DESIGN AND DEVELOPMENT OF AFFORDABLE TECHNOLOGIES FOR RAPID ENDOTOXIN DETECTION USING NANOBIOCONJUGATES

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by

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Submitted
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Dedicated to my family

(My Parents, Brother & Sister)

Certificate

This is to certify that the thesis entitled "***Design and Development of affordable technologies for rapid endotoxin detection using nanobioconjugates***" being submitted by **Mr. Prasanta Kalita** for the award of the degree of **Doctor of Philosophy** in Chemical Engineering is a record of bonafied work carried out by him under my guidance and supervision at the Indian Institute of Technology Delhi.

The result 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 he has pursued the prescribed course of research.

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Prasanata Kalita

Abstract

Septicemia is the leading cause of hospital deaths worldwide that kills more people than breast and prostate cancer combined, mainly due to lack of sensitive and affordable methods for its early detection. Treatment for sepsis is available if it is detected within a couple of hours after its onset. Major surgeries, trauma or acute bacterial infections alter the intestinal permeability resulting in the release of intestinal bacteria and their cell wall components like lipopolysaccharides (LPS) into the bloodstream. This triggers a systemic inflammatory response in the body complicated by organ dysfunction, an important hallmark of severe sepsis. Thus, LPS is an important toxin that needs rapid detection for early diagnosis of sepsis (typically within 2 h).

We explored three different strategies for efficient detection of LPS. In the first one, a homogeneous assay was developed in which endotoxin was detected in water via an agglutination approach. Next, a heterogeneous format was investigated in which modified glass-substrates were used to capture LPS from either water or human serum and subsequently detected using a label. This resulted in a facile benchtop bioassay of 3 h. To further eliminate the use of pipettes, centrifuges, water baths or any other auxiliary equipment, a membrane-based flow-through assay was developed that allowed endotoxin detection under 10 min, meeting our original objective of a rapid bedside assay. All the three assays were based on the novel use of an antibiotic drug, polymyxin B (PMB) conjugated to gold nanoparticles (GNPs) serving as ultrasensitive colorimetric detection probes and required only a few microliters of blood serum analyte for their final readout. The use of a drug in place of antibodies was not only novel, it significantly reduced the cost of the assay by as much as 2000 times. While the homogeneous format was found to be ultrasensitive, the glass bioassay provided a wide dynamic range without requiring bulky or expensive instruments. The membrane assay by far appeared to be the most advanced from a

translation point of view and can potentially make a huge impact on the way sepsis is diagnosed worldwide.

Overall, the three techniques developed are simple and cost-effective allowing endotoxin detection within the first 2 h of bacterial exposure. Their simplicity lends themselves to use in resource-poor settings and their design makes them adaptable for translation such that even a layman can use it with minimal guidance. The proof-of-concept validation studies on patient samples suggest that the assays have good utility in a clinical setting. We hope that the assays can provide the necessary therapeutic guidance to doctors who still overly rely on clinical symptoms.

संक्षिप्त सारांश

सैप्टिसीमिया दुनिया भर में अस्पताल में होने वाली मृत्यु के प्रमुख कारणो मे से एक माना जाता है जो संयुक्त स्तन और प्रोस्टेट कैंसर मे मरने वालो की तुलना में अधिक है और इसका कारण मुख्य रूप से इसकी प्रारंभिक पहचान के लिए संवेदनशील और सस्ती विधियों की कमी होना है| प्रमुख शल्यचिकित्सा, आघात या तीव्र बैक्टीरिया संक्रमण आंतों की पारगम्यता को बदलते हैं जिसके परिणामस्वरूप आंतों के बैक्टीरिया मे रेहने बाले लिपोपॉलीसेकेराइड (lipopolysaccharide) रक्तप्रवाह मे प्रवेश कर जाता है |यह शरीर में एक प्रणालीगत उत्तेजन प्रतिक्रिया पैदा करता है जिस से शरीर में गंभीर स्थिति उत्पन होती है और शरीर के आंतरिक अंग सामान्य रूप से कार्य करना बंद कर देते हैं| यह सेप्सिस (Sepsis) की एक महत्वपूर्ण पहचान है| एलपीएस एक विषाक्त अणु है जिसे सेप्सिस के शुरुआती निदान के लिए शीघ्र पहचान की आवश्यकता है (आमतौर पर 2 घंटे के भीतर)|

