



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF ALGINATE HYDROCOLLOID FILM PERMEATED WITH
VICENIN 2 ON WOUND HEALING IN STREPTOZOTOCIN-INDUCED
DIABETICS SPRAGUE DAWLEY RATS***

TAN WOAN SEAN

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By

TAN WOAN SEAN

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirements for the
Degree of Doctor of Philosophy**

March 2019

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

EFFECTS OF ALGINATE HYDROCOLLOID FILM PERMEATED WITH VICENIN 2 ON WOUND HEALING IN STREPTOZOTOCIN-INDUCED DIABETICS SPRAGUE DAWLEY RATS

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TAN WOAN SEAN

March 2019

Chair : Associate Professor Sharida Fakurazi, PhD
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Wound healing impairment is one of the debilitating complications caused by diabetes mellitus (DM) which leads to morbidity and mortality. Previous preliminary report of our team has showed that Vicenin-2 (VCN-2) bioactive compound which possesses potent wound healing properties is identified from *Moringa oleifera* extract (MO). The general objective of this study is to determine the wound healing efficacy of VCN-2 hydrocolloid film on diabetic rats. Firstly, the cytotoxicity and medicinal effects of VCN-2 was investigated through *in vitro* studies. A new sodium alginate (SA) film formulation contained VCN-2 was then optimized and developed, the various physicochemical tests were conducted on the developed VCN-2 film. Subsequently, the VCN-2 film was then tested in streptozotocin (STZ) induced male Sprague Dawley rats. The diabetes induced rat models were subjected to wound infliction and topical treatment of SA hydrocolloid film with VCN-2 in respective doses (12.5, 25 and 50 μ M) for 14 days. Finally, the animals were sacrificed for wound healing assessment. Results indicated that VCN-2 hydrocolloid film has significantly reduced the production of pro-inflammatory cytokines and mediators (IL-6, IL-1 β , TNF- α , NF- κ B, iNOS and COX-2), while enhancing the production of healing growth factors (VEGF and TGF- β) in wound samples. During diabetic wound healing, the VCN-2 hydrocolloid film had influence inflammation phase by suppressing pro-inflammatory cytokines and proliferation phase by inducing growth factor expression. It has ameliorated prolong inflammatory process and enhanced healing through VEGF and TGF- β pathways. Taken together, VCN-2 hydrocolloid film is a promising new treatment to enhance impaired wound healing in hyperglycemic condition which prompts further investigation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**HIDROKOLOID LAPISAN ALGINAT MENGANDUNGI VICENIN-2
MEMPERCEPATKAN PENYEMBUHAN LUKA DALAM DIABETIK TIKUS
SPRAGUE DAWLEY YANG DISUNTIK DENGAN STREPTOZOTOSIN**

Oleh

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Pengerusi : Profesor Madya Sharida Fakurazi, PhD
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Antara komplikasi daripada diabetes mellitus (DM) adalah gangguan dalam penyembuhan luka yang berlanjutan sehingga boleh mengakibatkan morbiditi dan kematian. Laporan awal dari kajian pasukan kami terdahulu telah membuktikan bahawa sebatian bioaktif Vicenin-2 (VCN-2) ditemui di dalam ekstrak *Moringa oleifera* (MO) mempunyai sifat penyembuhan luka yang berkesan. Objektif umum kajian ini adalah untuk mengkaji keberkesanan lapisan hidrokoloid alginat yang mengandungi VCN-2 terhadap proses penyembuhan luka pada tikus yang diaruh diabetik. Pada peringkat awal, kesan ketoksikan dan nilai perubatan VCN-2 dikaji secara *in vitro*. Kemudian, lapisan natrium alginat (SA) yang mengandungi VCN-2 telah dihasilkan melalui proses formulasi dan pengoptimuman, diikuti dengan pelbagai ujian fizikokimia untuk memastikan kualiti lapisan VCN-2. Selepas itu, lapisan VCN-2 diuji terhadap tikus jantan Sprague Dawley yang telah diaruh menggunakan streptozotosin (STZ). Model tikus diabetik yang telah dihasilkan akan dilukai dan diberi rawatan topikal dengan lapisan VCN-2 dengan dos masing-masing (12.5, 25 dan 50 μ M) selama 14 hari. Selepas tempoh rawatan, tikus tersebut akan dikorbankan untuk penilaian penyembuhan luka. Hasil kajian menunjukkan bahawa penggunaan lapisan VCN-2 bukan sahaja dapat mengurangkan pengeluaran sitokin dan perantara pro-radang (IL-6, IL-1 β , TNF- α , NF- κ B, iNOS dan COX-2), malahan ia juga telah meningkatkan pengeluaran faktor pertumbuhan penyembuhan (VEGF dan TGF- β) dalam sampel luka. Semasa penyembuhan luka diabetik, lapisan VCN-2 telah mempengaruhi fasa keradangan melalui pengurangan hasilan sitokin. Ia juga mendorong hasilan faktor pertumbuhan dalam fasa percambahan. Lapisan VCN-2 telah memperbaiki proses keradangan dan meningkatkan penyembuhan melalui mekanisme VEGF dan TGF- β . Kesimpulannya, lapisan VCN-2 berpotensi dalam rawatan pemulihan dan ia telah meningkatkan penyembuhan luka dalam keadaan hiperglisemia.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ALN	Allantoin
AP-1	Activator protein-1
ATCC	American type culture collection
BSA	Bovine serum albumin
CCK-8	Cell counting kit-8
COX-2	Cyclooxygenase-2
DEX	Dexamethasone
DFUs	Diabetic foot ulcers
DM	Diabetes Mellitus
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
ELISA	Enzyme linked immunosorbent assay
ERK	Extracellular signal-related kinase
HDF	Human dermal fibroblast
HPLC	High-performance liquid chromatography
Hs27	Human skin fibroblast cell line
HUVEC	Human umbilical vein endothelial cells
HIF1 α	Hypoxia inducible transcription factor-1 alpha
I κ B	Inhibitor kappa B
IKK	I κ B-kinase
iNOS	Inducible nitric oxide synthase
IL	Interleukin
LPS	Lipopolysaccharide
mTOR	Mammalian target of rapamycin
MMPs	Matrix metalloproteinases
MO	<i>Moringa oleifera</i>
MTT	3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MVTR	Moisture vapor transmission rate
NO	Nitric oxide
NF- κ B	Nuclear factor-kappa B
PBS	Phosphate buffered saline
PGE2	Prostaglandin E2
RPM	Room temperature
STZ	Streptozotocin
TLRs	Toll-like receptors
TNF- α	Tumor necrosis factor-alpha
TGF- β	Transforming growth factor-beta
VEGF	Vascular endothelial growth factor
VCN-2	Vicenin-2

