DEVELOPMENT OF CHITOSAN/FISH SCALES COLLAGEN/GLYCERIN/ NANO-ZnO 3D POROUS SCAFFOLDS FOR SKIN TISSUE ENGINEERING AND REGENERATION APPLICATIONS

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ABSTRACT

This study was aimed to develop three-dimensional (3D) porous scaffold from blending of chitosan/fish scales collagen/glycerin/nano zinc oxide for skin tissue engineering and skin regenerating template. 3D porous scaffolds were fabricated through freeze dry technique. The 3D porous scaffolds were subjected to phosphate buffer solution (PBS) washing while dehydrothermal (DHT) treatment and chemical treatment using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC)-N-hydroxysuccinimide (NHS) were used to create crosslinks. The mechanical properties, porosity and swelling ratio of the 3D porous scaffolds have been determined using general procedures. Crosslink density was calculated using the Fourier Transform Infrared (FTIR) technique while the crosslinking degree has been determined using 2,4,6-trinitrobenzenasulfonic acid (TNBS). Field emission scanning electron microscopy (FESEM) analysis was used to examine the morphology of 3D porous scaffolds. The biodegradation rate and cytocompatibility tests were performed using common procedure. The results showed that the 3D scaffolds were porous with interconnected pores with average pore sizes between 100 and 200 microns while mechanical properties and biostability fulfilled the requirements of the tissue engineering scaffold excepted for PBS washing treated scaffolds. In vitro analysis using human fibroblast and keratinocyte indicates that all 3D porous scaffolds were good cytocompatibility but scaffold containing nano zinc oxide and scaffold containing high fish scales collagen content were excellently facilitated cell proliferation and adhesion. In conclusions, the 3D porous scaffolds treated with DHT and EDAC-NHS and also the addition of zinc oxide nanoparticles and various chitosan/collagen/glycerin ratios showed excellent biostability. Furthermore, 3D scaffolds were also cytocompatible with mechanical strengths suitable for skin tissue engineering applications. The implication of this study indicate that porous 3D scaffolds produced most suitable to be used as skin tissue engineering and skin regeneration template applications.

PEMBANGUNAN TEMPLAT BERLIANG 3D KITOSAN/KOLAGEN SISIK IKAN/GLISERIN/NANO-ZnO UNTUK APLIKASI KEJURUTERAAN TISU DAN PERTUMBUHAN SEMULA KULIT

ABSTRAK

Kajian ini bertujuan membangunkan templat berliang tiga-dimensi (3D) hasil adunan kitosan/kolagen sisik ikan/gliserin/nano zink oksida untuk aplikasi kejuruteraan tisu dan pertumbuhan semula kulit. Templat berliang 3D dibangunkan melalui teknik pengering pembekuan. Templat 3D yang dibina dibasuh menggunakan larutan penampan fosfat (LPF) manakala teknik rawatan nyahhidroterma (RNH) dan rawatan kimia menggunakan 1-ethil-3-(3-dimethilaminopropil)karbodiimide (EDAC)-N-hidroksisuksinimid (NHS) digunakan untuk menghasilkan taut silang. Kekuatan mekanik, porositi, dan nisbah bengkakan templat 3D telah ditentukan menggunakan prosedur umum. Ketumpatan taut silang dikira menggunakan teknik spectrometer infra merah (FTIR) manakala darjah taut silang telah ditentukan menggunakan asid 2,4,6-trinitrobenzenasulfonik (TNBS). Analisis mikroskop electron pengimbas pancaran medan (FESEM) digunakan untuk mengkaji morfologi template 3D. Ujian kadar biodegradasi dan keserasian biologi telah dilakukan menggunakan teknik umum. Dapatan kajian menunjukkan bahawa templat 3D adalah poros dan mempunyai liang saling berhubung dengan purata saiz liang antara 100 dan 200 mikron manakala sifat mekanik dan kestabilan biologi memenuhi keperluan templat kejuruteraan tisu kecuali templat yang dibasuh menggunakan LPF. Analisis *in-vitro* menggunakan tisu fibroblas dan keratinosit manusia menunjukkan bahawa templat 3D adalah bioserasi tetapi templat yang mengandungi nano zink oksida dan templat yang mengandungi kandungan kolagen sisik ikan yang tinggi lebih menggalakan percambahan dan lekatan sel yang tinggi. Kesimpulan daripada kajian ini menunjukkan templat 3D berliang yang dirawat menggunakan RNH dan EDAC-NHS serta penambahan nano zarah zink oksida dan pelbagai nisbah kitosan/kolagen/gliserin menunjukkan kestabilan biologi yang sangat baik. Begitu juga templat 3D bersifat bioserasi dengan kekuatan mekanikal yang sesuai untuk aplikasi kejuruteraan tisu kulit. Implikasi daripada kajian ini adalah templat 3D berliang yang dihasilkan sangat sesuai digunakan sebagai templat dalam aplikasi kejuruteraan tisu dan pertumbuhan semula kulit.

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%	Percentage
°C	Degree Celsius
µL	Microliter
µm	Micrometer
3D	Three Dimensional
ANOVA	Analysis of Variance
cm	Centimeter
cm^{-1}	Reciprocal centimeter
cm^2	Centimeter Squared
CV	Coefficient of Variance
DDA	Degree of Deacetylation
DHT	Dehydrothermal
DMEM	Dulbecco's Modified Eagle's Medium
DNA	Deoxyribonucleic Acid
ECM	Extracellular Matrix
EDAC(EDC)	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetra Acetic Acid
EDX	Energy-Dispersive X-ray
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FE-SEM	Field Emission Scanning Electron Microscopy

FGF	Fibroblast Growth Factor
FT-IR	Fourier Transform Infrared
FTTC	Fluorescein Isothiocyanate
GAGs	Glycosaminoglycan's
GF-β	Growth Factor-β
h	Hour
H & E	Hematoxylin and Eosin
H_2O	Water
H_2O_2	Hydrogen Peroxide
HAp	Hydroxyapatite
HBEGF	Heparin Binding Epidermal Growth Factor
HCl	Hydrochloric Acid
HKGF	Keratinocyte Growth Supplement
IGF	Insulin Growth Factor
IL-1	Interleukin-1
KeV	KiloelectronVolt
KGF	Keratinocyte Growth Factor
KPa	Kilo Pascal
LL	Lower Left
LR	Lower Right
MES	2-(N-morpholino)ethanesulfonic Acid
Mg	Milligram
Ml	Millimeter
mM	Millimole
MPa	MegaPascal