Title	: DEVELOPMENT OF MATRIX FROM COLLAGEN/CHITOSAN OR COLLAGEN/PVA BLENDED FOR APPLICATION IN SKIN TISSUE ENGINEERING
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Abstract

This study was aimed to develop the matrix using the blends of collagen/chitosan or collagen/polyvinyl alcohol (PVA) for application in tissue engineering. The type I collagen used throughout the study was isolated from bovine tendon and characterized by fourier transform infrared spectroscopy (FTIR) and sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Various types of matrix were prepared by casting technique and proportions of isolated type I collagen and chitosan or PVA were varied in ratio of 9:1 to 6:4 (collagen/chitosan) and 9:1 to 8:2 (collagen/PVA), with various amounts of total polymer (0.5-4% w/v). The types of chitosan used in this study included shrimp chitosan with molecular weight 30,000 and degree of deacetylation (DD) 85-90% (shrimp chitosan MW 30,000), shrimp chitosan with molecular weight 100,000 and DD 90% by approximately (shrimp chitosan MW 100,000), shrimp chitosan with molecular weight 100,000-1,000,000 and DD more than 90% (shrimp chitosan MW 100,000-1,000,000), crab chitosan with molecular weight 100,000-1,000,000 and DD more than 90%(crab chitosan MW 100,000-1,000,000), and squid chitosan with molecular weight 100,000-1,000,000 and DD more than 90% (squid chitosan MW 100,000-1,000,000). In addition, PVA with different molecular weight including molecular weight of 72,000 (PVA MW 72,000) and 145,000 (PVA MW 145,000) were used in the blended polymeric solution. The effects of various types of crosslinker including

glutaraldehyde (GA) and β-glycerolphosphate (GP) on the physicochemical characteristics were also demonstrated. The crosslinkers were used in various concentrations; 0.05-0.15% of GA and 0.5-1.5% of GA by weight of total polymer. Physicochemical characteristics and surface morphology of the prepared matrices were determined by visualization and electromicroscopic technique, respectively. The mechanical properties including tensile strength and % elongation at break of the matrices were evaluated by the tensometer. Swelling and biodegradation studies were carried out in phosphate buffer saline (PBS, pH 7.4) containing collagenase enzyme. The selected matrix was evaluated the cytotoxicity including cytocompatibility, cell adhesion, cell proliferation, and genes expression. The obtained results indicated that the physicochemical properties of the matrix depended on the amount, MW and type of the polymer blended, and the amount and type of crosslinker. Introducing chitosan together with GA crossllinking could improve the physicochemical properties of the collagen matrix including tensile strength, % elomgation at break, swelling property and degradation time. The developed matrix, collagen/crab chitosan MW 100,000 - 1,000,000 in ratio of 7 to 3 crosslinked with 0.1% GA by weight of total polymer (3%), exhibited the suitable strength (tensile strength = 8.45 kgf/mm^2), flexibility (% elongation at break = 2.38) and swelling (76%) properties. Moreover, it could promote cell proliferation and prolongs 2 months expression of keratinocyte growth factor receptor (KGFR) and keratin (K4-14) genes, which are the important genes for keratinocyte growth. Therefore, the developed matrix from this study has potential for further application in skin tissue engineering.

ABBREVIATIONS

Abs.	absorbance
α	alpha
β	beta
BSA	bovine sreum albumion
°C	degree celsius
cm^2	Centrimetre
COH	aldehyde group
Cont.	continued
COOH	carboxyl group
DD	degree deatylation
DEPC	diethylpyrocarbonate
EDTA	ethylenediaminetetra-acetic acid
EGF	epidermal growth factor
FACIT	fibril-associated collagens with interupted triple helices
FTIR	fourier transform infrared spectroscopy
g	gram
GA	glutaraldehyde
GAG	glycosaminoglycan
GP	glycerolphosphate
γ	gramma
hr	hour(s)
HyP	hydroxyploline
K	keratin
KGF	keratinocyte growth factor
kgf	kilogram force
KGFR	keratinocyte growth factor receptor
KX	kilo-fold

ABBREVIATIONS (CONT.)

M	molar
MPa	mega Pascal
m^2	square meter
µm	micrometer
µl	micro liter
mg	milligram
min	minute
ml	millilitre
mm	millimetre
MW	molecular weight
N	newton
NaCl	sodium chloride
NH_2	amine group
NH_4OH	sodium hydroxide
nm	nanometre
$OPO(O^-)^2$	phosphate ion
OsO_4	osmium tetraoxide
PBS	phosphate buffer saline
PGA	polyglycolic acid
PLLA	poly –L- lactic acid
PVA	polyvinyl alcohol
RNA	ribosomal neucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
SD	standard deveation
SDS- PAGE	sodium dodecylsulphate polyacrylamide gel electrophoresis
SEM	scanning electron microscope
TGF	transforming growth factor

ABBREVIATIONS (CONT.)

w/v	weight by volume
w/w	weight by weight
UV	ultraviolet
XTT	sodium 3'-[1-(phenylaminocarbonyl)-3, 4-tetrazolium]-bis (4-methoxy-6nitro) benzene sulfonicacid hydrate