

STUDIES ON THE PREPARATION OF ANTIMICROBIAL POLYURETHANE FOR BIOMEDICAL APPLICATION

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MARCH, 2024

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Studies on the Preparation of Antimicrobial Polyurethane for Biomedical Application

by

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Submitted

in fulfillment of the requirements of the degree of

Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

NEW DELHI-110016, INDIA

MARCH, 2024

Dedicated to my Grandparents

Mrs. Manohar Devi Somani and Mr. Khyali Lal Somani

&

Mrs. Brijkuwar Maheshwari and Mr. Dulichand Maheshwari

CERTIFICATE

This is to certify that the thesis titled '*Studies on the Preparation of Antimicrobial Polyurethane for Biomedical Application*', being submitted by **Manali Somani** 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. She has worked under our guidance and supervision and fulfilled the requirements for the submission of thesis, which has attained the standard required for a Ph.D. degree of this Institute. The results contained in this 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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Date: 04th March 2024

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ACKNOWLEDGEMENTS

“Gratitude even sweetens the smallest moments”

I wish to take a small moment to express my gratitude to everyone, whose vital contributions played an important role in the accomplishment of this thesis. This odyssey would not have been possible without their constant support and encouragement. Their knowledgeable advice and their assistance were essential in turning this idea into a reality in the form of this thesis.

First and foremost, I want to convey my heartfelt gratitude to mentors, Prof. Bhuvanesh Gupta and Prof. Samrat Mukhopadhyay. This dissertation would not have been possible without their vision, proficiency and constant backing. Their guidance makes this journey smooth and knowledgeable. I would especially like to thank Prof. Bhuvanesh Gupta for his constant belief in me and teaching me always *“Thrive for knowledge”* attitude. A special thanks to Prof. Samrat Mukhopadhyay for always encouraging attitude and suggesting things beyond the box ideology. I will forever be grateful to them for dedicating their valuable contribution in shaping my research into a thesis.

I am also sincerely thankful to Dr. Rohit Sarda and Dr. Amlan Gupta (Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India) for helping in conducting the animal studies at SMIMS, Gangtok, Sikkim, India. I am thankful to Prof. Surya Bhan and Mr. Flavius Phrangsngi Nonglang (Department of Biochemistry, North Eastern Hill University, Shillong) for helping in conducting the biocompatibility studies at NEHU, Shillong, India.

I would like to appreciate Ministry of Human Resource Development (MHRD), Government of India, for the financial assistance throughout this journey. I am also thankful to Department of Textile and Fibre engineering, Indian Institute of Technology

Delhi for giving me this opportunity. I also want to extend my thanks Prof. R. Alagirusamy, the present Head of the Department of Textile Technology, as well as the previous Heads of Department, Prof. R.S. Rengasamy, Prof. B. K. Behara, and Prof. Ashwini K. Agrawal, for their cooperations in completing this study work effortlessly. In addition, I am great full to the members of my SRC (Student Research Committee), Prof. Harpal Singh, Prof. Mangal Joshi and Prof. Rajiv Srivastava, for their insightful comments.

I would like to express my regards to the department's staff, particularly Mr. Shiv Upadhyay, Mr. Amarjit, Mr. Kala, Mr. Rajinder Khatter, Dr. Vikas Khatkar, and Mr. Rohit Kumar for their technical assistance and their cooperation throughout my doctoral studies. I extend my appreciation to Central Research Facility and Nano Research Facilities for giving access to all research facilities. Mr. Kuldeep Sharma, Mr. Mahesh Soni, Mr. Dinesh Kumar, Ms. Kumud Arora, Mr. Rajesh Kumar, Mr. Animesh Bhowal, and Ms. Aastha Sharma for their support in conducting various experimental studies throughout my dissertation. work.

It gives me immense pleasure to extend my appreciation to my senior, Dr. Chetna Verma for her support and unwavering encouragement. A warm thanks to my lab mates Ms. Ankita Sharma, Ms. Pratibha Singh and Ms. Vandana Kumari for their constant support and availability for discussion whenever needed. A special thanks to my juniors Ms. Rohini Verma and Ms. Vipula Sethi for always being there and helping me whenever needed.

