A NOVEL ELECTROSPUN NANOFIBROUS COMPOSITE MEMBRANE FOR CHRONIC WOUND HEALING

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ABSTRACT

In the present work an electrospun nanofibrous membrane composed of polyurethane (PU), sodium bicarbonate ($NaHCO_3$) and pantothenic acid (PA) is developed for treating chronic wounds. Wounds are a common health problem and in particular, the chronic wounds such as vascular ulcers, diabetic ulcers and pressure ulcers cause a large number of morbidity and mortality. The main problems of the chronic wounds are prolonged inflammation phase and presence of acidic environment. These events deactivate the operation of growth factors and also the progression of natural healing mechanism. So, various types of advanced textile based dressings are developed to address the clinical complications associated with chronic wound management. The prepared electrospun scaffolds were characterised to study their physicochemical and hemocompatible properties. The SEM micrographs depicted continuous, smooth-interconnected nanofibrous morphology of PU-$NaHCO_3$-PA scaffolds. The FTIR spectra indicated the addition of $NaHCO_3$ and PA-based hydrophilic chemical groups which significantly enhanced the wettability of the composites. Further, the PU-$NaHCO_3$-PA composite membrane inferred to have a highly porous structure with the mean porosity of $79.4\% \pm 4.8\%$ which may provide a conducive environment for adherence and proliferation of skin cells. The composite scaffold also offers a highly hemocompatible surface by delaying coagulation of blood through contact activation pathways and by limiting red blood cells damage. Therefore, the excellent physicochemical properties, blood compatibility and the delivery of PA are anticipated to speed up the impaired healing process of chronic wounds.

ABSTRAK

Membran berfiber nano dengan kaedah pusingan elektro yang terdiri daripada polyurethane (PU), natrium bikarbonat ($NaHCO_3$) dan asid panthothenic (PA) telah dihasilkan bagi tujuan penyembuhan luka kronik. Membran komposit PU-$NaHCO_3$-PA yang dihasilkan telah diperincikan berdasarkan ciri-ciri fisiokimia menggunakan mikroskop electron pengimbas (SEM), spektroskopi transformasi fourier inframerah (FTIR), analisis keliangan dan asai sudut sentuhan. Analisis keserasian darah bagi komposit yang dihasilkan adalah melalui masa pengaktifan thromboplastin separa (APTT), masa prothrombin (PT) dan asai hemolisis. Struktur berfiber nano yang selanjar telah diperhatikan melalui SEM bagi fiber bersaiz dalam lingkungan 152 nm $\pm$ 63 nm. Analisis FTIR mengesahkan kehadiran bahan $NaHCO_3$ dan PA berdasarkan ciri-ciri titik tertinggi. Analisis keliangan menunjukkan bahawa membran komposit PU-$NaHCO_3$-PA mempunyai keliangan yang tinggi dengan purata sebanyak 79.4% $\pm$ 4.8% dan memberikan persekitaran yang kondusif untuk proses perkembangan sel. Selain itu, asai sudut sentuhan menunjukkan bahawa membran yang dihasilkan ini mempunyai ciri hidrofilik yang tinggi dengan purata sudut sentuhan sebanyak 74.9° $\pm$ 0.5°. Tambahan lagi, membran komposit ini juga menunjukkan keserasian yang tinggi dengan darah dengan melengahkan masa bagi APTT dan PT. Nisbah bagi ciri hemolisis dengan komposit ini kurang daripada 5%. Membran komposit PU-$NaHCO_3$-PA yang dihasilkan mempunyai ciri fisiokimia dan keserasian darah yang lebih baik dan boleh diekploitasi fungsinya bagi tujuan penyembuhan luka kronik.

