

**STUDIES ON THE DEVELOPMENT OF
BIOCOMPATIBLE & INFECTION RESISTANT
POLYPROPYLENE HERNIA MESH**

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**Studies on the Development of Biocompatible & Infection
Resistant Polypropylene Hernia Mesh**

by

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Dedicated to my parents

My Papa Coach

&

Mrs. Nirmala Rani and Mr. Sushil Kumar Sethi

CERTIFICATE

This is to certify that the thesis titled “**Studies on the Development of Biocompatible & Infection-Resistant Polypropylene Hernia Mesh,**” being submitted by **Vipula Sethi** to the Indian Institute of Technology Delhi for the award degree **Doctor of Philosophy**, is a record of bonafide research work carried out by her. She has worked under our guidance and supervision and fulfilled the requirements for submitting her thesis, which has attained the standard required for a Ph.D. degree at this Institute. The results of the thesis have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.



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ABSTRACT

The field of biomaterials has witnessed transformative growth, driven by the need for advanced solutions in biomedical applications, such as tissue engineering, wound healing, and surgical implants. Among these, polymer-based materials have gained prominence due to their versatility, biocompatibility, and potential for functional modification. Polypropylene (PP) is extensively used in medical devices, particularly in hernia repair meshes, owing to its chemical inertness and low cost. However, the inherent susceptibility of the mesh to post-surgical infections remains a significant challenge, often leading to adverse clinical outcomes, such as mesh rejection, chronic inflammation, seroma formation and the development of biofilms. Addressing the critical issue of infection and seroma formation in implanted devices requires the development of infection-resistant biomaterials that provide long-term protection without compromising biocompatibility or mechanical integrity. While traditional approaches, such as antibiotic coatings, have provided some relief, they are often limited by problems like antibiotic resistance, inconsistent drug release profiles, and loss of efficacy over time. To bridge these gaps, current literature lacks robust insights into optimizing long-term antimicrobial efficacy and stability in PP-based meshes while also addressing seroma prevention and biocompatibility. These limitations highlight the urgent need for innovative strategies that can ensure sustained antimicrobial activity while maintaining the structural integrity of PP meshes.

Surface modification techniques, such as plasma grafting, are highly effective for functionalizing polymer surfaces without altering their bulk properties. Plasma treatment enables the creation of reactive groups on the PP surface, facilitating the immobilization of bioactive molecules with desired functionalities. In this investigation, the plasma grafting of PP mesh was performed to create functional groups on the surface. Oxygen plasma was used to generate hydrophilic functionalities

(hydroperoxide groups) on the surface of the PP mesh. The plasma power, plasma time, and gas flow rate were varied to achieve maximum hydroperoxide functionality on the surface. The highest peroxide content from plasma was $0.74 \mu\text{mol}/\text{cm}^2$, achieved under optimized experimental conditions of 120 s, 100 W, and 30 sccm. The surface morphology of plasma functionalized PP was investigated using FESEM and AFM techniques, which showed the formation of microdomains on the PP surface. The XPS analysis showed the formation of oxidative species on the mesh surface.

This plasma functionalized surface was subsequently grafted with itaconic acid under varied temperatures, concentrations, and grafting time. The maximum degree of grafting obtained was $1.5 \mu\text{g}/\text{cm}^2$ at optimized experimental conditions of 10%, 6 h and 50°C . The influence of reaction parameters on the physicochemical properties was studied using Fourier-transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS), while field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM) were employed to assess the surface topography. These groups act as anchoring sites for the subsequent immobilization of bioactive molecules on the surface to impart antibacterial and antifouling activity.

Chitosan, a biodegradable and biocompatible polysaccharide, is recognized for its inherent antimicrobial properties and ability to form nanogels that serve as efficient carriers for bioactive agents. Along with chitosan, ZnO nanoparticles have garnered considerable attention due to their superior properties and diverse applications, making them exceptionally advantageous compared to other metal particles in various fields. The unique characteristics of ZnO nanoparticles, such as high biocompatibility and good antibacterial nature, make them well-suited for fighting against microbial infections and developing advanced wound-healing materials.

By integrating ZnO nanoparticles within chitosan nanogels, was exploited their broad-spectrum antimicrobial activity, driven by the quenching of reactive oxygen species (ROS) that, can inhibit a wide range of bacterial strains. The amino and hydroxyl functional groups of CS contribute significantly to their biological properties, while ZnO exhibits antimicrobial action. Following the modification of the PP surface by plasma grafting, the CS-ZnO nanogels were covalently immobilized on the surface by an EDC coupling reaction. The carboxyl groups present on the surface *via* surface grafting covalently bind with the amino group present in the chitosan to form the amide linkages. The formation of the amide group was controlled by varying the pH of the solution for 3, 7, and 9. The maximum amide content was achieved in an acidic medium because of a stable intermediate and lack of hydrolysis. FE-SEM, contact angle, and AFM measurements were used to characterize and validate the immobilization of CS-ZnO on the functionalized PP mesh.