I am grateful to all my friends who were there in my thick and thin, particularly, Dr. Rupali Dhiman, Dr. Gurneet Kaur, Ms. Meenal Agrawal, Ms. Mamta Chaudhary, Ms. Anjali Kumawat, Ms. Shreya Singh, Ms. Jayshree Pati, and Ms. Anchal Anand. They

stood by me as a supportive wall throughout my journey. Their presence has made this journey rememberable and joyful. Thanks to all for being such wonderful companions.

Finally, I want to express my heartfelt thanks to my entire family for their unwavering support and unselfish sacrifices during my journey. I would want to express my gratitude to my grandparents, Mrs. Manohar Devi Somani and Mr. Khyali Lal Somani, as well as my parents, Mrs. Suman Somani and Mr. Anil Kumar Somani for providing me with the encouragement and motivation I needed to pursue this degree. I would also want to thank my sister and brother, Mrs. Mitali Mundra and Mr. Avi Somani for their unending encouragement and support during this dissertation. I would want to thank my in-law's Mrs. Indu Maheshwari and Mr. Balkrishan Maheshwari for accepting me and my desires, wholeheartedly. Last but not most I would like to thank my husband Mr. Porav Maheshwari, for not only accepting my objectives and desires to pursue my Ph.D., but also for providing unconditional support and encouragement to keep trying despite all odds.

Finally, I would want to admit that no one can go alone. Thesis is genuinely effective when many people contribute meaningfully along the work. I would want to offer my heartfelt gratitude to everyone who has assisted me, directly or indirectly, throughout my study. Throughout my life, I will treasure each person's important time, contributions, and sacrifices toward the fulfilment of my goals.

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ABSTRACT

In the advanced era of biomedical engineering the implants reside within human bodies either temporarily or permanently for restoring body functionality, diagnostic, monitoring, and therapeutic purposes. The choice of implant material (natural or synthetic polymer) and duration of implantation depend on the final application. Polyurethane (PU) has gained prominence in the field of biomedical devices and implants due to its tailor-made properties, biocompatibility, hemocompatibility, and ability to mimic natural tissues. The challenging part with PU implants is their chemical inertness, thrombosis formation, and the lack of cell adhesive ligands. Chemical functionalization techniques can introduce different functional groups on the PU surface. The generated functionality helps to immobilize bioactive components which reduces the occurrence of thrombosis and enhances the interface-cell interaction of PU implants.

Through the use of hydrolysis and aminolysis, the PU surface has been chemically functionalized. Alkaline hydrolysis using sodium hydroxide (NaOH) was used for the generation of hydrophilic (amine and hydroxyl) functionality on polyurethane surface. The reactant concentration and reaction time were investigated in terms of the amine functionality. The amine content at optimized condition was found to be $\sim 0.07 \mu\text{M}/\text{cm}^2$ for 8 h reaction time after treating with 10% NaOH. The surface morphology of hydrolyzed polyurethane was characterized using SEM and AFM technique, which confirmed the formation microdomains on the PU surface due to hydrolysis. These microdomains altered the transparency of the films and made the film opaque in appearance.

PU film was subjected to aminolysis using ethylene diamine (EDA) under varied temperatures, time, and EDA concentrations. The influence of reaction parameters on the

formation of free amino (-NH_2) groups and the resulting changes in the physicochemical properties of PU were investigated further. The optimized amine content was $\sim 0.08 \mu\text{M}/\text{cm}^2$ after treatment with 10% EDA for 15 min. The surface was characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM) and contact angle measurement. Aminolysis process was selected over hydrolysis because similar amine content was achieved in less time and at a lower temperature with relatively less weight loss and tensile strength loss. The process parameter optimized based on least compromise in inherent properties were: 10% EDA; 15 min and 50°C .

Carboxymethyl Cellulose (CMC) is utilized as a coating material due to its superior water absorption, nontoxicity, pH sensitivity, biodegradability, and biocompatibility. Following the modification of the polyurethane surface *via* aminolysis, the CMC polymer is covalently immobilized via Schiff base chemistry. The periodic oxidation of CMC results in the formation of dialdehyde groups along the backbone of the CMC chain. An imine bond is created when these aldehyde groups OCMC interact with the amino groups on aminolyzed PU (PU-A) surface. After 6 h, the change in aldehyde content was not significant, so 6 h was finalized for immobilization of OCMC on PU-A surface. Scanning electron microscopy (SEM), contact angle, and X-ray photoelectron spectroscopy (XPS) measurements are used to characterize and validate the immobilization of OCMC on aminolyzed PU film (PU-O).

