

**ELECTROSPUN FIBER MATS CONTAINING SILVER NANOPARTICLES
WITH ANTIBACTERIAL ACTIVITY**



Pim-on Rujitanaroj

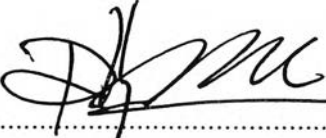
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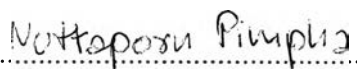
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ABSTRACT

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Silver has a long history as an antimicrobial agent, especially in the treatment of burns. Several products have incorporated silver for use as a topical antibacterial agent, such as silver nitrate, silver sulphadiazine (SSD), silver sulphadiazine/chlorhexidine. Moreover, nanotechnology has provided a way of producing pure silver nanoparticles (nanoAg⁰). This system also markedly increases the rate of silver ion release. NanoAg⁰ is one of the most effective antimicrobial agents because of the high specific surface or volume fraction so that a large proportion of metal atoms are directly contact with the environment and can kill a wide range of bacteria. In this work, mats of poly(acrylonitrile) (PAN; $M_w \approx 55,500$) and gelatine (GT; Bloom ≈ 180) fibers containing nanoAg⁰ were prepared by e-spinning and these e-spun fiber mats were prepared to be used as surgical mask and wound dressing pads, respectively. The nanoAg⁰-containing poly(acrylonitrile) and gelatin fiber mats were characterized for various properties (i.e., morphological, mechanical, swelling, and weight loss), the release characteristic of the as-loaded silver as well as their antibacterial activity. Moreover, *in vitro* and *in vivo* biological evaluation of neat and nanoAg⁰-containing e-spun gelatin fiber mats with intended uses as wound dressing materials were investigated by studying the cytotoxicity and cell spreading of human dermal fibroblast (NHDF) or monocytes/macrophage on materials. In addition, morphologies of NHDF and monocytes/macrophage attached on these fibers were also observed by scanning electron microscope (SEM) and confocal microscopy, respectively.

บทคัดย่อ

พิมพ์อร รุจิชนโรจน์ : การพัฒนาแผ่นเส้นใยอิเล็กโตรสปินที่มีอนุภาคซิลเวอร์นาโน สำหรับการประยุกต์ใช้เป็นวัสดุด้านเชื้อแบคทีเรีย (Electrospun Fiber Mats Containing Silver Nanoparticles with Antibacterial Activity) อ. ที่ปรึกษา : รศ. ดร. พิชญ์ สุขผล และ ดร. ณีฐพร พิมพ์ 258 หน้า