While these embedded nanogels prevent seroma formation and provide the desired antimicrobial properties with sustained drug release, the bacterial killing kinetics of these nanogels is very slow. Therefore, drug molecules with very fast-killing kinetics have been utilized to prevent instant surgical site infections. Among the widely used drugs, Doxycycline (DOX) is an antibiotic that plays a crucial role in inhibiting collagen synthesis, which can help reduce the amount of fluid accumulation in hernia. It possesses significant anti-inflammatory and antibacterial properties and inhibits matrix metalloproteinases (MMPs), promoting tissue healing and potentially reducing recurrence. The addition of doxycycline, a potent broad-spectrum antibiotic, further enhances the infection-resistant properties of the modified mesh, providing immediate and sustained antimicrobial defense during critical post-operative periods. The CS-ZnO immobilized mesh was loaded with DOX to create a bioactive infection-resistant hernia mesh. The loading of DOX in CS-ZnO resulted in a decrease in the water contact angle due to the presence of hydroxyl moieties on the drug surface.

The antimicrobial efficacy of the functionalized PP mesh is tested against clinically relevant pathogens, including *Staphylococcus aureus* and *Escherichia coli*, known for their role in post-operative infections. DOX has shown concentration-dependent bacteriostatic and bactericidal activity against Gram-positive and Gram-negative bacteria, which has resulted in a drastic reduction in bacterial adherence. The bacteria were significantly swollen and had ruptured the bacterial cell membrane, confirming that the adhered bacteria were deceased. *In vitro* release studies demonstrate the sustained release of doxycycline, ensuring prolonged antimicrobial activity for more than 10 days. Moreover, cell culture assays using fibroblasts confirm the biocompatibility of the modified mesh, showing improved cell adhesion and proliferation on the plasma-grafted surface with more than 95% cell viability. The synergistic effect of CS-ZnO nanogels and doxycycline creates a multi-functional mesh with dual antimicrobial action immediate response *via* antibiotic release, long-term bacterial inhibition and seroma prevention through ROS quenching by ZnO nanoparticles. The histopathology results for the DOX-loaded mesh showed minimal inflammation, no significant fibrosis, improved biocompatibility, no eosinophils, and good immunomodulating properties. In the dermis layer, there was a noticeable infiltration of neutrophils and macrophages. This dual mechanism not only combats infection but also prevents biofilm and seroma formation, which is a critical factor in the failure of many conventional mesh implants. The ongoing investigations thus offer valuable insights into the development of PP hernia mesh that prevents seroma formation, possesses antimicrobial properties and biocompatibility.

सार

बायोमटेरियल्स के क्षेत्र में व्यापक विकास देखा गया है, जिसे बायोमेडिकल अनुप्रयोगों जैसे कि टिश्यू इंजीनियरिंग, वाउंड हीलिंग, और सर्जिकल इम्प्लांट्स में उन्नत समाधान की आवश्यकता द्वारा प्रेरित किया गया है। इन सबके बीच, पॉलीमर आधारित सामग्री ने अपनी बहुमुखी प्रतिभा, जैव संगतता, और कार्यात्मक संशोधन की संभावनाओं के कारण प्रमुखता प्राप्त की है। पॉलीप्रोपाइलीन (PP) का चिकित्सा उपकरणों, विशेषकर हार्निया रिपेयर मेश में व्यापक रूप से उपयोग किया जाता है, इसकी मजबूती, रासायनिक जड़ता, और कम लागत के कारण। हालांकि, मेश की पोस्ट-सर्जिकल इन्फेक्शनों के प्रति स्वाभाविक संवेदनशीलता एक महत्वपूर्ण चुनौती बनी रहती है, जो अक्सर प्रतिकूल क्लिनिकल परिणामों जैसे मेश अस्वीकृति, क्रोनिक इन्फ्लेमेशन, सेरोमा निर्माण और बायोफिल्म्स के विकास की ओर ले जाती है। प्रत्यारोपित उपकरणों में इन्फेक्शन और सेरोमा गठन की इस महत्वपूर्ण समस्या को हल करने के लिए, संक्रमण-प्रतिरोधी बायोमटेरियल्स का विकास आवश्यक है जो लंबे समय तक सुरक्षा प्रदान करते हैं बिना जैव संगतता या यांत्रिक अखंडता से समझौता किए। जबकि पारंपरिक दृष्टिकोण, जैसे कि एंटीबायोटिक कोटिंग्स, ने कुछ राहत प्रदान की है, वे अक्सर एंटीबायोटिक प्रतिरोध, असंगत दवा रिलीज प्रोफाइल और समय के साथ प्रभावशीलता में कमी जैसी समस्याओं से सीमित रहते हैं। ये सीमाएँ नवाचार की रणनीतियों की तात्कालिक आवश्यकता को उजागर करती हैं जो PP मेश की संरचनात्मक अखंडता को बनाए रखते हुए निरंतर रोगाणुरोधी गतिविधि सुनिश्चित कर सकें।

