DEVELOPMENT OF CARRAGEENAN BASED BIOACTIVE HYDROGEL MEMBRANES AS WOUND DRESSING

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by

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DEDICATED TO

MY SUPERVISORS & PARENTS

CERTIFICATE

This is to certify that the thesis entitled **"Development of Carrageenan based Bioactive Hydrogel Membranes as Wound Dressing"** submitted by **Ms. Pratibha Singh** to the **Indian Institute of Technology Delhi** for the award of degree **Doctor of Philosophy**, is a record of bonafide research work carried out by her. **Ms. Pratibha** diligently worked under our guidance, meeting all requirements for the submission of this thesis, which meets the standards set for a Ph.D. degree at this institution. The results included within this thesis are entirely original and have not been previously submitted, either in part or whole, to any other academic institution for the conferral of a degree or diploma.

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ABSTRACT

Wound management is a crucial aspect of any post-operative care, or trauma. Achieving efficient wound management hinges on employing advanced dressing materials possessing exceptional biocompatibility, high exudate absorption capacity, robust mechanical attributes, and superior anti-inflammatory, antioxidant, and antibacterial properties. Additionally, an ideal wound dressing should facilitate rapid healing, maintain aesthetic appearance, and allow painless removal during changes while preserving healed tissue integrity. Focusing on these objectives, the current study aimed to design and develop carrageenan based bioactive hydrogel membranes, striving to incorporate all essential attributes of an ideal wound dressing.

This investigation involves developing hydrogel membranes comprising κ-carrageenan (CG) and polyethylene glycol (PEG) to establish a moist environment and achieve robust mechanical performance. The addition of PEG aids in plasticizing the membranes, thereby enhancing their flexibility and handling characteristics. Through a facile solution casting approach without employing any toxic solvents or chemical crosslinkers, CG was blended with PEG in various ratios to fabricate CG-PEG hydrogel membranes. A comprehensive examination of their morphological and mechanical properties was conducted, investigating the variation of these properties with increasing PEG content. Observations from scanning electron microscopy (SEM) and atomic force microscopy (AFM) revealed a smoother and homogenous surface with up to 30% PEG content. Rheological studies unveiled a decrease in viscosity as PEG concentration increased. DSC studies delved into the impact of PEG content on compatibility and interactions within the CG matrix, analyzing parameters like glass transition temperature (T_g), melting temperature (T_m), and heat of fusion (H_f) of the membranes. Notably, an increase in mechanical strength occurred up to 30% PEG content due to polymer chain interactions *via* hydrogen bonding, followed by a decline attributed to an anti-plasticizing effect that compromised tensile performance. Moreover, the modulus

decreased while elongation at break increased, signifying the plasticizing impact of PEG from 10% to 40%. The investigation also encompassed analyzing membrane swelling concerning PEG content.

In the subsequent phase of this research, the incorporation of Lecithin (LC) into CG-PEG membranes was conducted, resulting in the development of κ-carrageenan-PEG/lecithin ((CG-PEG)/LC) hydrogel membranes. Physicochemical analyses of (CG-PEG)/LC hydrogel membranes involved scanning electron microscopy (SEM), Fourier transforms infrared spectroscopy (FTIR), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and mechanical assessments. The morphology of these membranes was explored using scanning electron microscopy (SEM) and atomic force microscopy (AFM), revealing no microphase separation in the matrix. These (CG-PEG)/LC membranes exhibited notably enhanced mechanical properties with exceptional flexibility and displayed a high swelling capacity (>1000%), fostering a moist environment conducive to wound healing. Investigation into water vapor transmission rate (WVTR) and x-ray diffraction (XRD) in relation to LC content was also conducted. Furthermore, the (CG-PEG)/LC hydrogel membranes demonstrated reduced peel strength (26.5 N/m) due to the weakening of the hydrogel-gelatin interface in an *in vitro* gelatin peeling test. Additionally, the membranes exhibited potent antibacterial adhesion against both *S. aureus* and *E. coli.* Moreover, these hydrogel membranes displayed antiadhesive properties against NIH3T3 cells, indicating their potential as dressing materials for easy detachment from healed tissue without causing secondary damage.

