ELECTROSPUN POLYURETHANE WITH COMBINATION OF NICKEL OXIDE NANOPARTICLE AND GROUNDNUT OIL FOR FUTURE BONE APPLICATION

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DEDICATION

This thesis is dedicated to my mother, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my best friend Hasvin, who taught me that even the largest task can be accomplished if it is done one step at a time.

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ABSTRACT

The main challenge in bone tissue engineering is to fabricate a synthetic bone scaffold with mimicking the native extracellular matrix (ECM) structure of the human bone. Recently, the nanofibrous scaffold holds a great potential in the tissue engineering applications because of their structure resembling the original properties of ECM. This research is focussed to fabricate a synthetic scaffold based on polyurethane (PU) incorporated with ground nut oil (GO) and nickel oxide (NiO). Nickel oxide nanoparticles were synthesized using plant extract using *Plectranthus amboinicus* (PA) with 60% and 100% concentration. The synthesized nanoparticles using green extract showed particle size in the range of 800-960nm respectively. Particles with reduced particle diameter produced using 100% extract was used to fabricate the scaffolds. The electrospun membranes were characterized using FE-SEM, FTIR, and contact angle measurements, XRD, TGA and AFM. Further, the bone mineralisation test was carried out to examine minerals formation along with the amount of mineral particles deposition in the electrospun membranes. The fabricated nanocomposites showed reduced fiber diameter of 327nm than the pristine PU of 616nm. The presence of NiO nanoparticles were evident through FTIR analysis by hydrogen bond formation. Further, XRD analysis confirmed the existence of NiO in the polyurethane matrix by exhibiting various crystal planes. The contact angle measurements depicted that the contact angle of PU/NiO/GO was within the optimum range suitable for improved cell adhesion and proliferation. The addition of NiO and GO improved the thermal behaviour of the pure PU explained in the thermal study. Further, the AFM analysis showed the fabricated composites showed smooth surfaces compared to the pure PU with a remarkable difference in surface roughness. Bone mineralisation testing depicted the improved deposition of calcium in the developed composites than the pristine PU which is corroborated with SEM images and EDX analysis. Hence, the fabricated nanocomposites with improved physico-chemical and mineral deposition might be used as potential candidate for bone application.

ABSTRAK

Cabaran utama dalam kejuruteraan tisu tulang adalah membuat tiruan tulang sintetik dengan meniru struktur matriks ekstraselular asli (ECM) tulang manusia. Baru-baru ini, perancah nanofibrous memegang potensi besar dalam aplikasi kejuruteraan tisu kerana strukturnya menyerupai sifat asli ECM. Kajian ini bertujuan untuk mengarang perancah sintetik berdasarkan poliuretana (PU) yang digabungkan dengan minyak kacang tanah (GO) dan nikel oksida (NiO). Nanopartikel nikel oksida telah disintesis menggunakan ekstrak tumbuhan menggunakan *Plectranthus amboinicus* (PA) dengan kepekatan 60% dan 100%. Nanopartikel yang disintesis menggunakan ekstrak hijau menunjukkan saiz zarah dalam lingkungan 800-920nm masing-masing. Zarah dengan diameter yang dikurangkan yang dihasilkan menggunakan ekstrak 100% digunakan untuk mengarang perancah. Membran elektrospun dicirikan menggunakan FE-SEM, FTIR, pengukuran sudut sentuhan, XRD, TGA dan AFM. Selanjutnya, ujian penggenangan tulang dilakukan untuk meneliti pembentukan mineral dan jumlah pemendapan zarah mineral dalam membran electrospun. Nanocomposites fabricated menunjukkan diameter serat yang dikurangkan 327nm daripada PU murni 616nm. Kehadiran nanopartikel PA dan NiO terbukti melalui analisis FTIR oleh pembentukan ikatan hidrogen. Tambahan lagi, analisis XRD mengesahkan kewujudan NiO dalam matriks poliuretana dengan mempamerkan pelbagai pesawat kristal. Pengukuran sudut sentuhan menggambarkan bahawa sudut hubungan PU/NiO/ GO adalah dalam julat optimum sesuai untuk lekatan dan pembiakan sel yang lebih baik. Penambahan NiO dan GO meningkatkan tingkah laku haba PU tulen yang dijelaskan dalam kajian termal. Tambahan lagi, analisis AFM menunjukkan komposit fabrikasi menunjukkan permukaan licin berbanding PU tulen dengan perbezaan yang ketara dalam kekasaran permukaan. Ujian mineralisasi tulang menggambarkan pemendapan kalsium yang lebih baik dalam komposit yang dibangunkan daripada PU murni yang disokong dengan imej SEM dan analisis EDX. Oleh itu, nanocomposites yang dibuat dengan pemendapan fiziko-kimia dan mineral yang lebih baik mungkin digunakan sebagai calon yang berpotensi untuk aplikasi tulang.