CHAPTER 1

INTRODUCTION

1.1 Background

Wound healing process in patients with Diabetes Mellitus (DM) is deteriorated due to hyperglycemic condition and it leads to major chronic complications, such as risk of diabetic foot ulcers (DFUs) development (Boulton et al; 2005). Diabetes mellitus is described as a metabolic disorder characterized by hyperglycemia that is a result of inadequate insulin production or insensitivity of tissues to respond to insulin action, or both (Ozougwu et al., 2013). According to George et al., (2010), it was estimated that about 285 million people in the world were suffering from DM. More than 439 million victims are assumed to be having the disease by the year 2030 (Shaw et al., 2010). While, 25% of diabetic patients will develop DFUs during their lifetime and 85% of them will undergo lower extremity amputation (Singh et al., 2005, Shojaiefard et al., 2008).

Chronic DFUs is one of the most common complications in DM which occurs due to altered wound healing. Essentially, wound healing takes place at an optimal rate in healthy individuals but it is usually delayed or impaired in patients with diabetic conditions. Under persistent hyperglycemic condition, the mechanism of delayed wound healing is multifactorial, including prolong inflammatory stage and delayed proliferation and remodeling stage (Qiu et al., 2006). Moreover, open wounds of DFUs are highly susceptible to micro-bacterial infections especially *Staphylococcus*, *Enterococcus*, *Pseudomonas*, and *Proteus* species which will further deteriorate the impaired wound healing during hyperglycemic condition (James et al., 2008).

Wound dressings form a key part of wound management to achieve a rapid wound closure and minimal acceptable scar. Medicinal wound dressing is an effective approach for reaching an optimum milieu for wound healing through impregnation of therapeutic drugs of different pharmacological actions (Ovington, 2007; Pawar et al., 2013). Dressings cast from hydrocolloid films are the main choices among the popular wound dressings owing to their high absorption ability via strong hydrophilic gel formation characteristics (Lanel et al., 1997). Besides, they are environment-friendly and sustainable. Hydrocolloid is able to absorb exudates and form gel to provide a moist environment which is essential for healing. The simple, thin semi-permeable films also permit for the easy inspection of wound condition, water vapor and gases exchange for effective restoration of wound (Rezvanian et al., 2016).

