EVALUATION OF THE EFFECTS OF COLLAGEN/CHITOSAN SCAFFOLD ON WOUND HEALING

SIRINTIP INTARAPASIT

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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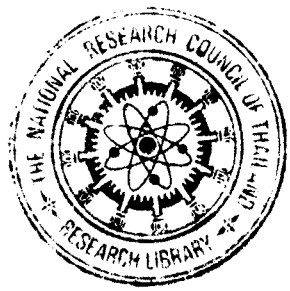

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This thesis entitled "Evaluation of the Effects of Collagen/Chitosan Scaffold on Wound Healing" submitted by Sirintip Intarapasit in partial fulfillment of the requirements for the Master of Science Degree in Pharmacology and Biomolecular Sciences (International Program) is hereby approved.

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Sirintip Intarapasit

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ABSTRACT

This study was aimed to evaluate the effects of the collagen/chitosan scaffold for application in wound treatment .Lyophilized collagen isolated from bovine tendon demonstrated white in color and spongy-like characteristic. DC protein assay Kit indicated the protein content in isolated collagen of 87.97%±3.4% w/w. Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) exhibited 3 bands representing one β chain, α_1 chain and α_2 chain. The fourier transform infrared spectroscopy (FT-IR) spectra of isolated collagen were displays at 3424, 2925, 2656, 1560 and 1241 cm^{-1} which are characteristic of the amide A, amide B, amide I, amide II and amide III bands, respectively. A spectrum of the isolated collagen was not different from that of the standard commercial collagen.

Isolated collagen and crab chitosan, with degree of deacetylation more than 90%, were blended in ratio 7 and 3 of 3% by weight of total polymer, 0.1% glutaraldehyde (GA) were used throughout in this study to crosslink collagen and chitosan. The collagen/chitosan scaffold was successively fabricated by casting technique. It provided about 60 μm of thickness and showed brown in color. Morphology of the scaffold was determined by scanning electron microscope (SEM).

Subsequently, the scaffold was investigated to the cytotoxicity toward Human Dermal Fibroblasts (HDFs) including cytocompatibility, cell adhesion, cell distribution, cell proliferation and cell functions including alpha-smooth muscle actin (α-SMA) and basic fibroblast growth factor (bFGF) proteins expression by

immunocytochemistry. The obtained results indicated that the developed scaffold showed good cytocompatibility, supporting cell attachment, accelerating cell proliferation and distribution, and retaining to express normally α-SMA and bFGF proteins.

Moreover, the efficacy of collagen/chitosan scaffolds on wound healing was evaluated *in vivo* by using the domestic pigs. The results revealed that the collagen/chitosan scaffolds with HDFs and Human keratinocytes (HaCaTs) co-cultured started to show the smallest wound area and to present the highest percentage of re-epithelialisation for 14 days of healing period compared to other treatments; povidone iodine treatment, non treatment, cell-free col/chi scaffold, the HDFs cultured on the col/chi scaffold.

From histological study, fibroblasts-kertinocyted co-cultured on the scaffold showed the most similarity of skin structure with normal porcine skin. Therefore, the collagen/chitosan scaffolds with HDFs and Human HaCaTs co-cultured may be a promising wound dressing to accelerate the wound healing potentially for acute wound patients.

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ABBREVIATIONS

aFGF	=	acidic fibroblast growth factor
α	=	alpha
bFGF	=	basic fibroblast growth factor
BSA	=	bovine serum albumin
β	=	beta
°C	=	degree celsius
CO_2	=	carbondioxide
Cont.	=	continue
Da		dalton
DD	=	degree of deacetylation
DMEM	=	dulbecco's Modified Eagle's Medium
DMSO	=	dimethysulfoxide
ECM	=	extracellular matrix
EGF	=	epidermal growth factor
FACIT	=	fibril associated collagens with interrupted triple helices
FBS	=	fetal bovine serum
FDA	=	the US Food and Drug Administration
FTIR	=	fourier transform infrared
g	=	gram
GA	=	glutaraldehyde
GAG	=	glycosaminoglycan
g	=	gram
HaCaT	=	human keratinocyte cell
HDF	=	human dermal fibroblast cell
hr	=	hour (s)
IGF	=	insulin-like growth factor
IL-1	=	interleukin-1

ABBREVIATIONS (CONT.)

kD	=	kilodalton
KGF	=	keratinocyte growth factor
kgf	=	kilogram force
M	=	molar
mg	=	milligram
m^2	=	square meter
mm	=	millimeter
μm	=	micrometer
μL	=	microliter
mg	=	miligram
mRNA	=	messenger ribonucleic acid
MTT	=	3-(4,5-Dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	=	molecular weight
NH_4OH	=	ammonium hydroxide
nm	=	nanometer
PBS	=	phosphate buffer saline
PDGF	=	platelet-derived growth factor
RGD	=	arginine-glycine-aspartate groups
SDS-PAGE	=	sodium dodecylsulphate polyacrylamide gel electrophoresis
S.D.	=	standard deviation
SEM	=	scanning electron microscopy
SMA	=	smooth muscle actin

ABBREVIATIONS (CONT.)

TIMP	=	tissue inhibitor of metallo-protease
VEGF	=	vascular endothelial growth factor
w/v	=	weight by volume
XTT	=	sodium 3'-[1-(phenylamino carbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate