STUDIES ON THE DEVELOPMENT OF BIOACTIVE POLYCAPROLACTONE BASED HYBRID NANOFIBERS

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by

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Dedicated to ...

My Father

-who always believe in me

Certificate

This is to certify that the thesis entitled "Studies on the Development of Bioactive Polycaprolactone Based Hybrid Nanofibers" submitted by Ms. Vandana Kumari 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. Vandana 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

Polycaprolactone is an electrospinnable polymer that holds significant importance in the healthcare field due to its biodegradable nature and biocompatibility. PCL makes an intriguing substrate for human healthcare applications because of its biodegradability and compatibility with the biological environment. The high viscoelastic properties and low melting temperature make it easy to fabricate into various shapes. The Food and Drug Administration (FDA) has approved it for different biomedical applications including contraceptive devices, meniscus regeneration, dental implants, osteoinductive, vascular biomaterial, tissue engineering and wound healing.

The use of polycaprolactone (PCL) is limited by its poor hydrophilicity, lack of functional groups, and inadequate cell affinity due to the absence of cell recognition sites. Without physical, chemical, or biological modifications, PCL's effectiveness is significantly reduced, which calls for the critical need for such modifications. This can be achieved by preparing PCL biocomposites, where polysaccharide, protein and bioactive agents bring favourable physiochemical changes to allow protein adsorption, cell adherence and proliferation on its surface.

Polycaprolactone (PCL) has been electrospun with various materials such as silk, gelatin, chitosan, collagen, and hyaluronic acid for wound healing applications. However, one of the least explored combinations has been a blend of PCL with kappa carrageenan (kC). kC is a sulphated linear polysaccharide derived from red seaweeds, known for its biocompatibility, non-immunogenicity, and biodegradability. Its structural similarity to native glycosaminoglycans (GAGs) found in the skin's extracellular matrix (ECM) makes kC a candidate which can be explored and was chosen for this study. The incorporation of the anionic

surfactant sodium dioctylsulfosuccinate (AOT) to enhance the miscibility between PCL and kappa carrageenan (kC) has been explored.

In order to provide antimicrobial, antioxidant and therapeutic properties, different herbal extracts were incorporated into PCL/ kC nanofiber. The presence of several phytochemicals helps in the eradication of bacteria, providing antimicrobial properties along with antioxidant properties. It also provides medicinal properties and facilitates the healing of wounds. The herbal- extracts investigated for this study include *T. arjuna* (hydroalcoholic extract), *B. monnieri* (methanolic extract) and *C. asiatica* (methanolic extract).

This study focuses on developing a matrix by blending PCL and kC. The matrix aims to combine the mechanical strength of PCL with the cytocompatibility of kC. AOT is employed to improve the miscibility between PCL and kC while serving as a co-spinning agent. This dual role helps reduce the mean fiber diameter and minimize bead defects during the electrospinning process. The herbal drug extracts were incorporated into optimized PCL/ kC /AOT nanofibers by blending them with a polymer solution and subsequently, electrospun them. The drug release from the matrix was examined, and the release mechanism was investigated by using the Korsmeyer-Peppas (KP) model. The antioxidant and antimicrobial properties were examined to assess the potential of the drug-loaded nanofibers as a suitable wound-healing matrix. Further, the biocompatibility of nanofibers was scrutinized by the MTT assay and cell staining. The biocomposite matrix demonstrated promising potential to be used as a wound healing application.

PCL is a hydrophobic synthetic polymer with good mechanical strength. kC is a hydrophilic natural polymer with good cytocompatibility. Nevertheless, it has insufficient mechanical strength and electrospinnability. Achieving homogeneous, bead-free nanofibers by blending hydrophobic PCL with hydrophilic kC presented a significant challenge. To enhance compatibility between these materials, a surfactant was incorporated. Simultaneously, it enhanced the physiochemical properties of nanofiber.

The herbal drugs have several medicinal properties that are suitable for wound healing, tissue engineering, and drug delivery. However, loading herbal extract without losing its bioactivity in nanofiber is difficult, as high voltage is applied to form nanofiber. Hence, the challenges in incorporation of herbal extract and its bioactivity were investigated and have been reported in the thesis.