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LIST OF ABBREVIATIONS

AFM	-	Atomic Force Microscopy
DMF	-	Dimethylformamide
DTG	-	Derivative Thermogravimetry
ECM	-	Extracellular Matrix
EDX	-	Energy Dispersive X-ray Analysis
FE-SEM	-	Field Emission Scanning Electron Microscope
FTIR	-	Fourier Transform Infrared
GO	-	Groundnut Oil
NP	-	Nanoparticle
PA	-	*Plectranthus amboinicus*
PU	-	Polyurethane
NiO	-	Nickel Oxide
SBF	-	Simulated Body Fluid
TGA	-	Thermogravimetric Analysis
THF	-	Tetrahydrofuran
XRD	-	X-Ray Diffraction Analysis

LIST OF SYMBOLS

cm	-	Centimeter
μm	-	Micrometer
μg	-	Microgram
μl	-	Microlitre
GHz	-	Gigahertz
mg	-	Milligram
mm	-	Millimeter
nm	-	Nanometer

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CHAPTER 1

INTRODUCTION

1.1 Research background

In recent years, the tissue engineering (TE) have been emerged as a well-established and promising technique for repairing the damaged tissue. The human body is a multifaceted network having different organ and tissue performing specified functions. The human organ or tissue will undergo changes when people become older which result in the damage to the tissue or loss of its function (Burg, Porter, & Kellam, 2000). The studies of TE aim to tissue maintaining, restoring or remodeling through engineered tissues. Tissue engineering focus on developing an appropriate substitute for damaged tissue by the combination of cell and produced extracellular matrix (ECM).

The components involved in the TE is classified into scaffolds, cells and growth factors. Among these, the scaffold play a vital role in tissue engineering for regeneration of damaged tissue. Tissue engineering involves isolating the cells form the human body and seeded on the developed substrate and utilized as an implant for patients. The substrate structure was named as scaffold and it is able to mimic the native properties of the human ECM which facilitates the cell attachment, proliferation and migration for new tissue growth (Wade & Burdick, 2014). In some cases like blood vessel, the scaffolds were incorporated with growth factor to provide mechanical support for the new tissue growth. A proper scaffold should provide a supporting structure for cell preservation on infected tissue to maintain the cell growth, maintenance and phenotype (Hall, Mohtaram, Willerth, & Edwards, 2017).

A scaffold to be used in TE applications should have several properties. Mainly, the materials used for fabricating a scaffold should be biodegradable after implanted, so that it starts to degrade once the suitable tissue regeneration is reached (Huang, Wang, & Luo, 2009).

The degraded material should not influence any adverse effects to the cells surrounding it. Moreover, the developed scaffold should be biocompatible so that it could provide a conducive environment (Forero, Roa, Reyes, Acevedo, & Osses, 2017) for cell adhesion and proliferation for new tissue growth. In addition, the fabricated scaffold should be cost effective and non-allergic.

The scaffold in certain clinical fields such as surgery and organ replacement is used as a substitute via artificial implants to treat the damaged human organ. Due to the limited natural bone grafts, the need for synthetic bone substitute with biological and physical properties similar to that of natural bone has been increased now-a-days (Farzadi, Bakhshi, Solati-Hashjin, Asadi-Eydivand, & Osman, 2014). The main motivation of this study is to meet this limitations by fabricating artificial composite materials as a bone substitute for repairing the damaged bone.

Previous studies have been reported that the biological responses can be influenced by fabricating a porous scaffold. There are various techniques (Eui Jong Lee, An, He, Woo, & Shon, 2016) used for fabricating the scaffolds were foaming, phase separation, particle leaching, electrospun, sintering and freeze drying. Particularly in tissue engineering, the nanofibrous scaffold has gained a wide attention recently. The widely used fibrous based techniques were gas forming, sintering, particle leaching, polymer phase separation, rapid prototyping, freeze drying and electrospinning. Among these, the scaffold based on electrospinning (Bhardwaj & Kundu, 2010) is observed to be wide spread owing to their many advantages like offering high surface area with interconnected pores which could able to mimic the native function of the human ECM. Further, nanofibers facilitates the cell adhesion, migration and proliferation.

