

**DEVELOPMENT AND IMPROVEMENT OF DUAL-LEACHED
POLYESTER POROUS SCAFFOLDS FOR BONE TISSUE ENGINEERING**

Napaphat Thadavirul

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By: Napaphat Thadavirul
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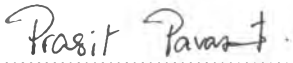
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..... College Dean
(Asst. Prof. Pomthong Malakul)

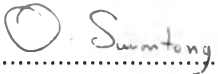
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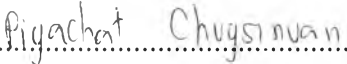

.....
(Asst. Prof. Pomthong Malakul)


.....
(Prof. Pitt Supaphol)


.....
(Prof. Prasit Pavasant)


.....
(Asst. Prof. Hathaikarn Manuspiya)


.....
(Dr. Orawan Suwantong)


.....
(Dr. Piyachat Chuysinuan)

ABSTRACT

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Keywords: Scaffold/ Polycaprolactone/ Solvent casting/ Particulate leaching method/ Polymer leaching/ Poly(hydroxybutyrate)/ Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) / Hydroxyapatite

Sodium chloride and polyethylene glycol (PEG) were used as water-soluble porogens for the formation of porous polycaprolactone (PCL) and their blends scaffolds. The main purpose was to prepare and evaluate in vitro efficacy of highly interconnected, three-dimensional, porous polymeric scaffolds, as obtained from the combined solvent casting and particulate-polymer leaching techniques. Evidently, the use of PEG as the secondary porogen not only improved the interconnectivity of the pore structures but also resulted in the scaffolds that exhibited much better support for the proliferation and differentiation of the cultured bone cells. Although increased porosity and interconnected network facilitate bone ingrowth, the result is a reduction in mechanical properties of scaffold. For these reason, the another purpose of this study was to improve the mechanical properties of the dual-leached PCL scaffold by the addition of hydroxyapatite and improve the hydrophilicity of dual-leached scaffolds by alkaline treatment. The potential for PCL-PHB, PCL-PHBV, PCL-PHB/HA, and PCL-PHBV/HA dual-leached scaffolds use as bone scaffolding materials were also evaluated in vitro mouse calvaria-derived preosteoblastic cells (MC3T3-E1). The results indicate that NaOH treated PCL/HA dual-leached scaffold possesses improvement in mechanical properties and hydrophilicity, and PCL-PHB, PCL-PHBV, PCL-PHB/HA, and PCL-PHBV/HA dual-leached scaffolds possess improvement in mechanical properties, degradation. All dual-leached scaffolds show their ability to support MC3T3-E1 cell attachment, proliferation, and mineralization for used as bone scaffolding materials.

บทคัดย่อ

ณปภัช ธาดาวิรุฬห์ : การพัฒนาการขึ้นรูปวัสดุโครงร่างพอลิเอสเทอร์ที่มีความเป็นรูพรุนสูงสำหรับใช้ในงานทางวิศวกรรมเนื้อเยื่อกระดูก (Development and Improvement of Dual-leached Polyester Porous Scaffolds for Bone Tissue Engineering) อ. ที่ปรึกษา: ศาสตราจารย์ ดร. พิษณุ สุภผล 195 หน้า