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LIST OF ABBREVIATIONS

APTT	-	Activated partial thromboplastin time
CA	-	Cellulose acetate
CDC	-	Center for Disease Control
FTIR	-	Fourier transform infrared spectroscopy
GO	-	graphene oxide
NO	-	Nitric oxide
PA	-	Pantothenic acid
PBS	-	Phosphate buffered solution
PLA	-	polylactic acid
PLLA	-	Poly-L-lactide
PT	-	Prothrombin time
PU	-	Polyurethane
PPP	-	Platelet poor plasma
RBC	-	Red blood cells
RVP	-	Retrograde venous perfusion
SEM	-	Scanning electron microscopy
SD	-	Standard deviation
WP	-	Work package
ZnSe	-	Zinc selenide

LIST OF SYMBOLS

B	-	Brightness
C	-	Contrast
d	-	Collector distance
k	-	Wavenumber
m_{solute}	-	Weight of the solute
m_{wet}	-	Weight of the wet sample
m_{dry}	-	Weight of the dry sample
$NaHCO_3$	-	Sodium bicarbonate
NC	-	Absorbance of the negative control
HR	-	Hemolysis ratio
P	-	Level of significance
PC	-	Absorbance of the positive control
ρ	-	Specific gravity
TS	-	Absorbance of the test sample
U	-	Voltage
V	-	Volume
$V_{solution}$	-	Volume of solution
$\dot{V}$	-	Flow rate
$wt/vol\%$	-	Weight per volume percentage solution
$wt\%$	-	Weight percent
λ	-	Wavelength

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CHAPTER 1

INTRODUCTION

1.1 Overview

A wound could be described as an injury on the skin surface by physical, chemical, mechanical or thermal damages. There are two types of wounds in case of wound healing: acute and chronic wounds. Acute wounds are caused by traumas and usually curable within 8 to 12 weeks. These wounds are often caused by mechanical damage, but can be also formed by exposure to extreme heat, irradiation, electrical shock or corrosive chemicals [1]. In addition, acute wounds pass well organized through all repair mechanism like coagulation, inflammation, formation of granulation tissue, and remodelling. In contrast, chronic wounds do not pass all phases of wound healing. They often stay in the inflammatory phase and are commonly originated due to specific diseases like diabetes or tumors [2]. Moreover, the healing takes more than 12 weeks and the recurrence of the wounds is possible [1]. Another classification of wounds is according to their appearance. There are moist and dry wounds [3]. The wound healing of these different types is affected by many parameters. One important factor is the pH value. Variations in pH may affect e.g. wound closure, microbial infection rates, bacterial virulence and biofilm formation [4]. Several researches show that wound healing occurs more effectively at low pH [5]. In case of moist chronic wounds studies show different results. Some research groups report that an alkaline environment is more advantageous and some refer that an acid environment is more beneficial for chronic wound healing. But it is proven that the pH value influences the wound healing [4]. Thus, the pH is one important parameter which can be recognized in wound treatment. In addition, the treatment of a wound varies depending on the type of the wound. Due to the difficulties in treatment of chronic wounds, traditional dressings which include cotton, wool, natural or synthetic bandages and gauzes are largely replaced by novel advanced dressings. Modern materials which are currently utilized for wound healing are natural inert polymers, natural bioactive materials, synthetic polymers and hydrogels [3]. Electrospinning is a widely used technique to

manufacture nanofibers from different natural or synthetic polymers, metal, ceramic or glass material. Thus, it has a broad range of applications such as filtration, textiles, energy, acoustics, as well as medicine [6].

1.2 Research background

In the past, traditional dressings such as bandages, cotton wool, lint and gauzes were used for the management of wounds. These dressings kept the wound dry by absorbing the wound exudate and prevented the entry of bacteria into the wound. But it has been shown in several studies that a warm and moist wound environment is more beneficial for successful wound healing [7]. Thus, advanced dressings are developed. They have biological activity on its own or release bioactive ingredients. In contrast, solutions, creams, and ointments which include bioactive molecules are not very effective for drug delivery to the wounds. Similarly, traditional dressings like gauze and cotton wool take no active part in the wound healing process. In advanced dressings, cleansing and debriding agents can remove necrotic tissue and have an antimicrobial effect which prevents or treats infection and proliferates growth factors to aid tissue regeneration. In addition, advanced dressings provide a moist environment [3]. Chronic wounds require a specific control of bacterial load and moisture level. These facts show that advanced dressings are suitable for the treatment of chronic wounds [8]. There are different types of advanced wound dressings which can be classified in a number of ways depending on e.g. the function in the wound (debridement, antibacterial, occlusive, absorbent, adherence), the type of material employed to produce the dressing (hydrocolloid, alginate, collagen) or the physical form of the dressing (ointment, film, foam, gel). Furthermore, dressings can be ranged into primary, secondary and island dressings. Primary dressings have physical contact with the wound, secondary dressings cover the primary dressing and island dressings have a central absorbent region which is surrounded by an adhesive portion. Classification criteria can be useful to select a dressing for specific wound treatment [7]. For instance, bioadhesive polymeric (synthetic, semisynthetic, or naturally derived) dressings are potentially useful in the treatment of local infection. They can achieve increased local concentrations of antibiotics and avoid high-systemic dose to reduce the patient exposure. Composite dressings which are made of synthetic and natural polymers are also known for controlled drug delivery. The degree of swelling, cross-linking density and degradation rate can be controlled, so that delivery kinetics are adapted according to the desired drug release. Basically, the drug release from polymeric formulations is influenced by some physical processes like hydration of the