Nitrofurantoin (NF) drug is used to incorporate within the OCMC gel and to immobilize on the PU surface for creating antimicrobial PU surface. The incorporation of NF in OCMC resulted in a moderate increase in WCA. The cross-sectional morphology of PU-ON films was investigated using FESEM. The incorporation of the drug is confirmed using EDX analysis. NF has shown the concentration dependent bacteriostatic and bactericidal activity against Gram-positive and Gram-negative bacteria. The PU-ON

showed a drastic reduction in bacterial adherence. The bacteria were vastly inflated and had ruptured the bacterial cell membrane, confirming that the adhered bacteria were dead.

The designing of nanosilver (nAg) has been carried out by *in-situ* reduction of silver nitrate using oxidized carboxymethyl cellulose (OCMC). The reduction process was also accompanied by the stabilization of nAg particles using the OCMC polymer chain, leading to the formation of a structure where nAg was entrapped within OCMC gel. The nAg particles were characterized using transmission electron microscopy (TEM) and were found to be ~22 nm. The nAg particles have exhibited prominent antimicrobial activity against both gram-negative and gram-positive bacteria. It is observed that a 3 mM concentration of nAg is active in inhibiting bacterial growth. The antibacterial activity of the synthesized nAg particles was dose-dependent, with 99.9% of *E. coli* and *S. aureus* destroyed within 5 h. The antibactericidal effect of the nAg particles was further investigated by TEM and reactive oxygen species (ROS) generation after nAg particle treatment. It showed strong intracellular fluorescence and also showed that OCMC-nAg particles produced intracellular ROS in *E. coli* and resulted in cell death.

There is a good possibility to use a blend of nAg particles and drug to address the concerns with cytotoxicity of nanoparticles and resistance to antibiotics. The synergistic effect of NF and nAg particles was assessed through *in-vitro* experiments. The NF and nAg particles were blended in different blend solutions, such as 100:0, 90:10, 80:20, 70:30 and 60:40. The PU-A films were immersed in the different blend solution for 6 h at 40 °C. To confirm the immobilization of blend on PU-A films on the surface, the cryo-fracture of PU-ON and PU-ON/nAg films was carried out. There was a layered structure with nAg particles distributed in the top layer. Further in EDX analysis, the emergence of peak at ~2.9 KeV, which is the characteristic peak of Ag, confirmed the presence of nAg

particles in the coating. The NF and nAg particles solemnly were not capable of giving the prolong antimicrobial activity, but the blends showed prolonged activity due to synergistic effects. The 70:30 blend ratio was carried forward for further study.

Urinary catheterization in hospitals leads to nosocomial infections and high mortality rate. The major cause of nosocomial infection is the development of encrustation and bacterial biofilm on the surface of the urinary catheter. The infection resistance coating having (70:30) NF:nAg particles, was utilized to design antimicrobial catheter. Initially, the catheter was functionalized from inner lumen using aminolysis technique and generated the $\sim 0.04 \mu\text{M}/\text{cm}^2$ amino functionality on the surface. Later, OCMC-NF/nAg blend were immobilized on the functionalized catheter, exploiting Schiff base chemistry. The bacterial colony formation was nearly completely suppressed by the PU-ON/nAg (70:30) catheter, whereas many colonies could still be found with PU catheter. PU-ON/nAg (70:30) catheter was found to be resistant to the bacterial colonization, and no trace of bacteria was detected.