การพัฒนากรรมวิธีการขึ้นรูปวัสดุโครงร่างโดยการนำกรรมวิธีการหล่อขึ้นรูปด้วยตัวทำละลาย (solvent casting), การชะล้างอนุภาคเกลือ (salt leaching) และการชะล้างพอลิเมอร์ (polymer leaching) มาประกอบกันเพื่อสร้างความเป็นรูพรุนสูงในวัสดุโครงร่างสำหรับกระดูกที่มีความเป็นรูพรุนสูง คุณสมบัติความเป็นรูพรุนสูงในวัสดุโครงร่างสำหรับกระดูกเป็นคุณสมบัติที่ดีสำหรับการเจริญของเนื้อเยื่อเพื่อที่เซลล์กระดูกจะสามารถแทรกซึมเจริญเข้าไปในโครงร่าง รวมทั้งรูพรุนสามารถเป็นทางถ่ายเทอาหารและของเสียสำหรับเซลล์กระดูก ในงานวิจัยนี้ อนุภาคเกลือโซเดียมคลอไรด์ และพอลิเอททิลีนไกลคอล ถูกใช้เพื่อสร้างความเป็นรูพรุนในวัสดุโครงร่างของพอลิคาโพรแลคโตน จากการศึกษาวัสดุโครงร่างโดยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด วัสดุโครงร่างนี้มีความเป็นรูพรุนและความเชื่อมโยงของรูพรุนสูง และยังมีการกระจายตัวของรูพรุนและขนาดของรูพรุนที่สม่ำเสมอ ถึงแม้ว่าความเป็นรูพรุนสูงเป็นคุณสมบัติที่ดีของโครงร่างสำหรับการเจริญของเนื้อเยื่อเซลล์กระดูก แต่คุณสมบัติเชิงกลก็ยังคงเป็นคุณสมบัติที่สำคัญเช่นกัน ในงานวิจัยนี้ จึงได้ทำการพัฒนาสมบัติเชิงกลของโครงร่างโดยการเติมผงไฮดรอกซีอะพาไทต์เข้าไปในโครงร่าง และทำการผสมพอลิไฮดรอกซีบิวทีเรต หรือ พอลิไฮดรอกซีบิวทีเรตโควาริเรตเข้าไปในเนื้อโครงร่างพอลิคาโพรแลคโตน นอกจากนี้ในงานวิจัยนี้ยังได้วิเคราะห์ความเป็นไปได้ในการใช้วัสดุโครงร่างในทางวิศวกรรมเนื้อเยื่อกระดูก โดยจากใช้เซลล์ MC3T3-E1 จากการทดสอบความเป็นพิษของวัสดุกับเซลล์ พบว่า ไม่มีสารที่ก่อให้เกิดความเป็นพิษกับเซลล์จากวัสดุโครงร่างนี้ และ เซลล์ MC3T3-E1 สามารถเจริญเติบโตและแผ่ขยายได้ดีบนวัสดุโครงร่างชนิดนี้ จากผลการทดสอบ โดยวัสดุดังกล่าวสามารถใช้เป็นโครงร่างเลี้ยงเซลล์กระดูกได้ดี วัสดุทั้งหมดสามารถนำไปใช้เป็นวัสดุเพื่อนำไปใช้ทางการแพทย์ที่มีประสิทธิภาพ

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ABBREVIATIONS

3D	Three dimensional
ANOVA	One-Way Analysis of Variance
ALP	Alkaline phosphatase
BCA	Bicinchoninic acid
BMP	Bone Morphogenetic Protein
BMSCs	Bone Marrow Stem Cells
BMUs	Basic multicellular units
BV	butyrate-co- hydroxyvalerate
cm	Centrimeter
CAD	Computer aided design
CAM	Computer aided manufacturing
CaCO ₃	Calcium carbonate
CaOH ₂	Calcium hydroxide
CO ₂	Carbon dioxide
d	Day
DI	Deionized water
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EDS	Energy dispersive spectrophotometer
FBS	Fetal bovine serum
FDA	Food and Drug Administration
FHA	Fluridated hydroxyapatite
h	Hour
HA	Hydroxyapaatite
HMDS	Hexamethyldisilazane
hMSC	Human bone marrow stem cell
H ₃ PO ₄	Phophoric acid

HSD	Honestly significant difference
L929	Murine dermal fibroblasts
LSD	Least-significant difference
MC3T3-E1	Mouse calvaria-derived pre-osteoblastic cells
MEM	Minimum Essential Medium
MgCl ₂	Magnesium chloride
MRI	Magnetic resonance Imaging
MTT	3-(4,5-Dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide
NaCl	Sodium chloride
NaOH	Sodium hydroxide
Pa	Pascal
PBS	Phosphate buffer saline
PEG	Polyethylene glycol
PCL	Polycaprolactone
PGA	Polyglycolic acid
P4HB	poly-4-hydroxybutyrate
PHB	Poly(3-hydroxybutyrate)
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PHH	polyhydroxyhexanoate
PHO	polyhydroxyoctanoate
PHV	polyhydroxyvalerate
PLA	Poly(lactic acid)
PLGA	Poly(lactide-co-glycolide)
PLLA	Poly(l-lactide)
pNPP	<i>p</i> -nitrophenyl phosphate
sec	Second
SEM	Scanning electron microscopy
SFF	Solid freeforming fabricatiion
SFM	Serum-free medium
TCP	Tricalcium phosphate

TCPS	Tissue-culture polystyrene plate
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
XRD	X-ray diffraction
UTM	Universal testing machine
UV-vis	UV-visible spectrophotometer

LIST OF SYMBOLS

D	Average crystallite size
β	Peak at half width
θ	Bragg angle of the peak
λ	X-ray wavelength
K	Shape factor
ρ_{PCL}	Density of PCL film
ρ_{polymer}	Density of the polymer from which the scaffolds were fabricated
ρ_{scaffold}	Apparent density of the scaffolds
W_0	Initial weight
W_d	Weight of the specimen before submersion in the medium
W_t	Weight of scaffold at single degradation time point
W_w	Weight of the specimen after submersion in the medium