To produce simple and bioinspired wound care system, natural bioactive agents were further integrated into CG-PEG-LC hydrogel membranes. The process involved casting a mixture of biopolymers in water at 55°C, followed by the addition of *Calendula officinalis* (CL), *Moringa oleifera* (MO), and *Vitis vinifera* (GS) at room temperature, resulting in CG-PEG-LC/CL, CG-PEG-LC/MO, and CG-PEG-LC/GS bioactive wound dressings. Assessment *via* DPPH analysis showcased remarkable antioxidant activity in a dose-dependent manner. The dressings were tested for antibacterial properties against both *S. aureus* and *E. coli* at varying

drug concentrations. Evaluation of water vapor transmission rate (WVTR) and swelling behavior of the membranes was conducted. The effectiveness of the bioactive dressings in microbial inhibition was evaluated using a plate/CFU count method over 24 h. A bacterial adherence test demonstrated the dressings' efficiency in preventing microbial invasion. Spectrophotometric monitoring was employed to study the drug release behavior of CG-PEG-LC/CL, CG-PEG-LC/MO, and CG-PEG-LC/GS bioactive dressings. Biocompatibility assessments using the MTT assay on NIH3T3 cells revealed >90% cell viability, indicating their cytocompatibility.

A comparative assessment of the wound healing efficacy among optimized dressings, including CL, MO, and GS (CG-PEG-LC/CL, CG-PEG-LC/MO, and CG-PEG-LC/GS), was conducted through an *in-vivo* wound contraction (%) test, focusing on their rapid wound healing activity. *In vivo* studies on wound healing and subsequent histological examinations were performed using male Swiss albino mice of Balb/C strain. CG-PEG-LC/CL dressings exhibited >80% wound healing within the initial day of treatment and accomplished complete healing within 7 days, notably preventing scar formation. Histological analysis validated complete wound healing, evident in well-structured epidermal layers, significant collagen deposition, and skin appendage formation in herbal drug-loaded bioactive dressings compared to CG-PEG and CG-PEG-LC dressings. This research culminated in a bioactive wound dressing combining biopolymer based hydrogel technology (CG-PEG-LC) with herbal bioactive agents (CL, MO, and GS), presenting a simple, affordable, and effective solution for painless, scarless, and faster wound healing, especially beneficial for economically disadvantaged populations.

सार

वाउंड प्रबंधन किसी भी सर्जरी के बाद की देखभाल या घायलता के किसी महत्वपूर्ण पहलुओं में से एक है। कुशल वाउंड प्रबंधन को उत्कृष्ट जीवसंगतता, उच्च एक्स्युडेट अवशोषण क्षमता, मजबूत मैकेनिकल गुण, और श्रेष्ठ एंटी-इन्फ्लेमेटरी, एंटीऑक्सीडेंट, और एंटीबैक्टीरियल गुणों से सुसज्जित उन्नत ड्रेसिंग सामग्रियों का उपयोग करके हो सकता है। साथ ही, एक आदर्श वाउंड ड्रेसिंग तेज़ गिलायबंदी, सौंदर्यिक रूप बनाए रखने, और परिवर्तन के दौरान दर्दहीन हटाने की अनुमति देना चाहिए, साथ ही ठीक हुए ऊतक की सांदर्भिकता को बनाए रखना चाहिए। इन उद्देश्यों पर ध्यान केंद्रित करते हुए, वर्तमान अध्ययन का उद्देश्य कारेजीनन आधारित बायोएक्टिव हाइड्रोजेल मेम्ब्रेन डिज़ाइन और विकसित करना था, जिसमें एक आदर्श वाउंड ड्रेसिंग की सभी आवश्यक विशेषताएं शामिल की गई हों।

इस अनुसंधान में, एक नमूना अधिशेषन दृष्टिकोण के साथ, कारेजीनन (सीजी) और पॉलीथीन ग्लाइकॉल (पीईजी) से बने हाइड्रोजेल मेम्ब्रेन का विकास किया गया था, जिससे एक गीले आवास बनाया जा सकता है और मजबूत मैकेनिकल प्रदर्शन प्राप्त किया जा सकता है। पॉलीथीन ग्लाइकॉल के संयोजन से मेम्ब्रेन को प्लास्टिसाइज़ करने में मदद होती है, जिससे उनकी लचीलाई और हैंडलिंग गुणवत्ता में सुधार होती है। किसी भी जहरीले या रासायनिक क्रॉसलिंकर का उपयोग किए बिना, सीजी को पॉलीथीन ग्लाइकॉल के साथ विभिन्न अनुपातों में मिलाया गया था ताकि सीजी-पीईजी हाइड्रोजेल मेम्ब्रेन बना सके। इनकी स्थूलयात्मक और यांत्रिक गुणों का विस्तृत परीक्षण किया गया, जिसमें इन गुणों की विभिन्नता का अध्ययन पूर्वतया किया गया। स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (एसईएम) और एटमिक फ़ोर्स माइक्रोस्कोपी (एएफएम) की अवलोकन से यह सामने आया कि 30% पीईजी साझेदारी के साथ एक सहमत और समृद्ध सतह है। रेयोलॉजिकल अध्ययन ने दिखाया

