

**DESIGN AND DEVELOPMENT OF
NANOTHERANOSTIC PLATFORMS FOR
DIAGNOSIS OF BLOODSTREAM BACTERIAL
INFECTIONS AND TREATMENT OF BACTERIAL
INFECTIONS IN CANCER**

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INFECTIONS AND TREATMENT OF BACTERIAL
INFECTIONS IN CANCER**

by

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Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy

to the



Indian Institute of Technology Delhi

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CERTIFICATE

This is to certify that the thesis entitled “**Design and development of nanotheranostic platforms for diagnosis of bloodstream bacterial infections and treatment of bacterial infections in cancer**” being submitted by **Ms. Rohini Singh** to the Indian Institute of Technology Delhi, New Delhi for the award of the degree of **Doctor of Philosophy** is a bonafide record of original research work carried out by her under my supervision in conformity with rules and regulations of the Institute. The results contained in the thesis have not been submitted elsewhere, either in part or in full, to any other University or Institute for the award of any degree or diploma.

I certify that she has pursued the prescribed course of research.

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ABSTRACT

The lethality of bacterial infections on the human population does not need any introduction and if we take a careful look at the statistics of global mortality, it is a major reason to worry. Data from World Health Organization (WHO) suggest that infectious diseases affect more than 300 million people worldwide, of which 7 million die annually. Disease-causing bacteria typically reside as localized infections inside or on the body and can occasionally translocate into the bloodstream to cause systemic inflammation that leads to severe medical conditions like sepsis. This happens more commonly in immunocompromised patients suffering from trauma, burns, wounds, injury, or in neonatal and geriatric populations. Localized bacterial infections inside the body can also have dire implications in the long term as bacteria tend to hide in the human body for indefinite periods of time both externally (skin, wounds) or internally (in specific organs like liver, lungs, cervix *etc.*) and this long-term association can alter the cellular growth mechanisms and physiochemical environment in mammalian cells leading to tumor-like conditions. *Helicobacter pylori* (*H. pylori*) causing stomach cancer is a classic example.

While antibiotics are available to fight bacterial infections, their untargeted intake and non-specific distribution greatly reduces their efficacy. Particular challenge lies for intracellular bacteria that escape antibacterial action altogether by seeking refuge inside cancer cells, also evading immune response of the body. Ultimately, higher doses of drugs have to be given which results into the bigger problem of antimicrobial resistance (AMR). It is also recently reported that the presence of bacteria can also lower the effectiveness of chemotherapeutic drugs causing resistance in cancer patients. While individual therapies are available for combating bacterial infections and cancer, no therapy exists for simultaneous targeting of both cancer and intracellular bacteria. This can be an issue as bacteria may escape unhurt from a

tumor site and become potential carriers of lethal infection or systemic inflammation. Thus, timely intervention is required both from the therapy and diagnostic viewpoint to address this issue. In my thesis, we have addressed both these unmet needs by developing two novel platforms - (1) *Dualosomes* or multifunctional drug delivery vehicles designed to co-target cancer and intracellularized bacteria residing inside cancer cells, and (2) *Septiflo-N*, a disposable, point-of-care (POC) bioassay for early (presymptomatic) detection of Gram-negative bacteremia (bacteria in blood) providing results under 10 min with a detection limit of 100 fg/mL by eye.