Bone is composed of both organic and inorganic tissue in which the organic component is collagen called ossein and the inorganic component are mineral salts (Yokoyama et al., 2005). Bone tissue is mineralized of two type's namely cortical and cancellous tissue. It also includes other tissue like bone marrow, endosteum, periosteum, nerves, blood vessels and cartilage.

Tissue engineering mainly focusing on the developed substrate for cancellous bone tissue and it is developed in the form of scaffold which could mimic the cancellous tissue and also stimulate the cellular response for new bone tissue formation. Moreover, the scaffold for bone tissue engineering should have enhanced physical and chemical properties, biocompatibility, biodegradability, and sufficient mechanical properties.

The most commonly used material in the bone tissue engineering were polycaprolactone, polyvinyl alcohol, polyurethane etc. (S. Agarwal, Greiner, & Wendorff, 2013). In this study, PU is used to fabricate the bone scaffold. Several studies have been reported the nanofibrous scaffold on PU widely used for tissue engineering, wound dressing and drug delivery. It possess properties like biodegradability and biocompatibility which are significant for tissue engineering. They also possess good oxidation stability which makes them widespread in biomedical applications.

Even though, PU has an excellent physicochemical property, the cellular response is quite low. Several modifications have been attempted for the past years to improve the cellular response. Polyurethane scaffold fabricated with olive oil, honey and propolis for bone tissue engineering (Jaganathan, Mani, Ismail, Prabhakaran, & Nageswaran, 2018) resulting the addition of olive oil, honey and propolis improved the cellular response of the pristine PU. Similar experiments on PU incorporated with ginger reported with improved cellular response than the control. It can be concluded that the natural substances play a vital role in influencing the cellular response.

Additionally, an ideal bone scaffold should possess good mechanical properties to support the cell growth. This can be improved by the addition of metallic particles into the scaffold. It was reported that the addition of Cu and Zn (Jaganathan, Basri, & Mani, 2018) resulted in the improvement of tensile strength by creating a strong interfacial bond with the polymer matrix. Studies revealed that the metallic particles have a significant impact on the mechanical properties (Kotela, Podporska, Soltysiak, Konsztowicz, & Blazewicz, 2009). Present work attempted a combination of groundnut oil and NiO nanoparticles were used to improve the biological and mechanical properties.

1.2 Problem Statement

Bone tissue engineering has emerged as a promising approach for remodelling the damaged bone tissue. Major components of the bone tissue engineering were cells, biodegradable scaffolds and growth factors (Wu, Yu, Chen, & Wang, 2017). Among these, the scaffolds plays an important role in mimicking the ECM of the human tissue and also facilitates the cell adhesion and proliferation (Dhandayuthapani, Yoshida, Maekawa, & Kumar, 2011). Among the techniques used for scaffold fabrication, electrospinning technique has emerged as a promising method for non-woven porous scaffold fabrication (Okada & Matsumoto, 2016). An ideal scaffold should have sufficient biochemical, mechanical and biological requirements that promote vascularization during bone regeneration. Several synthetic polymers have been used for biomaterial scaffold to improve the properties and enhance the cell proliferation rate (Park, 2010). Polyurethane is widely used in tissue engineering because of its biodegradability and biocompatibility. Even though, PU has an excellent physicochemical property, cellular response is quite low. Several modifications have been attempted for the past years to improve the cellular response. Introducing nanoparticles into the scaffolds have reported with excellent mechanical properties (Jaganathan, Mani, et al., 2018).