कि पीईजी अनुपस्थिति में चिकनाई में कमी होती है। डीएससी अध्ययन ने पीईजी साझेदारी के प्रभाव को खोलने के लिए सीजी मैट्रिक्स के भीतर संगतता और आपसी क्रियाओं पर परीक्षण किया, मेम्ब्रेन के ग्लास ट्रांजिशन तापमान (टीजी), पिघलाने का तापमान (टीएम), और मेम्ब्रेन की पिघलान की ऊर्जा (एचएफ) के पैरामीटरों की जाँच की। विशेष रूप से, पॉलिमर शृंगारिक बंधों के माध्यम से 30% पीईजी कंटेंट के साथ मैकेनिकल शक्ति में वृद्धि हुई, जिसे हाइड्रोजन बॉन्डिंग के माध्यम से पॉलिमर शृंगारिक कारण, जिसके बाद एक आंतर-प्लास्टिसाइजिंग प्रभाव ने खींची की प्रदर्शन की। इसके अलावा, मॉड्यूलस कम हुआ जबकि टूटने पर वृद्धि हुई, जिससे पीईजी के 10% से 40% तक का परिचय कराता है कि पॉलिसाइन इम्पैक्ट। अध्ययन ने इसके अलावा पीईजी साझेदारी के संबंध में मेम्ब्रेन स्वेलिंग की जाँच भी की।

इस अनुसंधान के इस पश्चात्तर चरण में, (सीजी-पीईजी) /लेसिथिन (एलसी) की मेम्ब्रेन्स में शामिल किया गया, जिससे कारेजीनन-पॉलीथीन ग्लाइकॉल/लेसिथिन ((सीजी-पीईजी)/एलसी) हाइड्रोजेल मेम्ब्रेन्स का विकास हुआ। (सीजी-पीईजी)/एलसी हाइड्रोजेल मेम्ब्रेन्स की भौतिक-रासायनिक विश्लेषण में स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (एसईएम), फोरियर ट्रांसफ़ॉर्म इंफ्रारेड स्पेक्ट्रोस्कोपी (एफटीआईआर), एक्स-रे डिफ्रैक्शन (एक्सआरडी), डिफरेंशियल स्कैनिंग कैलोरीमीट्री (डीएससी), और मैकेनिक आँकलनों की जाँच हुई। इन मेम्ब्रेन्स की मोर्फोलॉजी को स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (एसईएम) और एटमिक फ़ोर्स माइक्रोस्कोपी (एएफएम) का उपयोग करके खोजा गया, जिससे मैट्रिक्स में कोई माइक्रोफेज सेपरेशन नहीं था। इन (सीजी-पीईजी)/एलसी मेम्ब्रेन्स ने अद्वितीय लचीलाई और उच्च स्वेलिंग क्षमता (>1000%) प्रदर्शित की, जो घाव भराई के लिए सुबीक्ष्म पर्यावस्था को बढ़ावा देती है। जल वाष्प अंधाधुंध प्रेषण दर (डब्ल्यूवीटीआर) और एक्स-रे डिफ्रैक्शन (एक्सआरडी) की जाँच भी एलसी साझेदारी के संबंध में की गई। इसके अलावा, (सीजी-पीईजी)/एलसी हाइड्रोजेल मेम्ब्रेन्स ने इन विघटन टेस्ट में अवसाद की कमी (26.5 एन/एम) प्रदर्शित की थी, जिसका कारण एक इनविट्रो

जेलाटिन पीलिंग टेस्ट में हाइड्रोजेल-जेलाटिन इंटरफ़ेस की कमजोरी थी। साथ ही, मेम्ब्रेन्स ने सारीज़ और ई. कोली के खिलाफ प्रभावी एंटीबैक्टीरियल अडीशन दिखाई और निहायत ही, ये हाइड्रोजेल मेम्ब्रेन्स निखरी ऊतक से उबारकर उन्हें सेकेंडरी क्षति नहीं पहुंचाने की संभावना बताती हैं।