सारान्शु

मानव आबादी पर जीवाणु संक्रमण की घातकता को किसी भी परिचय की आवश्यकता नहीं है और अगर हम वैश्विक मृत्यु दर के आंकड़ों पर सावधानीपूर्वक विचार करते हैं, तो यह चिंता का एक प्रमुख कारण है। विश्व स्वास्थ्य संगठन (डब्ल्यूएचओ) के डेटा का सुझाव है कि संक्रामक रोग दुनिया भर में 300 मिलियन से अधिक लोगों को प्रभावित करते हैं, जिनमें से 7 मिलियन सालाना मर जाते हैं। रोग पैदा करने वाले बैक्टीरिया आम तौर पर शरीर के अंदर या शरीर में स्थानीय संक्रमण के रूप में रहते हैं और कभी-कभी रक्तप्रवाह में बदलकर प्रणालीगत सूजन पैदा कर सकते हैं जो सेप्सिस जैसी गंभीर चिकित्सा स्थिति की ओर जाता है। यह आघात, जलन, घाव, चोट, या नवजात और जराचिकित्सा आबादी में पीड़ित प्रतिरक्षा में अक्षम रोगियों में अधिक होता है। शरीर के अंदर स्थानीयकृत बैक्टीरियल संक्रमण के दीर्घकालिक प्रभाव भी हो सकते हैं, क्योंकि बैक्टीरिया मानव शरीर में बाहरी (त्वचा, घाव) या आंतरिक रूप से (यकृत, फेफड़े, ग्रीवा आदि जैसे विशिष्ट अंगों में) अनिश्चित काल के लिए छिप जाते हैं। और यह लंबे समय तक एसोसिएशन स्तनधारी कोशिकाओं में कोशिकीय वृद्धि तंत्र और फिजियोकेमिकल वातावरण को ट्यूमर जैसी स्थितियों के लिए बदल सकता है। हेलिकोबैक्टर पाइलोरी (एच. पाइलोरी) पेट के कैंसर का कारण है, इसका एक उत्कृष्ट उदाहरण है। जबकि जीवाणुरोधी संक्रमणों से लड़ने के लिए एंटीबायोटिक्स उपलब्ध हैं, लेकिन इनका असंगत सेवन और गैर-विशिष्ट वितरण उनकी प्रभावकारिता को बहुत कम कर देता है। विशेष रूप से चुनौती इंट्रासेल्युलर बैक्टीरिया के लिए है जो कैंसर कोशिकाओं के अंदर शरण लेने से जीवाणुरोधी कार्रवाई से बचते हैं, शरीर की प्रतिरक्षा प्रतिक्रिया भी विकसित करते हैं। अंततः दवाओं की अधिक खुराक दी जाती है, जिसके परिणामस्वरूप रोगाणुरोधी प्रतिरोध (एएमआर) की बड़ी समस्या पैदा हो जाती है। यह भी हाल ही में बताया गया है कि बैक्टीरिया की उपस्थिति कैंसर के रोगियों में प्रतिरोधक क्षमता बढ़ाने वाले कीमोथैरेप्यूटिक दवाओं की प्रभावशीलता को कम कर सकती है। जबकि व्यक्तिगत उपचार जीवाणु संक्रमण और कैंसर का मुकाबला करने के लिए उपलब्ध हैं, कैंसर और इंट्रासेल्युलर बैक्टीरिया दोनों के एक साथ लक्ष्यीकरण के लिए कोई भी चिकित्सा मौजूद नहीं है। यह एक मुद्दा हो सकता है क्योंकि बैक्टीरिया एक ट्यूमर साइट से अस्वास्थ्यकर बच सकते हैं और घातक संक्रमण या प्रणालीगत सूजन के संभावित वाहक बन सकते

हैं। इस प्रकार, इस मुद्दे को हल करने के लिए चिकित्सा और नैदानिक दृष्टिकोण दोनों से समय पर हस्तक्षेप की आवश्यकता होती है। मेरी थीसिस में, हमने दो प्लेटफार्मों को विकसित करके इन दोनों की जरूरत को पूरा किया है - (1) ड्यूलोसोम्स या मल्टीफंक्शनल ड्रग डिलीवरी वाहनों को कैंसर कोशिकाओं के अंदर रहने वाले कैंसर और इंट्रासेल्युलराइज्ड बैक्टीरिया के लिए डिज़ाइन किया गया है, और (2) सेप्टिफलो-एन, एक डिस्पोजेबल, पॉइंट-ऑफ-केयर डिवाइस (POC) ग्राम-नेगेटिव बैक्टीरिया (रक्त प्रवाह में बैक्टीरिया) का जल्दी पता लगाने के लिए डिज़ाइन किया गया है तथा 10 मिनट के तहत परिणाम प्रदान करते हैं, जो आँख से 100 fg / mL की पहचान सीमा के साथ होता है।

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ABBREVIATIONS

AuNPs – Gold nanoparticles

PMB – Polymyxin B

FA – Folic acid

LPS – Lipopolysaccharide

LTA – Lipoteichoic acid

TEM – Transmission electron microscope

DLS – Dynamic light scattering

OPA – ortho-phthaldehyde

L – Liposomes

SL – Sushi peptide S3 liposomes

SDL – Sushi peptide S3 Doxorubicin liposomes

FSDL – Folic acid sushi peptide S3 Doxorubicin liposomes

HEPES - 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid

UV – Ultraviolet

OD – Optical density

ELISA – Enzyme linked immunosorbant assay

PCR – Polymerase chain reaction

LAL – Limulus ameocyte assay

AMR – Antimicrobial resistance

Doxorubicin – Doxorubicinorubicin

IC₅₀ – Half maximal inhibitory concentration

PAMPs – Pathogen associated molecular markers

PCT – Procalcitonin

MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

LDH – Lactate dehydrogenase

S. typhi - *Salmonella typhi*

E. coli - *Escherichia coli*

WHO – World health organization
NCI – National cancer institute
DI – Deionized
FTIR – Fourier transform infrared spectroscopy
MQ – Milli Q
DMEM – Dulbecco’s modified eagle medium
ICU – Intensive care unit
PEG – Polyethylene glycol
POC – Point-of-care
EDTA – Ethylenediaminetetraacetic acid
DMSO – Dimethyl sulfoxide
DNA – Deoxyribonucleic acid
SDS – Sodium dodecyl sulfate
PBS – Phosphate buffer saline
FBS – Fetal bovine serum
ROC – Receiver operating characteristic
USFDA – United States Food and Drug Administration