Encrustation is a significant urinary catheter problem in addition to bacterial adhesion. Encrustation, *i.e.*, deposition of salt on the surface, was gradually developed with time in the urinary environment. After flowing synthetic urine for 7 days, encrustation was examined using SEM. The number of encrustations on the PU-ON and PU-ON/nAg catheter significantly decreased. The antibacterial migration assay was carried out through an *in-vitro* model. In PU catheter bacterial migration was prominent, as a dense biofilm was formed on the section in the vicinity of a bacterial suspension. These results indicated that both catheters may not be able to impede bacterial adhesion and migration in a nutrient-rich culture medium. In comparison with the above, the PU-ON and PU-ON/nAg catheters effectively inhibited bacterial migration and showed the lowest

biomass accumulation, giving a reduction of over ~70% and 95%, respectively. The histopathological evaluation of catheter in Swiss albino mice showed that the catheter is biocompatible in matrix. The present investigations therefore lead to the insight of the PU catheter development which is antimicrobial and biocompatible in nature.

सार

जैवचिकित्सा अभियांत्रिकी के उन्नत युग में शरीर की कार्यक्षमता, निदान, निगरानी और चिकित्सीय उद्देश्यों को बहाल करने के लिए प्रत्यारोपण अस्थायी या स्थायी रूप से मानव शरीर के भीतर रहते हैं। प्रत्यारोपण सामग्री (प्राकृतिक या कृत्रिम पॉलिमर) का चुनाव और प्रत्यारोपण की अवधि अंतिम उपयोग पर निर्भर करती है। पॉलीयुरेथेन (पीयू) ने अपने विशिष्ट गुणों, जैव अनुकूलता, हेमोकम्पैटिबिलिटी और प्राकृतिक ऊतकों की अनुकृति करने की क्षमता के कारण जैव चिकित्सा उपकरणों और प्रत्यारोपण के क्षेत्र में प्रमुखता प्राप्त की है। पीयू प्रत्यारोपण के साथ चुनौतीपूर्ण हिस्सा उनकी रासायनिक जड़ता, घनास्त्रता गठन और कोशिका चिपकने वाले संलग्न की कमी है। रासायनिक क्रियाशीलता तकनीक पीयू सतह पर विभिन्न प्रकार्यात्मक समूहों को पेश कर सकती है। उत्पन्न प्रकार्यात्मक समूह जैवसक्रिय घटकों को स्थिर करने में मदद करती है जो घनास्त्रता की घटना को कम करती है और पीयू प्रत्यारोपण के इंटरफ़ेस-सेल इंटरैक्शन को बढ़ाती है।

हाइड्रोलिसिस और एमिनोलिसिस के उपयोग के माध्यम से, पीयू सतह को रासायनिक रूप से कार्यात्मक बनाया गया है। पॉलीयुरेथेन सतह पर हाइड्रोफिलिक (अमाइन और हाइड्रॉक्सिल) कार्यक्षमता उत्पन्न करने के लिए सोडियम हाइड्रॉक्साइड (NaOH) का उपयोग करके क्षारीय हाइड्रोलिसिस का उपयोग किया गया था। अमीन कार्यक्षमता के संदर्भ में प्रतिक्रियाशील एकाग्रता और प्रतिक्रिया समय की जांच की गई। 10% NaOH के साथ उपचार के बाद 8 घंटे की प्रतिक्रिया समय के लिए अनुकूलित स्थिति में अमीन सामग्री $\sim 0.07 \mu\text{M}/\text{cm}^2$ पाई गई। हाइड्रोलाइज्ड पॉलीयुरेथेन की सतह को एसईएम (SEM) और एएफएम (AFM) तकनीक का उपयोग करके चित्रित किया गया था, जिसने हाइड्रोलिसिस के कारण पीयू सतह पर माइक्रोडोमेन के गठन की पुष्टि की। इन माइक्रोडोमेन ने फिल्मों की पारदर्शिता को बदल दिया और फिल्म को अपारदर्शी बना दिया।

