

STUDIES ON THE DEVELOPMENT OF DEXTRAN BASED BIONANOCOMPOSITE MEMBRANES FOR WOUND CARE

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STUDIES ON THE DEVELOPMENT OF DEXTRAN BASED BIONANOCOMPOSITE MEMBRANES FOR WOUND CARE

by

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“The key to life is accepting challenges”

*DEDICATED TO ALL THOSE WHO LIVE
WITH A STRONG SPIRIT AND HEAD HIGH*

CERTIFICATE

This is to certify that the thesis entitled “**Studies on the development of dextran based bionanocomposite membranes for wound care**” submitted by **Ms. Surabhi Singh** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her. Ms. Surabhi Singh has worked under my guidance and has fulfilled the requirements for the submission of this thesis which has attained the standard required for a Ph.D degree of this institute. The results contained in this thesis are original and have not been submitted in partial or full, to any other university or institute for the award of any degree or diploma.

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ABSTRACT

Wound repair is a complex process involving systemic and balanced functioning of inflammatory, connective, vascular tissue and epithelial cells. Effective management of wound infection requires prolonged prevention from microbial contamination along with accelerated wound healing and aesthetic closure of the wound. With these objectives in mind, an effort has been directed towards the development of herbal based bionanocomposite membranes for wound care in the current study.

This investigation is associated with the preparation of soy protein isolate nanoparticles (nanosoy) reinforced dextran based wound dressings, which are the nanocomposites based on naturally derived plant protein and a polysaccharide. Nanosoy (NS) was prepared by nanoprecipitation which enabled excellent control over protein aggregation, producing nanoparticles in the size range of 5-15 nm. The effect of various process parameters, such as the temperature, solvent/nonsolvent ratio, crosslinker content and type of surfactant on the particle size has been investigated. The structural and morphological features have been analyzed using dynamic light scattering (DLS), X-ray diffraction (XRD) and high resolution transmission electron microscopy (HRTEM), respectively.

Crosslinked nanocomposite membranes were then developed by *in-situ* reaction of dextran and nanosoy. The formation of covalent bond between the reducing end of dextran and amine groups of NS leads to the *in-situ* crosslinking. Glycerol addition assisted in the plasticization of membranes, thus improving their flexibility and handling features. The effect of polymer composition on the extent of crosslinking, morphology and flexibility of the films was investigated. Field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM) unveiled that single phase, homogeneous membranes are obtained within a specific composition of dextran/NS/glycerol (DNG). Increase in tensile strength and decrease in Young's modulus was observed with the increase in the NS content up to 28%

due to reinforcing effect of NS and plasticizing effect of glycerol playing roles simultaneously in the system.

Properties of DNG composite membranes as a function of increasing glycerol content were explored. Surface morphology of the membranes, as evidenced from FESEM and AFM, exhibited smoother surface with the increasing glycerol content up to 30%. Glycerol content also significantly led to diminishing water vapor transmission rate (WVTR) with increasing glycerol content. Influence of plasticization on rheological properties of the composite fluid was investigated. Although tensile strength and modulus decreased with increasing glycerol content up to 30%, a significant increment was observed in the values at a concentration higher than 30% due to the anti-plasticizing effect of glycerol. Similar behavior was reflected in XRD results.

In the third step of the study, chitosan incorporation into DNG membranes was carried out to develop dextran/nanosoy/glycerol/chitosan (DNG/Ch) membranes. The morphology of these membranes was investigated using scanning electron microscopy (SEM) and the results indicated no microphase separation in the matrix. Tensile strength showed a slight increase from 3.43 MPa to 4.81 MPa upto chitosan content of 21% beyond which the strength declined. Rise in the value of elongation at break at 14% chitosan content was observed. WVTR and swelling of the membranes as a function of the chitosan content were investigated. Mild antibacterial nature was introduced into the DNG/Ch membranes as compared to DNG membranes.

To fabricate dextran based bionanocomposite wound dressings, bioactive agents were incorporated into DNG/Ch membranes. Wound care membranes were produced by casting the mixed solutions of biopolymers in water at 70°C followed by subsequent addition of clove oil (CO), sandalwood oil (SO), aloe vera (AV) and manuka honey (MH) at room temperature to obtain DNG/Ch/CO, DNG/Ch/SO, DNG/Ch/AV and DNG/Ch/MH wound dressings. The antibacterial behavior of the dressings was studied against both *S. aureus*

and *E. coli* at different drug concentrations. Tensile, water sorption and swelling behavior of the membranes was studied. Drug release was measured using bacterial reduction count and serial plate transfer disk diffusion test over a period of 72 h for DNG/Ch/CO and DNG/Ch/SO dressings, whereas release was monitored spectrophotometrically for DNG/Ch/AV and DNG/Ch/MH dressings. Bacterial adherence test was performed to demonstrate the efficiency of dressings for arresting microbial invasion.

In vivo wound healing studies and histological examination was conducted using male swiss albino mice of Balb/C strain. DNG/Ch/AV dressings exhibited complete healing in just 14 days with remarkable efficacy in prevention of scar formation. Histological analysis on healed tissue revealed the deposition of ordered collagen along with fibroblast migration in drug loaded membranes as compared to DNG and DNG/Ch dressings.

सार

घाव की मरम्मत एक जटिल प्रक्रिया है, जिसमें उत्तेजक, संयोजी, संवहनी ऊतक और उपकला कोशिकाओं के व्यवस्थित और संतुलित कार्य पद्धति शामिल है। घाव संक्रमण के प्रभावी प्रबंधन के लिए घाव के त्वरित भराव और सौंदर्य बंद के साथ-साथ सूक्ष्मजीव संदूषण के लंबे समय तक रोकथाम की आवश्यकता है। इन उद्देश्यों को ध्यान में रखते हुए, वर्तमान अध्ययन के द्वारा घाव की देखभाल के लिए हर्बल आधारित बायोनैनोकम्पोजिट झिल्ली को विकसित करने का प्रयास किया गया है।

यह शोध सोया प्रोटीन ऐसो-लेट नैनो पार्टिकल्स (नैनोसोय) प्रबलित डेक्सट्रान आधारित घाव मरहम-पट्टी को विकसित करने से सम्बंधित है, जो प्राकृतिक रूप से प्राप्त पौधों के प्रोटीन और पॉलीसेकेराइड पर आधारित नैनोकम्पोजिट हैं। नैनोसोय (एन एस) नैनो. प्रेसिपीटेशन द्वारा तैयार किया गया था जो कि सक्षम प्रोटीन एकत्रीकरण पर उत्कृष्ट नियंत्रण के साथ-साथ 5 - 15 एनएम आकार की श्रेणी के नैनोकणों का उत्पादन प्राप्त किया गया था। इन कणों के आकार/ माप पर विभिन्न प्रक्रिया मापदंडों, जैसे कि प्रक्रिया का तापमान, सोलवेंट/ नॉनसोलवेंट अनुपात, क्रॉसलिंकर सामग्री और भिन्न-भिन्न प्रकार के सर्फैक्टेंट के प्रभाव की तहकीकात की गयी है। डायनामिक लाइट स्कैटरिंग (डीएलएस), एक्स-रे डिफ्रैक्शन (एक्सआरडी) और हाई रेसोलुशन ट्रांसमिशन इलेक्ट्रान माइक्रोस्कोपी (एचआरटीईएम) का उपयोग करके संरचनात्मक और रूपात्मक विशेषताओं का विश्लेषण किया गया है।

डेक्सट्रान और नैनोसोय की इन-सीटू प्रतिक्रिया द्वारा तिर्यक नैनोकम्पोजिट झिल्ली को विकसित किया गया था। डेक्सट्रान के रेडूसिंग समूह और एनएस के एमीन समूहों के बीच सहसंयोजक गठबंधन इन-सीटू क्रॉसलिंकिंग को बढ़ावा देता है। ग्लिसरॉल संयोजन से झिल्ली के प्लास्काइजेशन में सहायता प्रदान हुई, जो कि इसके लचीलेपन और हैंडलिंग फीचर्स में सुधार कर रहा है। झिल्ली के आकृति विज्ञान, क्रॉसलिंकिंग की मात्रा, और लचीलेपन पर बहुलक संरचना के प्रभाव की जांच की गई। फील्ड एमिशन स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (एफईएसईएम) और एटॉमिक फोर्स माइक्रोस्कोपी

(एएफएम) ने अनावरण किया कि डेक्सट्रान/ एनएस/ ग्लिसरॉल (डीएनजी) की विशिष्ट मात्रा से एकल चरण सजातीय झिल्ली को प्राप्त किया गया है। एनएस सामग्री के मजबूती प्रभाव और ग्लिसरॉल के प्लास्काइजेशन प्रभाव की प्रणाली में एक साथ भूमिका के कारण एनएस सामग्री के 28 % तक की वृद्धि से टेंसिल स्ट्रेंथ में वृद्धि और इनिशियल माॅडयूल्स में कमी पाई गयी ।

ग्लिसरॉल सामग्री की मात्रा को बढ़ने के कार्य में डीएनजी समग्र झिल्ली के गुणों का अन्वेषण किया गया। एफईएसईएम और एएफएम से प्रमाणित हुआ कि ग्लिसरॉल की मात्रा 30 % तक बढ़ने पर झिल्ली की आकारिकी सतह ओर भी चिकनी प्रदर्शित हो रही थी। ग्लिसरॉल की मात्रा में वृद्धि के साथ वाटर वेपर ट्रांसमिशन रेट (डब्लू वी टी आर) में काफी हद तक कमी पायी गयी। कम्पोजिट तरल पदार्थ की रियोलॉजिकल गुणों पर प्लास्काइजेशन के प्रभाव की जांच की गई थी। यद्यपि, ग्लिसरॉल सामग्री के 28 % तक की वृद्धि से टेंसिल स्ट्रेंथ और इनिशियल माॅडयूल्स में कमी पाई गयी । ग्लिसरॉल के एंटी- प्लास्काइजेशन प्रभाव के कारण से इसके 30 % से अधिक मात्रा होने पर इन गुणों में काफी हद तक वृद्धि देखी गई। एक्स आर डी परिणामों से भी समरूप व्यवहार प्रतिबिंबित हुआ था।

अध्यन के तीसरे चरण में, डेक्सट्रान/ नैनोसोय/ ग्लिसरॉल/ चितोसन (डीएनजी/ सीएच) झिल्ली को विकसित करने के लिए चितोसन को डीआईजी झिल्ली में मिश्रित किया गया था। स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (एसईएम) का उपयोग करके इन झिल्लियों की आकारिकी सतह की जांच की गई और परिणामस्वरूप मैट्रिक्स में कोई भी माइक्रोफेज अलग नहीं दिखे। चितोसन सामग्री के 28 % तक की वृद्धि से टेंसिल स्ट्रेंथ में 3.43 एमपीए से 4.81 एमपीए की मामूली वृद्धि पाई गयी उसके पार स्ट्रेंथ में गिरावट आई थी। चितोसन सामग्री की 28 % मात्रा पर एलाॅगेशन-अत-ब्रेक में वृद्धि देखी गई। चितोजान सामग्री ध्यान में रखते हुए, वीवीटीटीआर और झिल्ली की सूजन की जांच की गई। डीएनजी झिल्ली की तुलना में हल्के जीवाणुरोधी स्वभाव को डीएनजीध् सीएच झिल्ली में उत्पन्न किया गया।

डेक्सट्रान आधारित बियोनैनोकम्पोजिट घाव ड्रेसिंग को विकसित करने के लिए बायोएक्टिव एजेंटों को डीएनजी /सीएच झिल्ली में सम्मिलित किया गया था। पानी में 70 डिग्री सेल्सियस पर बायोपॉलिमर्स

के मिश्रित घोल की कास्टिंग करके घाव की देखभाल के लिए झिल्ली का उत्पादन किया गया, तत्पश्चात् डीएनजी/ सीएच/ सीओ, डीएनजी/ सीएच/ एसओ, डीएनजी/ सीएच/ एवी और डीएनजी/ सीएच/ एमएच घाव ड्रेस्सिंग्स विकसित करने के लिए सामान्य तापमान पर लौंग तेल (सीओ), चंदन तेल (एसओ), मुसब्बर वेरा (एवी) और मनुका शहद (एमएच) को संकलित किया गया। अलग-अलग औषध की सांद्रता पर एसओरियस और ईकोलाई दोनों के प्रतिकूल ड्रेस्सिंग्स के जीवाणुरोधी गतिविधि का अध्ययन किया गया था। झिल्ली की तन्यता, वाटर सॉर्प्शन और सूजन व्यवहार का अध्ययन किया गया। बैक्टीरियल रिडक्शन काउंट का उपयोग करके ड्रग रिलीज को मापा गया था, 72 घंटे की अवधि के दौरान डीएनजी/ सीएच/ सीओ और डीएनजी/ सीएच/ एसओ सीरियल प्लेट ट्रांसफर डिस्क प्रसार परीक्षण किया गया और जबकि डीएनजी/ सीएच/ एवी और डीएनजी/ सीएच/ एमएच रिलीज के लिए स्पेक्ट्रोफोटोमेट्रिकरी का उपयोग किया गया। सूक्ष्मजीव आक्रमण को अवरुद्ध करने वाली ड्रेस्सिंग्स की दक्षता को साबित करने के लिए बैक्टीरियल दृढ़ता जाँच का निष्पादन किया गया था।