The challenges faced in tissue engineering applications are microbial attacks and cell proliferation on biomaterials (Rout, Behera, Ojha, & Nayak, 2012). Cell proliferation rate is mainly depending on the structure of the scaffolds. These problems can be overcome by incorporating nanoparticles into polymer matrices through the development of unique properties in the overall performance of biomaterials with increasing antimicrobial and antibacterial activity. Such properties can be enhanced using the nanoparticles when synthesised using plant extracts (Din, Nabi, Rani, Aihetasham, & Mukhtar, 2018). Metal ions have a therapeutic effect if their levels or location is controlled in the body and metal nanoparticles have a strong antimicrobial activity (Dhivya, Ajita, & Selvamurugan, 2015). The structure of the scaffolds are influenced by various parameters used during fabrication. Recently, the nanofibers produced through electrospinning technique has found to be widespread in biomedical applications (Sridhar et al., 2015). The refined morphology and uniform distribution of the fibers in the electrospun membrane provides necessary support during cell growth (Bas et al., 2015). To produce a scaffold in order to achieve the above properties is still a challenging process.

Despite the extensive research in biomaterial, there is no single material provides the aforementioned desirable properties for the different type of bones. Further, the nanofibers added with additives like nanoparticles and natural ingredients would make them more productive for bone tissue engineering applications. In this research, a novel scaffold will be fabricated to support the cell adhesion and proliferation for effective repair and regeneration of the damaged bone.

1.3 Research Objectives

The objectives of the research are:

(a) To fabricate a novel scaffold of Polyurethane/Nickel Oxide/Groundnut oil using electrospinning technique.

(b) To evaluate physicochemical properties (SEM, FTIR, TGA, AFM, and contact angle) of the developed scaffold.

(c) To examine the apatite formation on the scaffold to reveal the ideal combination of polyurethane and nickel oxide nanoparticle scaffold.

1.4 Scope of the Study

PU scaffold blended with NiO nanoparticles and groundnut oil using electrospinning technique was fabricated. Nanoparticles were synthesized using green extract from *Plectranthus amboinicus* for different extract concentration of 60% and 100%. Then, PU scaffold was optimized for different weight concentrations namely 2wt%, 4wt% and 8wt% respectively. Next, the PU scaffold blended with 4 wt% of NiO nanoparticle and groundnut oil was fabricated with the final composition of 8:0.5:0.5 w/v.

Fabricated scaffolds were characterized using scanning electron microscopy (SEM), Fourier transform and infrared spectroscopy (FTIR), thermo gravimetric analysis (TGA), contact angle measurement, atomic force microscopy (AFM) and X-ray diffraction (XRD). The apatite formation of the scaffold was performed and corresponding morphology was obtained using SEM to validate the developed scaffold for biomedical applications.

1.5 Significance of the Study

Tissue engineering requires biomaterials in which physico-chemical and mechanical properties can be tailored. This can be achieved by means of a nanocomposite, where enhancement of different properties of scaffold is achieved through blending of polymers. Nowadays, polymer scaffolds incorporated with nanoparticles has been found to be useful in various tissue engineering applications (Forero et al., 2017).

Green synthesis methods are widely used for producing metal nanoparticles as antibacterial, antimicrobial and antioxidant properties are enhanced when plant extracts are used as a precursor (H. Agarwal, Venkat Kumar, & Rajeshkumar, 2017). Also, the yield of the nanoparticles are increased and reduction in particle size when using plant extracts.

A novel scaffold using PU with NiO nanoparticles and groundnut oil can be used for bone application. Developed scaffold has an excellent surface morphology with wide distribution of smaller fiber diameter could improve mechanical properties of the membrane to accelerate the cell growth.

To increase the strength of the scaffolds and improve the surface integrity nanoparticles were introduced. Also, the developed scaffold is expected to have great potential for bone applications due to its antimicrobial properties enhancement by the green synthesis of NiO using PA.

Finally, the novel scaffold is tested for its suitability for bone applications by mineralization testing, demonstrates the engineered scaffolds suitability for bone tissue engineering.

1.6 Thesis Outline

Chapter 1 provides the information of tissue engineering, followed by a brief introduction to bone tissue engineering, and defines the objectives of this research.

Chapter 2 elaborates the literatures review of relevant researches, towards the understanding and formulates the strategies adapted in this research.

Chapter 3 explains the materials and methods to carry-out the experiments along with the characterization techniques used for this research. The discussion provides detailed explanation of the parameters used and the need for characterization studies.

Chapter 4 reports the detailed results and discussion of results. In the first section, importance of green synthesis and the morphology of nanoparticles were discussed. In the second section, characterization of developed PU/NiO/GO scaffolds were explained. In the third section, the effect of nanoparticles and the oil towards the calcium formation in the materials testing were discussed.

Chapter 5 summarizes the conclusions of this research, and recommends the future directions of this work.

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