पीयू फिल्म को विभिन्न तापमान, समय और एथिलीन डायमाइन (ईडीए) सांद्रता के तहत एथिलीन डायमाइन का उपयोग करके एमिनोलिसिस के अधीन किया गया था। मुक्त अमीनो ($-\text{NH}_2$) समूहों के गठन पर प्रतिक्रिया मापदंडों के प्रभाव और पीयू के भौतिक रासायनिक गुणों में परिणामी परिवर्तनों की आगे जांच की गई। 15 मिनट के लिए 10% ईडीए के साथ उपचार के बाद अनुकूलित अमीन सामग्री $\sim 0.08 \mu\text{M}/\text{cm}^2$ थी। सतह की विशेषता स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (SEM), परमाणु बल माइक्रोस्कोपी (AFM) और संपर्क कोण माप (WCA) द्वारा की गई थी। हाइड्रोलिसिस के स्थान पर एमिनोलिसिस प्रक्रिया को चुना गया क्योंकि समान अमीन सामग्री कम समय में और कम तापमान पर अपेक्षाकृत कम वजन घटने और तन्य शक्ति हानि के साथ प्राप्त की गई थी। अंतर्निहित गुणों में कम से कम समझौते के आधार पर अनुकूलित प्रक्रिया पैरामीटर थे: 10% ईडीए; 15 मिनट और 50°C .

कार्बोक्सिमिथाइल सेलुलोज (सीएमसी) का उपयोग इसके बेहतर जल अवशोषण, गैर-विषाक्तता, पीएच संवेदनशीलता, बायोडिग्रेडेबिलिटी और बायोकम्पैटिबिलिटी के कारण कोटिंग सामग्री के रूप में किया जाता है। एमिनोलिसिस के माध्यम से पॉलीयुरेथेन सतह के संशोधन के बाद, सीएमसी पॉलिमर को शिफ बेस रसायन विज्ञान के माध्यम से सहसंयोजक रूप से स्थिर किया जाता है। सीएमसी के आवधिक ऑक्सीकरण के परिणामस्वरूप सीएमसी श्रृंखला की रीढ़ की हड्डी के साथ डायल्डिहाइड समूहों का निर्माण होता है। जब ये एल्डिहाइड समूह ओसीएमसी एमिनोलाइज्ड पीयू (पीयू-ए) सतह पर एमिनो समूहों के साथ प्रतिक्रिया करते हैं तो एक इमाइन बंध बनता है। 6 घंटे के

बाद, एलडिहाइड सामग्री में परिवर्तन महत्वपूर्ण नहीं था, इसलिए पीयू-ए सतह पर ओसीएमसी के स्थिरीकरण के लिए 6 घंटे को अंतिम रूप दिया गया था। स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (SEM), संपर्क कोण, और एक्स-रे फोटोइलेक्ट्रॉन स्पेक्ट्रोस्कोपी (XPS) माप का उपयोग एमिनोलाइज्ड पीयू फिल्म (पीयू-ओ) पर ओसीएमसी के स्थिरीकरण को चिह्नित करने और मान्य करने के लिए किया जाता है।

नाइट्रोफ्यूरेटोइन (एनएफ) दवा का उपयोग ओसीएमसी जेल के भीतर शामिल करने और रोगाणुरोधी पीयू सतह बनाने के लिए पीयू सतह पर स्थिर करने के लिए किया जाता है। OCMC में NF को शामिल करने से WCA में मध्यम वृद्धि हुई। एफईएसईएम (FESEM) का उपयोग करके पीयू-ओएन (PU-ON) फिल्मों की क्रॉस-सेक्शनल आकृति विज्ञान की जांच की गई। ईडीएक्स (EDX) विश्लेषण का उपयोग करके दवा के निगमन की पुष्टि की जाती है। एनएफ (NF) ने ग्राम-पॉजिटिव और ग्राम-नेगेटिव बैक्टीरिया के खिलाफ एकाग्रता पर निर्भर बैक्टीरियोस्टैटिक और जीवाणुनाशक गतिविधि दिखाई है। पीयू-ओएन (PU-ON) ने बैक्टीरिया के पालन में भारी कमी देखी। उन्होंने बैक्टीरिया की कोशिका झिल्ली को तोड़ दिया था, जिससे यह पुष्टि हो गई कि चिपके हुए बैक्टीरिया मर चुके थे।