polymer by fluids, swelling to form a gel, diffusion of drug through the polymer matrix and eventual erosion of the polymeric system. The contact of a dry polymeric dressing with a moist wound surface effects the permeation of the wound moisture into the polymer matrix [3].

Polymeric materials were used in the composition of wound dressings. They can be divided into natural inert, natural bioactive and synthetic polymers. Synthetic polymers commonly used in wound dressings include polyvinylalcohol, polyethylene oxide and PU [3]. Their hydrophilic nature procures important functional wound healing characteristics e.g. moisture absorption capacity and water vapour transmission [8] [3]. In addition, they are generally adhesive, biocompatible and has excellent mechanical strength which allows them to stay longer than the natural polymers. Hence, the natural or bioactive polymers are widely used in combination with synthetic materials which ultimately yields a better wound healing product [3]. In tissue engineering, many reports expose the fabrication of electrospun nanofibers by using biocompatible polymers for skin, vasculature, heart and cornea as well as bone substitutes and nerve regeneration [9], [3]. The suitable properties of electrospun nanofibers for biomedical applications consist in their highly porous structure which is similar to the structure of extracellular matrix, high levels of gas permeation and surface-to-volume ratio [10], [3]. These properties accelerate a lot of healing processes such as cell respiration, skin regeneration, moisture retention, removal of exudate and hemostasis [10]. Especially, the presence of a high porosity with small pores enables a good permeability for oxygen and water, a good adsorption of liquid and protects the wound from bacterial penetration and dehydration. In addition, the high surface area of the nanofibers support the migration of keratinocytes on the wound surface and can improve the healing process. Moreover, nanofibrous materials offer a local delivery of therapeutic agents, such as antibiotics or growth factors [3].

Modern materials for chronic wound therapy include hydrocolloids, alginates and hydrogels. These ingredients afford a moist environment stimulating wound healing. There are several new techniques for the healing of infected ulcers. For these methods triphala incorporated collagen is used. Another possibility of treatment is the application of an electric field to a flowing medium which forms microscale and nanoscale structures to compose functional wound healing materials. In more advanced applications, commercial tissue engineered skin agents based on acelluar (AllodermTM) or cell containing membranes (Dermagraft TM, ApligraftTM, EpicelTM, TransCyteTM, etc.) are developed. But this type of treatment is expensive and limited available. The application of biopolymer nanofibers provides a cheap and

effective alternative for wound treatment and can be used more easily standardized [6]. There are already different PU composite membranes for wound healing in research e.g. PU-propolis, PU-olive oil/copper oxide nanocrystals, and PU-nitric oxide [11, 12, 13]. But there is a research gap regarding particular dressings for chronic wounds. In this project, a novel electrospun nanofibrous composite membrane which is especially suited to chronic wound healing is developed. The novel membrane includes the agents pantothenic acid (PA) and sodium bicarbonate ($NaHCO_3$) which have cell proliferating and antibacterial effects and are already used in chronic wound treatment [14, 15, 16].

1.3 Problem statement

Chronic wounds are a global healthcare problem. For instance, in 2011 the Center for Disease Control (CDC) assessed that 25.8 million people in the US are affected by diabetes mellitus. Fifteen percent of them developed lower extremity ulcers and up to 25% of foot ulcer patients will eventually undergo amputation [10]. Each year about 1.7 million new cases of diabetes in adults are determined. Thus, it is expected that in 2050 one out of every three adults in the United States will have diabetes [17]. For this reason it can be inferred that the amount of people affected by chronic wounds will also increase.