इन-विवो बाल्ब / सी श्रेणी के स्विस् अल्बिनो चूहों का उपयोग करके घाव चिकित्सा अध्ययन और हिस्टोलॉजिकल परीक्षा को संचालित किया था। सिर्फ 14 दिनों में डीएनजी/ सीएच/ एवी ड्रेसिंग ने घाव के निशान की संरचना को रोकने में उल्लेखनीय प्रभाव से उपचार किया। अरोग्य ऊतक पर हिस्टोलॉजिकल विश्लेषण दर्शा रहा था कि डीएनजी और डीएनजी/ सीएच ड्रेसिंग की तुलना में औषधि भरी हुई झिल्ली में फाइब्रोब्लास्ट प्रवासन के साथ-साथ श्रृंखलबद्ध कोलेजन डेपोजिशन है।

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Chapter 4

Table 4.1. Composition of components in DNG/Ch bionanocomposite membranes

LIST OF ABBREVIATIONS

Polyvinylpyrrolidone	PVP
Polyethylene glycol	PEG
Silver	Ag
Soy protein isolate	SPI
Poly(lactic-co-glycolic acid)	PLGA
Polyvinyl alcohol	PVA
Extracellular matrix	ECM
Escherichia coli	<i>E. coli</i>
Staphylococcus aureus	<i>S.aureus</i>
Methicillin-resistant staphylococcus aureus	<i>MRSA</i>
Bacillus subtilis	<i>B. subtilis</i>
Salmonella typhimurium	<i>S. typhimurium</i>
Staphylococcus albus	<i>S. albus</i>
Vibrio vulnificus	<i>V. vulnificus</i>
Pseudomonas aeruginosa	<i>P. aeruginosa</i>
Klebsiella pneumoniae	<i>K. pneumoniae</i>
Poly lactide	PLA
Bovine serum albumin	BSA
Endothelial growth factor	EGF
Cleome droserifolia	CE
Silver nanoparticles	AgNPs
Titanium dioxide	TiO ₂
Zinc oxide	ZnO
Oxidised pectin-gelatin	OP-Gel
Halloysite nanotubes	HNTs
Halloysite nanoclay	HNC

Sodium-montmorillonite	Na-MMT
SPI nanoparticles	NS
Dextran/NS/Glycerol	DNG
Dextran/NS/Glycerol/Chitosan	DNG/Ch
Dextran/NS/Glycerol/Chitosan/Clove oil	DNG/Ch/CO
Dextran/NS/Glycerol/Chitosan/Sandalwood oil	DNG/Ch/SO
Dextran/NS/Glycerol/Chitosan/Aloe vera	DNG/Ch/AV
Dextran/NS/Glycerol/Chitosan/Manuka honey	DNG/Ch/MH
Sodium hydroxide	NaOH
Sodium chloride	NaCl
Hydrochloric acid	HCl
Dynamic light scattering	DLS
High resolution transmission electron microscopy	HRTEM
X-ray diffraction	XRD
Isoelectric point	pI
Critical micelle concentration	CMC
Field emission transmission electron microscopy	FESEM
Scanning electron microscopy	SEM
Atomic force microscopy	AFM
Acid orange 7	AO7
Attenuated total reflectance	ATR
Water vapour transmission rate	WVTR
Thermogravimetric analysis	TGA
UV-Protection factor	UPF
Phosphate buffer	PBS
Root mean square value	Sq
Tensile strength	TS
Young's modulus	YM

Colony forming unit	CFU
Zone of inhibition	ZOI
Essential oils	EO
Polyurethane	PU