नैनोसिल्वर (nAg) की डिजाइनिंग ऑक्सीकृत कार्बोक्सिमिथाइल सेलुलोज (OCMC) का उपयोग करके सिल्वर नाइट्रेट की इन-सीटू रासायनिक अपचयन द्वारा की गई है। रासायनिक अपचयन की प्रक्रिया के साथ OCMC पॉलिमर श्रृंखला का उपयोग करके nAg कणों का स्थिरीकरण भी किया गया, जिससे एक संरचना का निर्माण हुआ जहां nAg OCMC जेल के भीतर फंस गया था। ट्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोपी (TEM) का उपयोग करके एनएजी (nAg) कणों की विशेषता निर्धारित की गई और उन्हें ~ 22 एनएम पाया गया। एनएजी (nAg) कणों ने ग्राम-नकारात्मक और ग्राम-पॉजिटिव दोनों बैक्टीरिया के खिलाफ प्रमुख रोगाणुरोधी गतिविधि प्रदर्शित की है। यह देखा गया है कि nAg की 0.3 मिलिमोल्स सांद्रता बैक्टीरिया के विकास को रोकने में सक्रिय है। संश्लेषित एनएजी कणों की जीवाणुरोधी गतिविधि खुराक पर निर्भर थी, जिसमें 99.9% *ई. कोली* और *एस. ऑरियस* 5 घंटे के भीतर नष्ट हो गए। एनएजी कणों के जीवाणुरोधी प्रभाव की एनएजी (nAg) कण उपचार के बाद टीईएम (TEM) और प्रतिक्रियाशील ऑक्सीजन प्रजातियों (ROS) पीढ़ी द्वारा आगे की जांच की गई। इसने मजबूत इंटरसेल्युलर प्रतिदीप्ति दिखाई और यह भी दिखाया कि ओसीएमसी-एनएजी (OCMC-nAg) कणों ने *ई. कोली* में इंटरसेल्युलर आरओएस का उत्पादन किया और परिणामस्वरूप कोशिका मृत्यु हुई।

नैनोकणों की साइटोटॉक्सिसिटी और एंटीबायोटिक दवाओं के प्रतिरोध से संबंधित चिंताओं को दूर करने के लिए एनएजी कणों और दवा के मिश्रण का उपयोग करने की एक अच्छी संभावना है। एनएफ और एनएजी कणों के सहक्रियात्मक प्रभाव का मूल्यांकन इन-विट्रो प्रयोगों के माध्यम से किया गया था। एनएफ और एनएजी कणों को अलग-अलग मिश्रण समाधानों में मिश्रित किया गया था, जैसे कि 100:0, 90:10, 80:20, 70:30 और 60:40। पीयू-ए फिल्मों को 40 डिग्री सेल्सियस पर 6 घंटे के लिए अलग-अलग मिश्रण समाधान में डुबोया गया। सतह पर PU-A फिल्मों पर मिश्रण के स्थिरीकरण की पुष्टि करने के लिए, PU-ON और PU-ON/nAg फिल्मों का क्रायो-फ्रैक्चर किया गया। शीर्ष परत में वितरित nAg कणों के साथ एक स्तरित संरचना थी। इसके अलावा ईडीएक्स (EDX) विश्लेषण में, ~2.9 केवी पर शिखर का उद्भव, जो कि एजी की विशेषता शिखर है, ने कोटिंग में एनएजी (nAg) कणों की उपस्थिति की पुष्टि की। एनएफ और एनएजी कण पूरी तरह से लंबे समय

तक रोगाणुरोधी गतिविधि देने में सक्षम नहीं थे, लेकिन मिश्रणों ने सहक्रियात्मक प्रभावों के कारण लंबे समय तक गतिविधि दिखाई। आगे के अध्ययन के लिए 70:30 मिश्रण अनुपात को आगे बढ़ाया गया।