Previous literature reviews have shown that the treatment of chronic wounds is very time-consuming in medical care. The wound healing needs about several months or years or never occurs so that amputation could be necessary. In all cases, the therapy of chronic wounds is expensive. Although standard materials like gauzes and bandages are cheap to manufacture, they have to be changed frequently [18]. Moreover, standard materials could be only used if wound healing does not need longer than four weeks. Otherwise, alternative methods would be needed [19]. For instance, oxygen-associated therapies offer an alternative solution to improve wound healing. In this therapy, a bag with pure oxygen which encases the wound is used. Due to the immobility during the treatment the oxygen-associated method is very time-consuming and inconvenient for the patient. The negative pressure therapy provides a further treatment option. It uses PU foam combined with a vacuum pump to reduce the wound area, to provide a moist wound environment and to support blood circulation. This method is only usable in small wounds. Another disadvantage is arised out of the immobility and potential pain during the regular therapy performed by medical staff. Stem cell application provides a further solution to treat wounds. It uses a natural material

which does not result in any side effect but it involves an invasive and very painful procedure [3]. Due to the mentioned disadvantages a novel nanofibrous membrane suited to the nature of chronic wounds was developed in this study. The production of nanofibers could be done using various processing techniques like drawing out, molecular self-assembly, thermally induced phase separation or electrospinning. But by manufacturing continuous polymeric nanofibers electrospinning is the only method which is able to manipulate and control the surface area, fiber diameter, porosity and base weight of the membrane [6].

In the present project, a novel nanofibrous composite membrane was developed using electrospinning technique. This model was suited to chronic wounds by the use of the antibacterial material $NaHCO_3$ and the cell-proliferating material PA. The antibacterial part may help the wound to merge from the inflammatory phase into the following proliferation phase. The cell-proliferating part of the novel product may support the proliferation phase, so that it may offer promising properties to improve the wound healing mechanism and the patient's compliance, shorten the medical care and reduce treatment costs. Therefore, the following problem statements reveal:

1. There is a continuous research to improve nanofibrous composite membranes for wound healing.
2. While developing a novel membrane for wound healing the physico-chemical properties have to be examined.
3. Blood compatibility is one of the important qualities which has to be evaluated for a wound healing membrane.

1.4 Research objectives

Main objectives are:

1. To fabricate and optimize a composite membrane (PU-$NaHCO_3$-PA) for wound treatment.
2. To characterize the physico-chemical changes of the composite membrane.
3. To analyse the hemocompatible nature of the composite membrane in order to assess its efficacy.

1.5 Scope of the work

Chronic wound healing is a major health issue faced by millions of people. The problem of slow healing makes it difficult for patients to recover completely and it also involves a continuous medical surveillance [18]. Hence, to reduce the economic burden on chronic wounds and improve the patient compliance a cost effective nanofibrous composite membrane is proposed in this research. Initially, the PU beads will be dissolved using dimethylformamide (DMF) solvent by adapting appropriate concentration. Then, the $NaHCO_3$ and PA composite solution will be prepared using suitable solvents. After preparing the PU and PU composite mixtures, the electrospinning process will be carried out using optimized flow rate, voltage and collector distance. Further to display the physico-chemical properties of prepared membranes, characterization tests like scanning electron microscopy (SEM) analysis, Fourier transform infrared spectroscopy (FTIR), porosity measurement and contact angle assay will be carried out. Finally, the hemocompatibility improvement of the composite membrane will be explored through *in vitro* assays of activated partial thromboplastin time (APTT), prothrombin time (PT) and hemolysis.

1.6 Significance of the study

The number of people affected by chronic wounds grows rapidly due to the increasing age of population [20][3]. Thus, the risk of delayed wound healing increases and the possibility of necessary amputations and in severe cases mortality rises [3]. Reports in the United States estimate that $25 billion are spent annually on the treatment of chronic wounds. The morbidity and costs of wound care create a need for cost-effective wound healing products [20]. A novel membrane suited to chronic wounds may improve the success rate of chronic wound healing and thus reduce hospital stays, nursing time and costs.

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