ELECTROSPUN FIBROUS AND SOLVENT-CAST FILM SCAFFOLDS FOR TISSUE ENGINEERING APPLICATION

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ABSTRACT

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Tissue engineering is an emerging technology in the contemporary human health care administration, in which the basic understanding of cellular biology and bioengineering are combined together for developing feasible substitutes to aid in the clinical treatment. The primary objectives of these substitutes are to restore, maintain and/or improve tissue functions by mimicking the structure and biological function of native extracellular matrix (ECM) proteins. In the present contribution, natural biocompatible polymer, Chitosan and synthetic biocompatible polymer, poly(3-hydroxybutyrate) were fabricated into fibrous membranes by electrospinning technique. The 3D structure and topography of the obtained electrospun fibrous membranes resemble those of the collagen bundles in the natural ECM. The potential for use of the electrospun fibrous membranes as tissue scaffolds was evaluated with different cell types in terms of the cytotoxicity, attachment and the proliferation of the cells as well as the morphology of the seeded and the cultured cells. For enhancing the cell-scaffold interaction, the surface treatment was performed. These treatments not only improve the hydrophilicity on the surface substrates, but also provide the necessary active sites for interacting with cell-adhesive molecules such laminin. The results from *in vitro* cell studies suggested that the surface topography and surface chemistry have a significant influence on the particular cell responding. All of these results emphasized the importance of the surface properties on the cellular behaviour.

บทคัดย่อ

ผกากรอง สังข์เสนาะ : เส้นใยอิเล็คโตรสปันและแบบแผ่นฟิล์มสำหรับการประยุกต์ด้านวิศวกรรมเนื้อเยื่อ (Electrospun Fibrous and Solvent-Cast Film Scaffolds for Tissue Engineering Application) อ. ที่ปรึกษา: ศาสตราจารย์ ดร. พิชญ์ ศุภผล 170 หน้า

วิศวกรรมเนื้อเยื่อเป็นเทคโนโลยีที่มีความเร่งด่วนในการพัฒนาความรู้เพื่อใช้ในการรักษาผู้ป่วย ซึ่งในการศึกษาพัฒนาจำเป็นต้องมีความรู้พื้นฐานทางด้านชีววิทยาของเซลล์และวิศวกรรมชีวภาพ เพื่อประยุกต์ใช้ในการพัฒนาวัสดุทดแทนเนื้อเยื่อสำหรับใช้ในทางการรักษา เป็นหมายหลักในการใช้งานวัสดุทดแทนเนื้อเยื่อคือการนำไปทดแทน หรือปรับปรุงการทำงานของเนื้อเยื่อโดยการจำลองแบบโครงสร้างของเนื้อเยื่อโปรตีนตามธรรมชาติ ในงานวิจัยนี้เราได้ทำการขึ้นรูปแผ่นเส้นใยไคโตซานซึ่งเป็นพอลิเมอร์จากธรรมชาติ และแผ่นเส้นใยพอลิไฮดรอกซิบิวทิเรตซึ่งเป็นพอลิเมอร์สังเคราะห์ด้วยกระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิตย์ โครงสร้างสามมิติและลักษณะพื้นผิวของแผ่นเส้นใยอิเล็คโตรสปันที่เตรียมได้สามารถจำลองลักษณะของเส้นใยคอลลาเจนที่ธรรมชาติ ความเป็นไปได้ในการใช้แผ่นเส้นใยอิเล็คโตรสปันเพื่อใช้เป็นวัสดุโครงร่างเทียมสำรับการเพาะเลี้ยงเซลล์ได้ถูกประเมินโดยใช้เซลล์ทดสอบชนิดต่างๆ ซึ่งประกอบด้วยเซลล์ชวานน์ เซลล์ออสทิโอบลาสต์ เซลล์คีราติโนไซต์ เซลล์ไฟโปรบลาสต์ เซลล์มูรีนนิวโรบลาสต์โตมา และ สเต็มเซลล์ของเซลล์ประสาท ในแง่ของความเป็นพิษต่อเซลล์ การยึดเกาะของเซลล์ การเพิ่มจำนวนเซลล์ และสัณฐานวิทยาของเซลล์ และเพื่อเพิ่มความเข้ากันได้ระหว่างเซลล์ทดสอบกับวัสดุโครงร่างเทียม แผ่นเส้นใยจะถูกนำไปผ่านกระบวนการไลซิสที่พื้นผิวของเส้นใย กระบวนการไลซิสนี้ไม่เพียงแต่ช่วยเพิ่มความชอบน้ำบนพื้นผิวของเส้นใย แต่ยังช่วยเพิ่มหมู่ฟังชั่นในการทำปฏิกิริยากับโปรตีนที่ช่วยส่งเสริมการยึดเกาะของเซลล์ได้อีกด้วย จากผลการทดสอบกับเซลล์ในห้องทดลองพบว่าลักษณะพื้นผิวและลักษณะทางเคมีของพื้นผิวมีอิทธิพลอย่างมากต่อการตอบแสดงของเซลล์ทดสอบ และจากการศึกษาเหล่านี้แสดงให้เห็นถึงความสำคัญของสมบัติของพื้นผิวที่มีผลต่อพฤติกรรมของเซลล์