सतह संशोधन तकनीकें, जैसे कि प्लाज्मा ग्राफ्टिंग, पॉलीमर सतहों को उनके बल्क गुणों को बदले बिना कार्यात्मक बनाने के लिए अत्यधिक प्रभावी हैं। प्लाज्मा उपचार PP सतह पर प्रतिक्रियाशील समूहों के निर्माण

को सक्षम बनाता है, जिससे वांछित कार्यक्षमताओं वाले जैव सक्रिय अणुओं की स्थिरीकरण की सुविधा मिलती है। इस जांच में, PP मेश की प्लाज्मा ग्राफ्टिंग को सतह पर कार्यात्मक समूह बनाने के लिए किया गया। ऑक्सीजन प्लाज्मा का उपयोग PP मेश की सतह पर हाइड्रोफिलिक कार्यक्षमताओं (हाइड्रोपरोक्साइड समूह) उत्पन्न करने के लिए किया गया। प्लाज्मा पावर, प्लाज्मा समय, और गैस प्रवाह दर को सतह पर अधिकतम हाइड्रोपरोक्साइड कार्यक्षमता प्राप्त करने के लिए भिन्न किया गया। 120 सेकंड, 100 वाट, और 30 एससीसीएम के अनुकूलित प्रायोगिक परिस्थितियों के तहत प्लाज्मा से उच्चतम पेरोक्साइड सामग्री 0.74 माइक्रोमोल/सेमी² प्राप्त हुई। प्लाज्मा कार्यात्मक PP की सतह रूपरेखा का जांच FESEM और AFM तकनीकों का उपयोग करके किया गया, जिसमें PP सतह पर माइक्रोडोमेन्स के निर्माण को दिखाया गया। XPS विश्लेषण ने मेश सतह पर ऑक्सीडेटिव प्रजातियों के गठन को दिखाया।

इस प्लाज्मा कार्यात्मक सतह को बाद में इटैकोनिक एसिड के साथ विभिन्न तापमानों, सांद्रणों, और ग्राफ्टिंग समय के तहत ग्राफ्ट किया गया। अनुकूलित प्रायोगिक परिस्थितियों के तहत अधिकतम ग्राफ्टिंग डिग्री 1.5 माइक्रोग्राम/सेमी² प्राप्त हुई जो 10%, 6 घंटे और 50°C थी। Fourier-transform infrared spectroscopy (FTIR) और X-ray photoelectron spectroscopy (XPS) का उपयोग करके भौतिक-रासायनिक गुणों पर प्रतिक्रिया मापदंडों के प्रभाव का अध्ययन किया गया, जबकि फील्ड एमिशन स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (FESEM) और एटॉमिक फोर्स माइक्रोस्कोपी (AFM) का उपयोग सतह टोपोग्राफी का मूल्यांकन करने के लिए किया गया। ये समूह सतह पर जैव सक्रिय अणुओं के स्थिरीकरण के लिए एंकरिंग साइट के रूप में कार्य करते हैं ताकि जीवाणुरोधी और एंटीफूलिंग गतिविधि प्रदान की जा सके।

चिटोसन, एक बायोडिग्रेडेबल और बायोकम्पैटिबल पॉलीसैकराइड, अपनी स्वाभाविक जीवाणुरोधी गुणों और जैव सक्रिय एजेंटों के लिए कुशल वाहक के रूप में सेवा करने वाली नैनोगेल्स बनाने की क्षमता के लिए मान्यता

