

**DEVELOPMENT OF THE SILVER NANOPARTICLE LOADED GELATIN
AND SILK FIBROIN HYDROGEL PADS FOR ANTIMICROBIAL WOUND
DRESSING APPLICATIONS**



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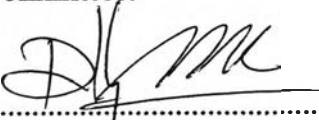
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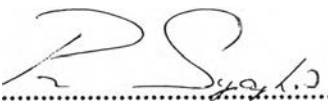
Thesis Title: Development of the Silver Nanoparticle Loaded Gelatin and Silk Fibroin Hydrogel Pads for Antimicrobial Wound Dressing Applications
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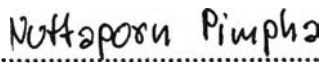
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ABSTRACT

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Gelatin hydrogels and silk fibroin (SF) films were prepared from gelatin and SF solutions containing different amounts of AgNO_3 by a solvent-casting technique. The AgNO_3 -containing gelatin and SF solutions were aged under a mechanical stirring for various times to allow for the formation of silver nanoparticles (nAgs). The formation and the size of nAgs in these solutions were characterized by a UV-vis spectrophotometer and transmission electron microscopy (TEM), respectively. To improve the water resistance of the gelatin hydrogels, various contents of glutaraldehyde (GTA) were added to the AgNO_3 -containing gelatin solution to cross-link the nAg-loaded gelatin hydrogels. These hydrogels were tested for their water retention and weight loss behavior, release characteristic of the as-loaded silver by using an atomic absorption spectroscope (AAS), the antibacterial activity against Gram-positive and Gram-negative bacteria and the indirect cytotoxicity. Moreover, we further studied the effect of the initial concentration of AgNO_3 that would be loaded in the gelatin solution to finally obtain the nAg-loaded gelatin hydrogel pads. The indirect cytotoxicity, the cell attachment and the cell proliferation were investigated. Furthermore, SF films were treated with 90 vol.-% of methanol aqueous solution to improve the water resistance. The nAg-loaded SF films were tested for their *in vitro* degradation behavior, release characteristic of the as-loaded silver and the antimicrobial activity.

บทคัดย่อ

วิทยุรัตน์ รัตนเรืองศรีกุล : การพัฒนาแผ่นเจลลาตินไฮโดรเจลและแผ่นซิลค์ไฟโบรอินฟิล์มที่มีอนุภาคซิลเวอร์นาโนผสมอยู่เพื่อใช้เป็นวัสดุปิดแผลที่มีฤทธิ์ในการยับยั้งเชื้อแบคทีเรีย (Development of the Silver Nanoparticle Loaded Gelatin and Silk Fibroin Hydrogel Pads for Antimicrobial Wound Dressing Applications) อ. ที่ปรึกษา : ศ. ดร. พิชญ์ สุภผล และ ดร. ณัฐพร พิมพ์ 155 หน้า