เป็นที่ทราบกันดีว่า ซิลเวอร์มีคุณสมบัติในการฆ่าเชื้อโรคได้เป็นอย่างดี ซิลเวอร์จึงถูกนำมาผสมในผลิตภัณฑ์ต่างๆเพื่อใช้เป็นแผ่นฆ่าเชื้อโรคหรือวัสดุปิดแผล ไม่ว่าจะเป็นซิลเวอร์ไนเตรด ซิลเวอร์ซัลไฟด์ และอื่นๆ นอกจากนี้นาโนเทคโนโลยีได้ถูกพัฒนามาใช้ในการขึ้นรูปซิลเวอร์หรือที่เรียกกันว่า อนุภาคซิลเวอร์นาโน (silver nanoparticles; nanoAg⁰) ด้วยขนาดของอนุภาคซิลเวอร์นาโนที่เล็กมาก ส่งผลให้ประสิทธิภาพในการฆ่าเชื้อโรคสูงยิ่งขึ้น งานวิจัยนี้จึงเป็นการเตรียมเส้นใยพอลิอะคริลาไมด์และเส้นใยเจลลาตินที่ผสมอนุภาคซิลเวอร์นาโนด้วยกระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิต โดยเส้นใยทั้งสองชนิดนี้สามารถนำไปประยุกต์ใช้เป็นแผ่นกรองอากาศหรือวัสดุปิดแผลตามลำดับ นอกจากนี้ในงานวิจัยนี้ได้มีการศึกษาสมบัติพื้นฐานต่างๆ เช่น ลักษณะพื้นผิวของเส้นใย ขนาดอนุภาคซิลเวอร์นาโนในเส้นใย รวมทั้งศึกษาสมบัติเชิงกล การบวมน้ำและการสูญเสียน้ำหนักของแผ่นเส้นใยเหล่านั้น และยังสามารถทดลองเพื่อศึกษาการปลดปล่อยของอนุภาคซิลเวอร์นาโนจากแผ่นเส้นใย โดยใช้วิธีการแซ่ในสารละลายบัฟเฟอร์ เนื่องจากความต้องการที่จะประยุกต์ใช้แผ่นเส้นใยอิเล็กโตรสปินเหล่านี้สำหรับเป็นวัสดุที่ฆ่าเชื้อโรคได้ จึงได้ศึกษาถึงความสามารถในการฆ่าเชื้อโรคของเส้นใยที่ผสมอนุภาคซิลเวอร์นาโน อีกทั้งเส้นใยเจลลาตินสามารถประยุกต์ใช้เป็นวัสดุปิดแผล จึงได้ศึกษาความเข้ากันได้ทางชีวภาพของวัสดุ กับเซลล์ผิวหนัง (NHDF) หรือเซลล์เม็ดเลือดขาว (monocyte/macrophage) โดยทดสอบความเป็นพิษ, การเกาะของเซลล์, การเจริญเติบโตของเซลล์ นอกจากนี้ยังได้ศึกษาลักษณะของเซลล์ผิวหนังที่เกาะบนแผ่นเส้นใยอิเล็กโตรสปิน โดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด และเซลล์เม็ดเลือดขาว โดยใช้กล้องคอนโฟคอลไมโครสโคปอีกด้วย

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ABBREVIATIONS

nanoAg ⁰	Silver nanoparticles
AgNO ₃	Silver nitrate
PAN	Poly(acrylonitrile)
GT	Gelatin
PLLA	Poly(L-lactic acid)
PCL	Polycaprolactone
PBSu-DCH	Poly (1,4-butylene succinate) extended with 1,6-diisocyanatohexane
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
E.coli	<i>Escherichia coli</i>
<i>B.subtilis</i>	<i>Bacillus subtilis</i>
M_w	Molecular weight
PV	Pervaporation
D	Dialysis
GS	Gas separation
ED	Electrodialysis
NF	Nanofiltration
UF	Ultrafiltration
MF	Microfiltration
RO	Reverse Osmosis
AFM	Atomic force microscope
SEM	Scanning electron microscope
TEM	Transmission electron microscope
EDX	Energy dispersive X-ray
TG-DTA	Thermogravimetric/differential thermal analyzer
AAS	Atomic absorption spectroscope
GTA	Glutaraldehyde
SBF	Simulated body fluid
PBS	Phosphate buffer solution

DMF	<i>N,N</i> -dimethylformamide
DMSO	Dimethylsulfoxide
NHDF	Normal human dermal fibroblasts
ECM	Extracellular matrix
TCPS	Tissue-culture polystyrene plate
DMEM	Dulbecco's modified Eagle's medium
FBS	Fetal bovine serum
SFM	Serum-free medium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
RGD	Fibronectin-like protein polymer
<i>RNAse</i>	Ribonuclease A
H&E	Hematoxylin and Eosin
h	Hour

LIST OF SYMBOLS

γ	Surface tension
ρ	Density
V^*	Critical Potential
V_c	Critical Voltage
DC	Direct current
M	Weight of sample after submersion in the testing solution
M_i	Initial weight of the sample in its dry state
M_d	Weight of the sample after submersion in the testing solution in its dry state
R	Percentage of reduction
A	The number of bacteria recovered from the incubated treated test specimen (GT/AgNO ₃) after 37°C for 24 hr
B	The number of bacteria recovered from the incubated untreated control specimen (Neat GT) after incubation at 37°C for 24 hr