एक सरल और जैव इंस्पायर्ड वाउंड केयर सिस्टम बनाने के लिए, प्राकृतिक बायोएक्टिव एजेंट्स को सीजी-पीईजी-एलसी हाइड्रोजेल मेम्ब्रेन्स में भी मिलाया गया। इस प्रक्रिया में, जल में बायोपॉलिमर्स का मिश्रण 55°C पर बनाया गया, जिसके बाद कैलेंडुला ऑफिसिनालिस (सीएल), मोरिंगा ओलीफेरा (एमओ), और वाइटिस विनिफेरा (जीएस) को कमर के तापमान पर जोड़ा गया, जिससे सीजी-पीईजी-एलसी/सीएल, सीजी-पीईजी-एलसी/एमओ, और सीजी-पीईजी-एलसी/जीएस बायोएक्टिव वाउंड ड्रेसिंग बने। डीपीपीएच विश्लेषण के माध्यम से आंतरराष्ट्रीय गतिविधि में अद्भुत एंटीऑक्सीडेंट गतिविधि दिखाई गई, जिसमें ड्रेसिंग्स को विभिन्न औषधि अनुक्रमों में जाँच किया गया। विभिन्न औषधि अनुक्रमों के साथ सी. और ई. कोली के खिलाफ एंटीबैक्टीरियल गुणों का परीक्षण किया गया। मेम्ब्रेन्स की जल वाष्प प्रेषण दर (डब्ल्यूवीटीआर) और स्वेलिंग व्यवहार की मूल्यांकन भी किया गया। ड्रग समुच्चय के व्याप्ति की अध्ययन के लिए स्पेक्ट्रोफोटोमेट्रिक मॉनिटरिंग का उपयोग किया गया, जिसने सीजी-पीईजी-एलसी/सीएल, सीजी-पीईजी-एलसी/एमओ, और सीजी-पीईजी-एलसी/जीएस बायोएक्टिव ड्रेसिंग्स की औषधि मुक्ति व्यवहार का अध्ययन किया। एमटीटी टेस्ट पर जीवसंगतता की जाँच में >90% कोश जीवता दिखाई दी, जिससे उनकी साइटोकॉम्पैटिबिलिटी की सूचना मिली।

समृद्धि किए गए ड्रेसिंग्स, समृद्धित ड्रेसिंग्स, और जीएस (सीजी-पीईजी-एलसी/सीएल, सीजी-पीईजी-एलसी/एमओ, और सीजी-पीईजी-एलसी/जीएस) में शामिल अनुकूलित ड्रेसिंग्स के बीच घाव भराई प्रभावी तरीके से एक तुलनात्मक मूल्यांकन, उनकी शीघ्र घाव भराई गतिविधि पर केंद्रित हुआ। बालब/सी के महस्वर एल्बिनो

माउस पर इन-विवो घाव भराई (%) परीक्षण और उसके बाद की सांदर्भिक जांचें की गईं। सीजी-पीईजी-एलसी/सीएल ड्रेसिंग्स ने प्रारंभिक उपचार के पहले ही दिन में >80% घाव भराई दिखाई और 7 दिनों में पूरी तरह ठीक हो गई, खासकर त्वचा में खारिश नहीं होने दिए। इसके बादी विवेचना ने उपयुक्त हुए मेम्ब्रेन्स के साथ तुलना करते हुए दिखाया कि सीजी-पीईजी और सीजी-पीईजी-एलसी ड्रेसिंग्स की तुलना में सांदर्भिक स्थायिता, महत्वपूर्ण कॉलेजन संचय, और त्वचा संलग्न गोली में तत्परता है। इस अनुसंधान ने से होकर बायोएक्टिव वाउंड ड्रेसिंग ने बायोपॉलिमर आधारित हाइड्रोजेल प्रौद्योगिकी (सीजी-पीईजी-एलसी) को हर्बल बायोएक्टिव एजेंट्स (सीएल, एमओ, और जीएस) के साथ मिलाकर एक सरल, किफायती, और प्रभावी समाधान प्रस्तुत किया है, जो दर्दमुक्त, निशान रहित, और त्वरित घाव भराई के लिए विशेष रूप से लाभकारी है, विशेषकर आर्थिक रूप से कमजोर जनता के लिए।

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LIST OF ABBREVIATIONS

Expanded Forms	Abbreviations
(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)	MTT
Acridine orange	AO
Calendula officinalis	CL
Carrageenan	CG
Colony forming unit	CFU
Differential scanning calorimetry	DSC
Diphenyl picrylhydrazyl	DPPH
Dulbecco's modified Eagle's medium	DMEM
Energy-Dispersive X-ray Spectroscopy	EDX
Escherichia coli	E. coli
Ethylene diamine tetra acetic acid	EDTA
Extracellular matrix	ECM
Fetal bovine serum	FBS
Field emission scanning electron microscopy	FESEM
Fourier transform-infrared spectroscopy	FTIR
Moringa oleifera	MO
Phosphate buffer saline	PBS
Phosphotidylcholine	PC
Poly(-L-glutamic acid)	PGA
Polycaprolactone	PCL
Polyethylene glycol	PEG

Polyurethane	PU
Polyvinyl alcohol	PVA
Polyvinylpyrrolidone	PVP
Propidium iodide	PI
Reactive oxygen species	ROS
Scanning electron microscopy	SEM
Soy Lecithin	LC
Staphylococcus aureus	S. aureus
Transmission electron microscopy	TEM
Vitis vinifera	GS
X-ray diffraction	XRD
Zone of inhibition	ZOI