แผ่นเจลลาตินไฮโดรเจลและแผ่นซิลค์ไฟโบรอินฟิล์มที่มีอนุภาคซิลเวอร์นาโนสามารถเตรียมได้จากขั้นรูปโดยใช้ตัวทำละลาย จากสารละลายเจลลาตินและสารละลายซิลค์ไฟโบรอินที่มีอนุภาคซิลเวอร์นาโนผสมอยู่ในปริมาณต่างๆ ที่ผ่านการปั่นกวนอย่างต่อเนื่องเป็นเวลานาน โดยการเกิดอนุภาคซิลเวอร์นาโนและขนาดของอนุภาคซิลเวอร์นาโนในสารละลายเจลลาตินและซิลค์ไฟโบรอินสามารถตรวจสอบได้โดยใช้เครื่องตรวจวัดสารด้วยการดูดกลืนแสงและการถ่ายภาพด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องผ่าน ตามลำดับ เนื่องจากแผ่นเจลลาตินไฮโดรเจลละลายน้ำได้ง่าย ดังนั้นแผ่นเจลลาตินไฮโดรเจลจึงถูกเชื่อมขวางด้วยสารเชื่อมขวางกลูตารัลดีไฮด์และได้ศึกษาผลของปริมาณกลูตารัลดีไฮด์ที่มีผลต่อการบวมน้ำและการสูญเสียน้ำหนักแผ่นเจลลาตินไฮโดรเจล การปลดปล่อยของซิลเวอร์แผ่นเจลลาตินไฮโดรเจลที่มีอนุภาคซิลเวอร์นาโนในสารละลายบัฟเฟอร์ ความสามารถในการยับยั้งเชื้อแบคทีเรียทั้งแกรมบวกและแกรมลบ และความเป็นพิษต่อเซลล์ของกลูตารัลดีไฮด์ที่มีในแผ่นเจลลาตินไฮโดรเจล นอกจากนี้ยังศึกษาผลของปริมาณสารตั้งต้นซิลเวอร์นาโนที่มีผลต่อการปลดปล่อยของซิลเวอร์ในสารละลายบัฟเฟอร์และความสามารถในการยับยั้งเชื้อแบคทีเรียของแผ่นเจลลาตินไฮโดรเจลที่มีอนุภาคซิลเวอร์นาโนผสมอยู่ ความเป็นพิษ ลักษณะการเกาะและการเจริญเติบโตของเซลล์บนแผ่นเจลลาตินไฮโดรเจลที่มีอนุภาคซิลเวอร์นาโนผสมอยู่ จากผลการทดสอบความเป็นพิษกับเซลล์พบว่าแผ่นเจลลาตินไฮโดรเจลที่มีอนุภาคซิลเวอร์นาโนผสมอยู่ผ่านการจุ่มในสารละลายโซเดียมเมตาไบซัลไฟด์จะช่วยลดความเป็นพิษของแผ่นไฮโดรเจลนี้ได้ นอกจากนี้แผ่นซิลค์ไฟโบรอินฟิล์มถูกนำมาจุ่มในสารละลายเมทานอลเข้มข้น 90 เปอร์เซ็นต์โดยปริมาตรเพื่อลดความสามารถในการละลายน้ำของแผ่นซิลค์ไฟโบรอินฟิล์ม และได้ศึกษาการย่อยสลายด้วยเอนไซม์ของแผ่นฟิล์ม การปลดปล่อยของซิลเวอร์ในสารละลายบัฟเฟอร์ แผ่นซิลค์ไฟโบรอินฟิล์มที่มีอนุภาคซิลเวอร์นาโนและความสามารถในการยับยั้งเชื้อแบคทีเรียของแผ่นซิลค์ไฟโบรอินฟิล์มที่มีอนุภาคซิลเวอร์นาโนผสมอยู่ ด้วยวิธีการทดสอบเดียวกัน

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ABBREVIATIONS

nAg	Silver nanoparticle
SF	Silk fibroin
SEM	Scanning electron microscope
TEM	Transmission electron microscope
AAS	Atomic absorption spectroscope
UV-vis	UV-visible spectrophotometer
NHDF	Normal human dermal fibroblasts
GTA	Glutaraldehyde
SBF	Simulated body fluid
PBS	Phosphate buffer saline solution
MIC	Minimal inhibition concentration
O.D.	Optical density
TCPS	Tissue-culture polystyrene plate
DMEM	Dulbecco's modified Eagle's medium
SFM	Serum-free medium
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

LIST OF SYMBOLS

W_i	Initial weight of the sample in its dry state
W_s	Weight of the sample in its wet, swollen state
W_d	Weight of the dried sample after immersing in buffer
M_c	The number-average molecular weight of the chain segments between cross-linking points
ρ_G	Density of gelatin
ρ_w	Density of water
V_1	Molar volume of water
χ	Flory-Huggins interaction parameter between water and gelatin
v_G	Volume fraction of gelatin in hydrogel specimens in their equilibrium swollen state
W_0	Initial dry weight of the specimens
W	Weight of the specimens in their equilibrium swollen state
N_A	Avogadro's number
V_e	The cross-link density in terms of the number of elastically effective chains per unit volume of the gelatin hydrogel network
$O.D._{sample}$	Optical density of sample
$O.D._{control}$	Optical density of control
A	Numbers of bacterial colonies for the plates that had been smeared with washing solutions from the nAg-loaded gelatin hydrogel specimens
B	Numbers of bacterial colonies for the plates that had been smeared with washing solutions from the neat gelatin hydrogel specimens