अस्पतालों में मूत्र कैथीटेराइजेशन से नोसोकोमियल संक्रमण और उच्च मृत्यु दर होती है। नोसोकोमियल संक्रमण का प्रमुख कारण मूत्र कैथेटर की सतह पर मूत्र का जम जाना और जीवाणु बायोफिल्म का विकास है। (70:30) एनएफ:एनएजी कणों वाली संक्रमण प्रतिरोधी कोटिंग का उपयोग रोगाणुरोधी कैथेटर को डिजाइन करने के लिए किया गया था। प्रारंभ में, कैथेटर को एमिनोलिसिस तकनीक का उपयोग करके आंतरिक लुमेन से क्रियाशील किया गया था और सतह पर $\sim 0.04 \mu\text{M}/\text{cm}^2$ एमिनो कार्यक्षमता उत्पन्न की गई थी। बाद में, OCMC-NF/nAg मिश्रण को सिर्फ बेस रसायन विज्ञान का उपयोग करते हुए कार्यात्मक कैथेटर पर स्थिर कर दिया गया। बैक्टीरियल कॉलोनी का गठन पीयू-ओएन/एनएजी (70:30) कैथेटर द्वारा लगभग पूरी तरह से दबा दिया गया था, जबकि कई कॉलोनियां अभी भी पीयू कैथेटर के साथ पाई जा सकती थीं। PU-ON/nAg (70:30) कैथेटर को बैक्टीरिया उपनिवेशन के प्रति प्रतिरोधी पाया गया, और बैक्टीरिया का कोई निशान नहीं पाया गया।

जीवाणु आसंजन के अलावा एन्क्रस्टेशन एक महत्वपूर्ण मूत्र कैथेटर समस्या है। एन्क्रस्टेशन, यानी, सतह पर नमक का जमाव, मूत्र वातावरण में समय के साथ धीरे-धीरे विकसित हुआ। 7 दिनों तक सिंथेटिक मूत्र बहने के बाद, SEM का उपयोग करके पपड़ी की जांच की गई। PU-ON और PU-ON/nAg कैथेटर पर एन्क्रस्टेशन की संख्या में उल्लेखनीय रूप से कमी आई। जीवाणुरोधी प्रवासन परख इन-विट्रो मॉडल के माध्यम से की गई थी। पीयू कैथेटर में जीवाणु प्रवासन प्रमुख था, क्योंकि जीवाणु निलंबन के आसपास के खंड पर एक घने बायोफिल्म का गठन किया गया था। इन परिणामों ने संकेत दिया कि दोनों कैथेटर पोषक तत्वों से भरपूर संस्कृति माध्यम में बैक्टीरिया के आसंजन और प्रवासन को बाधित करने में सक्षम नहीं हो सकते हैं। उपरोक्त की तुलना में, PU-ON और PU-ON/nAg कैथेटर ने बैक्टीरिया के प्रवास को प्रभावी ढंग से रोका और सबसे कम बायोमास संचय दिखाया, जिससे क्रमशः $\sim 70\%$ और 95% से अधिक की कमी आई। स्विस् अल्बिनो चूहों में कैथेटर के हिस्टोपैथोलॉजिकल मूल्यांकन से पता चला कि कैथेटर मैट्रिक्स में बायोफिल्म है। इसलिए वर्तमान जांच से पीयू कैथेटर विकास की अंतर्दृष्टि प्राप्त होती है जो प्रकृति में रोगाणुरोधी और जैव-अनुकूल 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	Milli gram
μ g	Micro gram
L	Liter
mL	Milli liter
MPa	Mega pascal
Wt. %	Weight percentage
kV	kilo volt
ppm	Parts per million
eV	Electron volt
T	temperature
PU	Polyurethane
PVDF	Poly (vinylidene fluoride)
PMMA	Poly(methyl methacrylate)
PP	Polypropylene
PCL	Polycaprolactone
PLA	Poly lactide
HDI	1,6-hexamethylene diisocyanate
IPDI	Isophorone diisocyanate

HEMA	2-hydroxyethyl methacrylate
PEGMA	Poly(ethylene glycol) methacrylate
PEG	Polyethylene glycol
PEI	Polyethyleneimine
PNIPAAm	Poly(N-isopropyl acrylamide)
DA	3,4-dihydroxyphenylalanine
SF	Silk fibroin
ECM	Extracellular matrix
THF	Tetrahydrofuran
WCA	Water contact angle
ATRP	Atom transfers radical polymerization
RATRP	Reverse atom transfers radical polymerization
CuAAC	Copper-catalyzed alkyne-azide cycloaddition
FE-SEM	Field emission scanning electron microscopy
XRD	X-Ray Diffraction
DSC	Differential scanning calorimeter
FTIR	Fourier transform infrared
AFM	Atomic force microscopy
XPS	X-ray photoelectron spectroscopy