सार

पॉलीकैप्रोलैक्टोन एक इलेक्ट्रोस्पिनेबल पॉलिमर है जो अपनी बायोडिग्रेडेबल प्रकृति और बायोकम्पैटिबिलिटी के कारण स्वास्थ्य सेवा क्षेत्र में महत्वपूर्ण महत्व रखता है। पीसीएल अपनी बायोडिग्रेडेबिलिटी और जैविक पर्यावरण के साथ अनुकूलता के कारण मानव स्वास्थ्य देखभाल अनुप्रयोगों के लिए एक दिलचस्प सब्सट्रेट बनाता है। उच्च विस्कोइलास्टिक गुण और कम पिघलने का तापमान विभिन्न आकारों में निर्माण करना आसान बनाता है। खाद्य एवं औषधि प्रशासन (एफडीए) ने गर्भनिरोधक उपकरणों, मेनिस्कस पुनर्जनन, दंत प्रत्यारोपण, ऑस्टियोइंडक्टिव, संवहनी बायोमटेरियल, ऊतक इंजीनियरिंग और घाव भरने सहित विभिन्न बायोमेडिकल अनुप्रयोगों के लिए इसे मंजूरी दे दी है। पॉलीकैप्रोलैक्टोन (पीसीएल) का उपयोग इसकी खराब हाइड्रोफिलिसिटी, कार्यात्मक समूहों की कमी, सैल्स की चिपकने की शक्ति की अनुपस्थिति के कारण और अपर्याप्त सेल आत्मीयता के कारण सीमित है। भौतिक, रासायनिक या जैविक संशोधनों के बिना, पीसीएल की प्रभावशीलता काफी कम हो जाती है, जिसके लिए ऐसे संशोधनों की महत्वपूर्ण आवश्यकता होती है। इसे पीसीएल बायोकंपोजिट तैयार करके प्राप्त किया जा सकता है, जहां पॉलीसेकेराइड, प्रोटीन और बायोएक्टिव एजेंट, प्रोटीन चिपकने की शक्ति, कोशिका पालन और इसकी सतह पर प्रसार की अनुमति देने के लिए अनुकूल भौतिक रासायनिक परिवर्तन लाते हैं। पॉलीकैप्रोलैक्टोन (पीसीएल) को घाव भरने के अनुप्रयोगों के लिए रेशम, जिलेटिन, काइटोसन, कोलेजन और हाइलूरोनिक एसिड जैसी विभिन्न सामग्रियों के साथ इलेक्ट्रोइस्पिन किया गया है। हालाँकि, सबसे कम खोजे गए संयोजनों में से एक कप्पा कैरेजेनन (केसी) के साथ पीसीएल का मिश्रण रहा है। केसी लाल समुद्री शैवाल से प्राप्त एक सल्फ़ेटेड रैखिक पॉलीसेकेराइड है, जो अपनी जैव-अनुकूलता, गैर-इम्यूनोजेनेसिटी और बायोडिग्रेडेबिलिटी के लिए जाना जाता है। त्वचा के बाह्य मैट्रिक्स (ईसीएम) में पाए जाने वाले मूल ग्लाइकोसामिनोग्लाइकेन्स (जीएजी) के साथ इसकी संरचनात्मक समानता केसी को एक