Bioactive compounds derived from natural products have been widely used as a source of prospective therapeutic agents for various diseases including alternated wound healing due to its safety, efficacy and lesser side effect properties. Bioactive compound Vicenin-2 (VCN-2) is a potential pharmaceutical

drug that is found in many species of plants such as *M. oleifera* (MO), *U. circularis*, *C. subternata*, *A. capillaris*, *C. aurantium*. This compound possesses many prospective anti-diabetics, anti-oxidant and anti-inflammatory properties (Islam et al., 2014; Verspohl et al., 2013; Grael et al., 2005; Muhammad et al., 2013; Tan et al., 2015). Previous studies by our group members have demonstrated that VCN-2 bioactive compound from MO leaves enhance the *in vitro* cell proliferation and cell migration of human dermal fibroblast (HDF) (Muhammad et al., 2013). Furthermore, VCN-2 active compound accelerates *in vivo* animal wound healing models in hyperglycemic condition (Muhammad et al., 2015). Fakurazi et al. (2012) reported that ethanolic extracts of MO flower contains higher phenolic content and antioxidant capacity than its leaves. Thus, anti-inflammatory properties of MO flower are examined and explored in the present study.

Therefore, the main goal of this study is to investigate the wound healing effect of a new formulation hydrocolloid dressing, a sodium alginate biomaterial scaffold with synthetic VCN-2 for wound healing through human dermal fibroblast by *in vitro* and Type 1 diabetes Sprague Dawley rat by *in vivo* assays. This study offers an alternative new formulation in wound therapy and is especially useful to facilitate wound healing in hyperglycemic condition.

1.2 Problem statement

World Health Organization (2016) has declared the prevalence of DM among adults 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. The percentage was observed to rise more rapidly in middle, low-middle and low-income countries which includes Malaysia (Tee and Yap 2017). In the Asia-Pacific Region, China has the highest prevalence of DM followed by India, while the prevalence in Malaysia was reported to be 17.6%, similar incidence was also observed in others Southeast Asia countries (Nanditha et al., 2016). According to the National Health and Morbidity Surveys (NHMS, 2011), the statistical data has shown that prevalence rate of DM in Malaysia has risen much faster than expected for the past two decades, with the increase in trend being more than double from year 1996 to 2015. Furthermore, there is also a dramatic increase of prevalence in DM disease in younger groups, 20–24 years old (5.9%) and 25–29 years old (8.9%) in 2015. More worrisome there might be about a third (or 36%) of undiagnosed in Malaysia.

Diabetes is leads to long term complications such as cardiovascular disease, retinopathy, nephropathy, neuropathy, and impaired wound healing. Out of all the diabetic chronic complications, diabetic foot ulcers (DFUs) take the greatest toll. The gravest outcome from DFUs is lower extremity amputation, which may also be accompanied by pain, disability, risk of depression and decreased quality of life (Singh et al., 2005). According to Ismail et al., (2007), such amputations due infected ulcers consequently lead to an increase of threefold risk of death within 18 months. In addition, International Diabetes Federation (IDF, 2015) has reported that one lower limb amputation in diabetic patients occurred around the world every 30 seconds.

Diabetes mellitus is a public health challenge worldwide that imparts loss in health and devastating economic burden on patients, families, healthcare machinery and society. DM patients have poor health related quality of life and often need prolonged hospital admission days for care. The cost care for a DFUs patient is 5.4 times higher when compared to a patient without ulcer (Driver et al., 2010). Deterioration of DFUs due to impaired wound healing can be preventable through a good wound care management. Thus, an ideal wound dressing with affordable price and superior effect on healing is urgently needed as an alternative treatment.

1.3 Justification

Vicenin-2 has been identified as a potential compound responsible to enhance healing in wound of diabetic animal model (Muhammad et al., 2013; Muhammad et al., 2015). In wound management, ideal wound dressing with potential medicinal drug and optimum drug release control ability can provide rapid wound closure and leave minimal scar. The proper and immediate coverage of wound surface with adequate dressing allow wound protection and speedy healing. This study offers an alternative new formulation in wound therapy especially in hyperglycemic condition, through developing wound dressing of VCN-2 hydrocolloid film based on alginate polymer. The developed hydrocolloid film is able to absorb wound exudate and provide hydration for healing. It will also act as a drug vehicle to modulate VCN-2 concentration, sustain the release of VCN-2 to wound site and enhance the treatment efficacy overall.

1.4 Hypothesis

The hypothesis of study is topical application VCN-2 hydrocolloid film enhances wound healing in hyperglycemic condition using *in vitro* and *in vivo* wound models.

1.5 Objectives

1.5.1 General objective

To evaluate the wound healing efficacy of topical administration of VCN-2 hydrocolloid film in diabetes using *in vitro* and *in vivo* experimental wound models.

1.5.2 Specific objectives

- To investigate anti-inflammatory activity of 80% hydro-ethanolic extract of MO flower on lipopolysaccharide (LPS) induced RAW 264.7 macrophages
- To determine wound healing potential of VCN-2 in human dermal fibroblast (HDF) through *in vitro* experiment model
- To optimize and develop a novel hydrocolloid topical formulation with VCN-2 that accelerates wound healing
- To evaluate efficacy of hydrocolloid film containing VCN-2 in healing the wound of diabetes animal model.

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