इन लक्ष्यों को प्राप्त करने के लिए हमने कई रणनीतियों की कोशिश की| सर्वप्रथम एग्लुटिनेशन (agglutination) विधि के माध्यम से पानी में एंडोटोक्सिन (endotoxin) का पता लगाया गया है | इसके बाद, सिलेन (silane) संशोधित कांच से मानव सीरम (serum) में से एलपीएस को निकाल लिया गया और बाद में नैनोकणों (nanoparticle) का उपयोग करके इसकी उपस्थिति 3 घंटे के भीतर पुष्टि कि गई| परख के समय को कम करने के लिए, एक झिल्ली (membrane) आधारित प्रवाह-माध्यम परख विकसित की गई जो 10 मिनट मे एंडोटोक्सिन का पता लगाने मे समर्थ है | इस तकनीक से हमारा मूल उद्देश्य पूरा हुआ|

सभी तीन तकनीकें एंटीबायोटिक दवा पॉलीमीक्सिन बी (पीएमबी) संयुग्मित स्वर्ण नैनोकणों का उपयोग करती हैं| इन तनों तकनीकों से मानव सीरम में एंडोटॉक्सिन सरल तरीके से, सस्ते और कम समय में पता लगाई जा सकती हैं| इन कारणों की वजह से, ये तीनों तकनीकें कम सुविधा वाली जगह में भी इस्तेमाल की जा सकती हैं| डिजाइन में ये तकनीकें सरल एंव अनुकूलनीय बना दि गई हैं जिससे की एक आम आदमी भी इसे न्यूनतम मार्गदर्शन के साथ उपयोग कर सके| इन तीनों तकनीकों की सीमा यह है कि ये केवल ग्राम-ऋणात्मक जीवाणुओं का एंडोटोक्सिन पता लगाने मे समर्थ हैं| यह अभी भी विकासशील देशों में लगभग ६0% मामलों का समाधान करती हैं और विकसित देशों में लगभग ४0% मामलों का समाधान करता है|

मेरी थीसिस उल्लेख किया तीनों में से प्रत्येक की अपनी विशिष्ट विशेषताएं हैं और व्यावहारिक क्षेत्र के अनुसार प्रयोग करने योग्य है। एक तरफ एग्लुतिनेशन परिक्षण अति संवेदनशील तो दूसरी तरफ ग्लास बायोसाय भारी या महंगे इंस्ट्रूमेंटेशन की आवश्यकता के बिना ही पानी तथा सीरम में इस्तेमाल की जा सकती है| झिल्ली परख अभी तक व्यावसायीकरण के परिप्रेक्ष्य से सबसे उन्नत प्रतीत होती है| इस तरह पूरे विश्व में सेप्सिस का परीक्षण होने के तरीके पर एक बड़ा प्रभाव पड़ सकता है।

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Abbreviations

µTAS	:	Micro total analysis system	LOD	:	Limit of detection
AMR	:	Antimicrobial resistant	LPS	:	Lipopolysaccharide
CMC	:	Critical micelle concentration	LTA	:	Lipoteichoic acid
CRP	:	C- reactive protein	MDR	:	Multidrug resistant
CTAB	:	Cetyltrimethylammonium bromide	NP	:	Nanoparticle
DLS	:	Dynamic light scattering	OPA	:	O-phthalaldehyde
DNA	:	Deoxyribonucleic acid	PAMP	:	Pathogen associated molecular pattern
DTH	:	Dithiol alkane aromatic PEG6 hydrazide	PCT	:	Procalcitonin
EAA	:	Endotoxin activity assay	PEG	:	Polyethylene glycol
ELISA	:	Enzyme-linked immunosorbent assay	PMB	:	Polymyxin B sulfate
EU	:	Endotoxin unit	POC	:	Point-of-care
FDA	:	Food and drug administration	PVDF	:	Polyvinylidene
FTA	:	Flow through assay	rcf	:	Relative centrifugal force
FTIR	:	Fourier Transform Infrared	SAM	:	Self-assembled monolayer
GLA	:	Glutaraldehyde	SD	:	Standard deviation
GNP	:	Gold nanoparticle	TEM	:	Transmission electron microscopy
ICU	:	Intensive care unit	TNF	:	Tumor necrosis factor
ICU	:	Intensive care unit	WBC	:	White blood cells
KDO	:	3-Deoxy-D-manno-octulosonic Acid	WFI	:	Water for injection
LAL	:	Limulus amebocyte lysate			
LOD	:	Limit of detection			