उम्मीदवार बनाती है जिसे खोजा जा सकता है और इस अध्ययन के लिए चुना गया था। पीसीएल और कप्पा कैरेजेनन (केसी) के बीच मिश्रणीयता को बढ़ाने के लिए आयनिक सर्फेक्टेंट सोडियम डाइऑक्टिलसल्फोस्यूसिनेट (एओटी) के समावेश का पता लगाया गया है। रोगाणुरोधी, एंटीऑक्सीडेंट और चिकित्सीय गुण प्रदान करने के लिए, विभिन्न हर्बल अर्क को पीसीएल/केसी नैनोफाइबर में शामिल किया गया था। कई फाइटोकेमिकल्स की मौजूदगी बैक्टीरिया के उन्मूलन में मदद करती है, जो एंटीऑक्सीडेंट गुणों के साथ रोगाणुरोधी गुण प्रदान करती है। साथ ही, यह औषधीय गुण प्रदान करता है और घावों के उपचार में सहायता करता है। इस अध्ययन के लिए जांचे गए हर्बल अर्क में टी. अर्जुन (हाइड्रोअल्कोहलिक अर्क), बी. मोनिएरी (मेथनॉलिक अर्क) और सी. एशियाटिका (मेथानॉलिक अर्क) शामिल हैं। यह अध्ययन पीसीएल और केसी को मिश्रित करके एक मैट्रिक्स विकसित करने पर केंद्रित है। मैट्रिक्स का लक्ष्य पीसीएल की यांत्रिक शक्ति को केसी की साइटोकंपैटिबिलिटी के साथ जोड़ना है। एओटी को सह-कताई एजेंट के रूप में कार्य करते हुए पीसीएल और केसी के बीच मिसिबिलिटी में सुधार करने के लिए नियोजित किया जाता है। यह दोहरी भूमिका इलेक्ट्रोस्पिनिंग प्रक्रिया के दौरान फाइबर व्यास को कम करने और बीड बनने की प्रक्रिया के दोष को कम करने में मदद करती है। हर्बल दवा के अर्क को पॉलिमर के साथ मिश्रित करके अनुकूलित पीसीएल/केसी/एओटी नैनोफाइबर में शामिल किया गया और बाद में, उन्हें इलेक्ट्रोइस्पिन किया गया। मैट्रिक्स से दवा रिलीज की जांच की गई, और कोर्समेयर-पेप्पस (केपी) मॉडल का उपयोग करके रिलीज तंत्र की जांच की गई। उपयुक्त घाव भरने वाले मैट्रिक्स के रूप में दवा से भरे नैनोफाइबर की क्षमता का आकलन करने के लिए एंटीऑक्सीडेंट और रोगाणुरोधी गुणों की जांच की गई। इसके अलावा, एमटीटी परख और सेल स्टेनिंग द्वारा नैनोफाइबर की जैव अनुकूलता की जांच की गई। बायोकम्पोजिट मैट्रिक्स घाव भरने वाले अनुप्रयोग के रूप में उपयोग किए जाने की आशाजनक क्षमता प्रदर्शित करता है। पीसीएल अच्छी यांत्रिक शक्ति वाला एक हाइड्रोफोबिक सिंथेटिक पॉलिमर है। केसी अच्छी साइटोकोम्पैटिबिलिटी वाला एक हाइड्रोफिलिक प्राकृतिक बहुलक है। फिर भी, इसमें

अपर्याप्त यांत्रिक शक्ति और इलेक्ट्रोस्पिनबिलिटी है। हाइड्रोफोबिक पीसीएल को हाइड्रोफिलिक केसी के साथ मिश्रित करके सजातीय, मनका-मुक्त नैनोफाइबर प्राप्त करना एक महत्वपूर्ण चुनौती पेश करता है। इन सामग्रियों के बीच अनुकूलता बढ़ाने के लिए, एक सर्फेक्टेंट को शामिल किया गया था। साथ ही, यह नैनोफाइबर के भौतिक रासायनिक गुणों को बढ़ाता है। हर्बल दवाओं में कई औषधीय गुण होते हैं जो घाव भरने, ऊतक इंजीनियरिंग और दवा वितरण के लिए उपयुक्त होते हैं। हालाँकि, नैनोफाइबर में अपनी जैव सक्रियता खोए बिना हर्बल अर्क को लोड करना मुश्किल है, क्योंकि नैनोफाइबर बनाने के लिए उच्च वोल्टेज लागू किया जाता है। इसलिए, हर्बल अर्क और इसकी जैव सक्रियता को शामिल करने में आने वाली चुनौतियों की जांच की गई।

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Abbreviations

AOT	Surfactant (bis (2-ethylhexyl) sulfosuccinate
BM	*Bacoppa monnieri*
CA	Cellulose acetate
CA	*Centella asiatica*
CFU	Colony forming unit
Col	Collagen
CS	Chitosan
DCM	Dichloromethane
DLS	Dynamic light scattering
DMEM	Dulbecco's modified Eagle's medium
DPPH	Diphenyl picrylhydrazyl
E. coli	*Escherichia coli*
ECM	Extracellular matrix
EDX	Energy-Dispersive X-ray Spectroscopy
FDA	Food and Drug Administration
FDD	Fiber Diameter Distribution
FESEM	Field emission scanning electron microscopy
FTIR	Fourier transform-infrared spectroscopy
Ge	Gelatin

kC	kappa Carrageenan
MFD	Mean Fiber Diameter
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
NFs	nanofibers
PBS	Phosphate Buffer solution
PCL	Polycaprolactone
PEG	Polyethylene glycol
PLA	Polylactic acid
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
Ra	Average roughness
Rq	Root mean square roughness
S. aureus	*Staphylococcus aureus*
SEM	Scanning electron microscopy
SF	Silk Fibroin
TA	*Terminalia arjuna*
TEM	Transmission electron microscopy
TX 100	Triton X 100
XRD	X-ray diffraction
ZOI	Zone of inhibition