प्राप्त है। चिटोसन के साथ, ZnO नैनोपार्टिकल्स ने भी अपनी उत्कृष्ट गुणों और विविध अनुप्रयोगों के कारण पर्याप्त ध्यान आकर्षित किया है, जो उन्हें विभिन्न क्षेत्रों में अन्य धातु कणों की तुलना में अत्यधिक लाभदायक बनाते हैं। ZnO नैनोपार्टिकल्स की अनूठी विशेषताएँ, जैसे उच्च जैव संगतता और अच्छी जीवाणुरोधी प्रकृति, उन्हें माइक्रोबियल इन्फेक्शनों से लड़ने और उन्नत वाउंड हीलिंग सामग्रियों के विकास के लिए उपयुक्त बनाती हैं।

चिटोसन नैनोगेल्स में ZnO नैनोपार्टिकल्स को एकीकृत करके, हम उनकी व्यापक-स्पेक्ट्रम जीवाणुरोधी गतिविधि का लाभ उठाते हैं, जो प्रतिक्रियाशील ऑक्सीजन प्रजातियों (ROS) के बुझाने से संचालित होती है, जो विभिन्न प्रकार के जीवाणु उपभेदों को रोक सकती हैं। CS के अमीनो और हाइड्रॉक्सिल कार्यात्मक समूह उनके जैविक गुणों में महत्वपूर्ण योगदान देते हैं, जबकि ZnO जीवाणुरोधी क्रिया का प्रदर्शन करता है। प्लाज्मा ग्राफ्टिंग द्वारा PP सतह के संशोधन के बाद, CS-ZnO नैनोगेल्स को EDC कपलिंग प्रतिक्रिया द्वारा सतह पर सहसंयोजक रूप से स्थिरीकरण किया गया। सतह पर उपस्थित कार्बोक्सिल समूह सतह ग्राफ्टिंग के माध्यम से चिटोसन में उपस्थित अमीनो समूह के साथ सहसंयोजक रूप से बंधकर अमाइड बंधन बनाते हैं। अमाइड समूह का गठन 3, 7, और 9 के pH को बदलकर किया गया था। अम्लीय माध्यम में अधिकतम अमाइड प्राप्त हुई क्योंकि एक स्थिर इंटरमीडिएट और हाइड्रोलिसिस की कमी थी। FE-SEM, संपर्क कोण, और AFM मापों का उपयोग CS-ZnO के कार्यात्मक PP मेश पर स्थिरीकरण की पुष्टि और चरित्रण के लिए किया गया।

ये एम्बेडेड नैनोगेल्स सेरोमा गठन को रोकते हैं और वांछित जीवाणुरोधी गुणों के साथ निरंतर दवा रिलीज प्रदान करते हैं, लेकिन इन नैनोगेल्स की जीवाणु हत्या की गतिशीलता बहुत धीमी होती है। इसलिए, सर्जिकल साइट इन्फेक्शनों को रोकने के लिए बहुत तेज़ हत्या गतिशीलता वाली दवा अणुओं का उपयोग किया जाता है। व्यापक रूप से उपयोग की जाने वाली दवाओं में, डॉक्सीसाइक्लिन (DOX) एक एंटीबायोटिक है जो हर्निया में तरल

पदार्थ संचय को कम करने में मदद कर सकता है, जिसमें कोलाजेन संश्लेषण को रोकने की महत्वपूर्ण भूमिका होती है। यह महत्वपूर्ण एंटी-इन्फ्लेमेटरी और जीवाणुरोधी गुणों का प्रदर्शन करता है और मैट्रिक्स मेटालोप्रोटीनास (MMPs) को रोकता है, जिससे टिशू हीलिंग और संभावित रूप से पुनरावृत्ति में कमी होती है। डॉक्सीसाइक्लिन का जोड़, एक शक्तिशाली व्यापक-स्पेक्ट्रम एंटीबायोटिक, संशोधित मेश की संक्रमण-प्रतिरोधी गुणों को और बढ़ाता है, महत्वपूर्ण पोस्ट-ऑपरेटिव अवधि के दौरान तात्कालिक और निरंतर जीवाणुरोधी रक्षा प्रदान करता है। CS-ZnO स्थिरीकरण मेश को DOX से लोड किया गया था ताकि एक जैव सक्रिय संक्रमण-प्रतिरोधी हर्निया मेश का निर्माण किया जा सके। DOX को CS-ZnO में लोड करने से जल संपर्क कोण में कमी आई, जो दवा सतह पर हाइड्रॉक्सिल मूर्तियों की उपस्थिति के कारण थी।