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ABBREVIATIONS

A-PHB	Aminolysis PHB fibrous scaffold
BHK21(C13)	Baby hamster kidney cells
DCM	Dichloromethane
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
ECM	Extracellular matrix
EDAC	N-Ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy dispersive X-ray
FBS	Fetal bovine serum
GAGs	Glycosaminoglycans
HaCaT	Human keratinocytes
HB	d,l-β-hydroxybutyrate
hESFs	human embryo skin fibroblasts
HFF	Human foreskin fibroblasts
HFP	1,1,1,3,3,3-hexafluoro-2-propanol
HMD	1,6-hexamethylenediamine
HMDS	Hexamethyldisilazane
H-PHB	Hydrolysis PHB fibrous scaffold
IPA	Isopropyl alcohol
LA-PHB	Laminin immobilized on aminolyzed PHB fibrous scaffold
LH-PHB	Laminin immobilized on hydrolyzed PHB fibrous scaffold
L929	Mouse fibroblasts
MC3T3-E1	Mouse calvaria-derived preosteoblastic cells
MES	(N-morpholino) ethanesulfonic acid
mNSCs	Mouse brain-derived neural stem cells

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay
NAHDF	Normal adult human dermal fibroblasts
Neuro2a	Murine neuroblastoma Neuro 2a cell line
NHS	N-Hydroxysuccinimide
PAAm	Polyacrylamide
PBS	Phosphate-buffered saline
PCLA	Poly(chitosan-*g*-DL-lactic acid)
PEO	Poly(ethylene oxide)
PET	Poly(ethylene terephthalate)
PHAs	Polyhydroxyalkanoates
PHB	Poly(3-hydroxybutyrate)
PLA	Poly(lactic acid)
PVA	Poly(vinyl alcohol)
PVP	Poly(vinyl pyrrolidone)
RT4-D6P2T	Schwannoma cell line derived from a *N*-ethyl-*N*-nitrosourea (ENU)-induced rat peripheral neurotumor
SEM	Scanning electron microscopy
SFM	Serum-free medium
TCPS	Tissue culture polystyrene plate
TFA	Trifluoroacetic acid
TGA	Thermogravimetric analysis
XRD	X-ray diffraction
XPS	X-ray photoelectron spectroscopy

LIST OF SYMBOLS

°C	Degree celsius
cP	Centipoise
%DD	The degree of deacetylation
%DDA	The degrees of acetylation
emu	Electromagnetic unit
G	Gauss
H_c	Coercive field
M	Magnetization
M_r	Remnant magnetization
M_n	Number average molecular weight
M_w	Weight average molecular weight
M_v	Viscosity average molecular weight
nm	Nanometer
μm	Micrometer
μS	Microsiemens
ppm	Part per million
w/v	Weight by volume