DEVELOPMENT OF FIBROIN/ALOE GEL EXTRACT FILM FOR APPLICATION IN WOUND HEALING

PAICHIT INPANYA

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science Degree
in Pharmacology and Biomolecular Sciences (International Program)
May 2011

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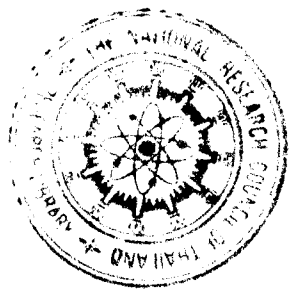

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This thesis entitled "Development of Fibroin/Aloe Gel Extract Film for Application in Wound Healing" submitted by Paichit Inpanya in partial fulfillment of the requirements for the Master of Science Degree in Pharmacology and Biomolecular Sciences (International Program) is hereby approved.

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Paichit Inpanya

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ABSTRACT

The purpose of the present study was to develop the fibroin film containing aloe gel extract for application in wound healing. Silk fibroin degummed fibers were firstly prepared by dissolving them in calcium chloride solution. The fibroin solution was subsequently lyophilized. DC protein assay kit indicated the protein content in an amount of 94% w/w for the fibroin extract. Additionally, the chemical properties of lyophilized fibroin extract were characterized by SDS-PAGE and FTIR. SDS-PAGE analysis showed the specific band of light chain at approximately 40 kDa, which indicated that the extraction procedure was performed correctly. FTIR spectra of the isolated fibroins showed the silk I structure, which indicates its property for the water-soluble state and easily converts to β-sheet structure. For aloe gel extraction, protein containing in aloe gel solution initially precipitated with $(NH_4)_2SO_4$ until achieve 35% saturation [of $(NH_4)_2SO_4$] at 4 °C. The remaining fraction of aloe gel solution was then precipitated until $(NH_4)_2SO_4$ reaching to 55% saturation at 4 °C. SDS-PAGE and FTIR were used to characterize the chemical properties of lyophilized aloe gel extracts obtaining from both 35% and 55% saturation of $(NH_4)_2SO_4$. The amount of protein contents were 6.8 and 4.8% w/w for 35% $(NH_4)_2SO_4$ and 55% $(NH_4)_2SO_4$ protein fractions, respectively. SDS-PAGE of isolated aloe gel extracts provided the bands of molecular weight of glycoprotein at approximately 22, 24, and 35 kDa.

The lyophilized fibroin and aloe gel extracts were dissolved in lactic acid (pH 3.8-4.0) prior to prepare the developed film by casting technique. The fibroins were

blended either with aloe gel extracted from 35% or 55% saturation of $(NH_4)_2SO_4$. The developed films were prepared into three formulations including 1) fibroin 2% w/v (F2%), 2) fibroin 1.95% w/v blended with 0.05% w/v of aloe gel extract with 35% $(NH_4)_2SO_4$ (F1.95%/A35), and 3) fibroin 1.95% w/v blended with 0.05% w/v of aloe gel extract with 55% $(NH_4)_2SO_4$ (F1.95%/A55). All formulations of the developed films were studied for the protein content, SDS-PAGE, physicochemical properties, stability properties, and biological studies. Tensile strength values of all developed films were in range of 21-23 MPa. Moreover, the water uptake and swelling ratios of the developed films provided in range of 37-43% and 0.6-0.8 times of their dry weights, respectively. These films showed the retain ability that is important for application in wound healing. The biological studies of developed films were carried out on the human primary skin fibroblasts. Cell viability, cell adhesion, and cell migration on the developed films are quantified by XTT assay. The biological functions of the fibroblasts on the developed film were investigated by determination of the expression of α-SMA and bFGF by immunocytochemistry. The obtained results showed that all developed films were non-toxic to human primary fibroblasts and acted as a good promoting matrix for cell growth.

Streptozotocin induced-diabetic rats were used as animal model to investigate the potential of the developed film on wound healing capability. All developed films were sterilized by ultraviolet light exposure under laminar cabinet for 16 hrs before application to the wound healing on the induced-diabetic rats. Diabetic rat with non-treatment, a small dented wound was still appeared indicating that epithelialization was not well completed. Interestingly, the wounds were completely healed at day 21 after treatment with all developed films. Histology study at day 21 indicated that the dermis layer of diabetic animals treated with the developed film especially F1.95%/A35 film had an increase of collagen content. The fibers showed similar fibrillar arrangement as seen in the normal rat. In additional, the fibroblast cells were regular arrangement and loosely distribution. It provided indications that it could recover the wound nearly to normal skin. Taken together, this study reveals the potential of the blended fibroin/aloe gel extract film for application in would healing.

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ABBREVIATIONS

2-D	=	two dimensional
3-D	=	three dimensional
α	=	alpha
β	=	beta
γ	=	gamma
μm	=	micrometer
μl	=	microliter
bFGF	=	basic fibroblast growth factor
°C	=	degree Celsius
CH_3COO	=	acetyl
cm^{-1}	=	inverse centimeter
cm^2	=	square centimeter
Cont.	=	continued
CO_2	=	carbon dioxide
COOH	=	carboxyl
Da	=	dalton
dl	=	deciliter
DMEM	=	Dulbecco's Modified Eagle Medium
DNA	=	Deoxyribonucleic acid
ECM	=	extracellular matrix
EDTA	=	ethylenediaminetetraacetic acid
EGF	=	epidermal growth factor
FBS	=	fetal bovine serum
FITC	=	fluorescein isothiocyanate
FTIR	=	Fourier transform infrared spectroscopy
g	=	gram
GAG	=	glycosaminoglycan
H-chain	=	heavy-chain
hr	=	hour(s)

ABBREVIATIONS (CONT.)

IGF	=	insulin like growth factor
IgG	=	immunoglobulin G
IR	=	infrared spectroscope
KBr	=	potassium bromide
kDa	=	kilodalton
kg	=	kilogram
KGF	=	keratinocyte growth factor
l	=	liter
L-chain	=	light-chain
M	=	molar
mg	=	milligram
min	=	minute
ml	=	milliliter
mm	=	millimeter
mm^2	=	square millimeter
mM	=	millimolar
MPa	=	megapascal
MTT	=	thiazolyl blue tetrazolium bromide or 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
MWCO	=	molecular weight cut-off
N	=	newton
$(NH_4)_2SO_4$	=	ammonium sulfate
nm	=	nanometer
NO	=	nitric oxide
OH	=	hydroxyl
PBS	=	phosphate buffer saline
PBST	=	phosphate buffer saline containing 0.1% Triton-X 100
PDGF	=	platelet-derived growth factor
PDGF	=	platelet-derived growth factor

ABBREVIATIONS (CONT.)

pH	=	power of hydrogen ion concentration
RGD	=	Arginine-Glycine-Aspatic acid
rpm	=	round per minute
SD	=	standard deviation
SDS-PAGE	=	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
sec	=	second(s)
SEM	=	Scanning Electron Microscope
SM	=	smooth muscle
SMA	=	smooth muscle actin
TGF	=	transforming growth factor
TNF	=	tumor necrosis factor
UV	=	ultraviolet
VEGF	=	vascular endothelial growth factor
V	=	volt
v/v	=	volume by volume
w/v	=	weight by volume
w/w	=	weight by weight
XTT	=	sodium 3'-[-1-(phenylaminocarbonyl)-3,4-tatrazolium]-bis (4-methoxy-6-nitro) benzene sulfonicacid hydrate