कार्यात्मक PP मेश की जीवाणुरोधी प्रभावकारिता को *Staphylococcus aureus* और *Escherichia coli* जैसे क्लिनिकल रूप से प्रासंगिक रोगजनकों के खिलाफ परीक्षण किया गया, जो पोस्ट-ऑपरेटिव इन्फेक्शनों में अपनी भूमिका के लिए जाने जाते हैं। DOX ने ग्राम-पॉज़िटिव और ग्राम-नेगेटिव बैक्टीरिया के खिलाफ सांद्रण-निर्भर जीवाणुस्थैतिक और जीवाणुनाशक गतिविधि का प्रदर्शन किया। उन्होंने जीवाणु चिपचिपाहट में जबरदस्त कमी दिखाई। बैक्टीरिया काफी सूज गए और उन्होंने जीवाणु कोशिका झिल्ली को फाड़ दिया, यह पुष्टि करते हुए कि चिपके हुए बैक्टीरिया मृत थे। इन विट्रो रिलीज अध्ययन निरंतर डॉक्सीसाइक्लिन रिलीज का प्रदर्शन करते हैं, जो 10 दिनों से अधिक समय तक निरंतर जीवाणुरोधी गतिविधि सुनिश्चित करता है। इसके अलावा, फाइब्रोब्लास्ट्स का उपयोग करके सेल कल्चर परखों ने संशोधित मेश की जैव संगतता की पुष्टि की, जो प्लाज्मा-ग्राफ्टेड सतह पर 95% से अधिक सेल जीवन शक्ति के साथ बेहतर सेल चिपचिपाहट और प्रसार दिखा रहे हैं। CS-ZnO नैनोगेल्स और डॉक्सीसाइक्लिन का तालमेलपूर्ण प्रभाव एक दोहरी जीवाणुरोधी क्रिया के साथ एक बहुआयामी मेश बनाता है, तत्काल प्रतिक्रिया एंटीबायोटिक रिलीज के माध्यम से, लंबी अवधि के बैक्टीरियल अवरोध और ROS बुझाने के माध्यम से सेरोमा की रोकथाम ZnO नैनोपार्टिकल्स द्वारा। DOX

लोडेड मेश के हिस्टोपैथोलॉजी परिणामों ने न्यूनतम सूजन, महत्वपूर्ण फाइब्रोसिस नहीं, उन्नत जैव संगतता, कोई ईसिनोफिल नहीं, और अच्छे प्रतिरक्षामॉडुलिंग गुण दिखाए। डर्मिस परत में, न्यूट्रोफिल्स और मैक्रोफेज का उल्लेखनीय प्रवेश देखा गया। यह दोहरा तंत्र न केवल संक्रमण से लड़ता है बल्कि बायोफिल्म और सेरोमा गठन को भी रोकता है, जो कई पारंपरिक मेश इम्प्लांट्स की विफलता में एक महत्वपूर्ण कारक है। वर्तमान जांचें इस प्रकार PP हर्निया मेश के विकास में मूल्यवान अंतर्दृष्टि प्रदान करती हैं जो सेरोमा गठन को रोकता है, जीवाणुरोधी गुण और जैव संगतता रखता है।

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol/Abbreviation	Expanded Form/Term
°C	degree Celsius
s	Second
N	Newton
g	Gram
mg	Milligram
µg	Microgram
L	Liter
mL	Milli liter
MPa	Mega pascal
Wt. %	Weight percentage
kV	kilo volt
mol	Molarity
W	Watt
eV	Electron volt
T	Temperature
MHz	Megahertz
PP	Polypropylene
PVDF	Poly (vinylidene fluoride)
PMMA	Poly(methyl methacrylate)

ND	Nanodiamonds
PCL	Polycaprolactone
CS	Chitosan
IA	Itaconic acid
ZnO	Zinc Oxide
CHX	Chlorohexidine
PVA	Polyvinyl Alcohol
AMP	Antimicrobial Peptide
EDC	N-(dimethyl aminopropyl)-N'-ethyl carbodiimide hydrochloride
PEGMA	Poly(ethylene glycol) methacrylate
PEG	Polyethylene glycol
DOX	Doxycycline hydrochloride
PNIPAAm	Poly(N-isopropyl acrylamide)
ECM	Extracellular matrix
CA	Contact angle
RAFT	Atom transfers radical polymerization
RATRP	Reverse atom transfers radical polymerization
TOF-SIIMS	Time of flight secondary ion mass spectroscopy
FE-SEM	Field emission scanning electron microscopy
XRD	X-Ray Diffraction
DSC	Differential Scanning Calorimeter
FTIR	Fourier transform infrared spectroscopy
AFM	Atomic force microscopy

XPS	X-ray photoelectron spectroscopy
ESR	Electron Spin Resonance
ROS	Reactive oxygen species
RF	Radio Frequency
DPPH	2,2-diphenyl-1-picrylhydrazyl
DBD	Dielectric barrier discharge