi orbital
R - Resistance
t - thickness
V - Voltage
V_d - Apparent volume
V_p - Pore volume
W_d - Surplus weight of scaffold after degradation
W_i - Initial weight of scaffold before degradation

CHAPTER 1

INTRODUCTION

1.1 Overview

Scaffolds in tissue engineering refer to biodegradable materials which are highly porous that can act as template for tissue regeneration (Yang *et al.*, 2001). Synthetic biodegradable scaffold such as polylactic acid (PLA) has found wide range of pharmaceutical applications in the tissue regeneration of skin (Mohiti-Asli *et al.*, 2015), cartilage (Muhonen *et al.*, 2015), blood vessel (Li *et al.*, 2015) and cardiac valve (Iop and Gerosa 2015). The advantages of PLA are its synthetically controllable degradation rate (Cui *et al.*, 2015), good mechanical properties (Shi *et al.*, 2015) and biocompatibility (Abdal-hay *et al.*, 2015) plus it can be produced from renewable resource (Yang *et al.*, 2015).

The methods of preparing a porous PLA scaffold are diverse which includes, thermally induced phase separation (Mannella *et al.*, 2015), 3D printing (Rosenzweig *et al.*, 2015), porogen leaching (Choudhury *et al.*, 2015), the highly popular freeze drying (Salerno *et al.*, 2015) and electrospinning (Morelli *et al.*, 2015). Another method to produce polymeric porous scaffold is the simple freeze extraction (Adeli *et al.*, 2011).

Though there are few reports on PLA scaffold prepared by freeze extraction method with the inclusion of other fillers or reinforcements such as carbon nanotubes (Adeli *et al.,* 2011), chitosan and alginate (Yuan *et al.,* 2009), bioactive glass (El-Kady *et al.,* 2010), to date there are no reported studies on the preparation of freeze extracted porous conductive scaffold of PLA with the inclusion of conducting polymers such as of polyaniline (PANI).

PANI is one of the most promising conducting polymers for wide range of applications (Li *et al.,* 2008) mainly due to its ease of synthesis and preparation (Bhadra *et al.,* 2009), excellent electrical properties (Wang *et al.,* 2015) and being biocompatible (Bidez *et al.,* 2006). Inclusion of conductive PANI filler in the PLA scaffold might open up opportunities in many biomedical applications such as tissue engineering. It is only quite recently that the tuneable electroactivity of PANI has been explored in the area of diverse biomedical applications, such as for scaffolds in tissue engineering (Qazi *et al.,* 2014).

Earlier in vivo test revealed that various forms of PANI caused minimal inflammation after 50 weeks of implantation beneath the dorsal skin of rats (Wang *et al.,* 1999). It was also shown that PANI can be a good reducing agents and effective scavengers of free radicals when present in biological media (Gizdavic-Nikolaidis *et al.,* 2004). Investigation on adhesion and proliferation of cardiac myocytes on PANI concluded that PANI potential usefulness as an electroactive conductive polymer in cell-culture experiments (Bidez *et al.,* 2006), able to stimulate cell differentiation to cardiomyocites (Borriello *et al.,* 2011) and biocompatible for both healthy and cancer cells after some modifications (Yslas *et al.,* 2015). However, due to its brittleness and nonprocessability (Saini *et al.,* 2012), it should be incorporated into other polymers that able to be fabricated into a tissue engineered scaffold.

Therefore, the main aim of this study is to prepare and investigate the effects of PANI addition on the properties of PLA scaffold prepared using freeze extraction. This new type of conductive composite scaffold is expected to exhibit new and enhanced properties including the ease of processing and low cost. Such conductive scaffold may be usable in many applications in tissue engineering and biomedical implants such as controllable electrically responsive cell growth scaffold, controllable drug delivery sites and skin graft for wounds.

1.2 Problem Statements

Most of the research works on PLA composite scaffold are focused on the mechanical and morphology improvement. Nonetheless it was shown that certain material can enhance Schwann cell growths for neural tissue engineering upon applied voltage (Baniasadi *et al.*, 2015). This could decrease the time taken for the cells to fully mature and it could lessen the time for patients to wait for their new regenerate tissue. Thus it seems feasible to induce a certain degree of electrical conductivity to a scaffold material in order to obtain cell responsive properties for tissue engineering. Though being reported, the study on conductive scaffold is still limited to some extent.

Freeze drying is a widely used method to prepare porous scaffold but it is time and energy consuming (Baldino *et al.*, 2015). Plus the resulting freeze dried scaffold usually produced unwanted surface skin which requires additional process thus becomes economically uncompetitive (Sachlos and Czernuszka, 2003). In regards to conductive scaffolds, they have been fabricated using the electrospinning method mainly due to their nanofiber formation which led to high porosity (McKeon 2010, Shokry *et al.*, 2015). Though the electrospinning process seemed feasible, various cumbersome factors should be taken into consideration to obtain its nanofiber form such as applied voltage, solvent mixtures, distance between the tip and the collector, viscosity of the polymer solution, flow rate and even humidity/temperature of the spinning chamber (Subbiah *et al.*, 2005).

Being relatively new in the tissue engineering field, conductive scaffold prepared using freeze extraction has many unexplored features and characteristics. Many aspects that should be studied which includes the electrical conductivity enhancement, morphology, pore size and porosity, electronic transitions, biodegradability and bioactivity.

1.3 Objectives

This study was conducted in order to fulfil the following objectives:

1. To prepare conductive composite scaffold of PLA/PANI via freeze extraction
2. To characterize the electrical, physical and morphological properties of the PLA/PANI scaffold
3. To evaluate the preliminary in-vitro degradation and preliminary bioactivity test

1.4 Scope of Study

In order to satisfy all the outlined objectives, the scopes of this research are undertaken according to the following:

Initially, PANI was synthesized according to conventional method as reported in literatures. The synthesized PANI was characterized for its morphology, color appearance, DC electrical conductivity and UV-vis spectroscopy. Following that, the as synthesized PANI will be used as fillers in the preparation of conductive scaffold.

Next step was to prepare the scaffold by the inclusions of PANI within the PLA using freeze extraction. Amount of PANI used were (0.5, 1, 2, 3, 4, 5 wt%). The resulting conductive composite scaffolds were evaluated in terms of its DC conductivity, tensile properties, porosity, pore size and degree of swelling. Scaffold of PLA/PANI with a suitable electrical conductivity value and good physical characteristics were identified and further tested using UV-vis spectroscopy and scanning electron microscope.

Consequently the conductive composite scaffold was tested for in-vitro degradation; evaluating the weight loss and the resulting morphology. Bioactivity test of the conductive scaffold was done by immersion in simulated body fluid solution (SBF), followed by the evaluation of hydroxyl apatite growth on the sample.

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