DESIGN AND DEVELOPMENT OF NUCLEIC ACID-BASED DETECTION SYSTEM FOR PROSTATE CANCER DIAGNOSIS

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Dedicated to

My Mother, Father, Brother, and Alpha

Design and Development of Nucleic Acid-Based Detection System for Prostate Cancer Diagnosis

by

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*“**T**he mind must be made calm and wide,*

free from the noise of desire and fear.

*In that stillness, knowledge reveals itself**.***

***P**rogress is a slow ascension,*

built by patience, effort, and aspiration

towards a greater truth.”

– **S**ri **A**urobindo

PREFACE

This journey was never about just completing a degree - it was about becoming. I chose to walk the longer, uncertain path, not out of indecision, but because I believe in building something that matters. I know this is hard. I know I'm tired. ***But I'm not here to just finish - I'm here to become something.***

Throughout this PhD, I've been drawn not only to solve problems in cancer diagnostics but to immerse myself in real-world, interdisciplinary challenges-because that's where true science lives. When others advised me to wrap up, I listened respectfully, but within me, a deeper voice always rose: "***I don't care if I have to stay a little longer - I will do the projects that I am given.***"

This isn't ambition alone - it's alignment. And I know that when the time comes, I would be outstanding with my experiences, not just for the degrees I hold but for the resilience, purpose, and passion I've lived through.

- **D**ebashree **K**ar

CERTIFICATE

This is to certify that the thesis entitled **'Design and Development of Nucleic Acid Based Detection System for Prostate Cancer Diagnosis'** is being submitted by **Ms. Debashree Kar** to the Indian Institute of Technology Delhi for the award of **Doctor of Philosophy** in Biochemical Engineering and Biotechnology, is a record of the authentic research work carried out by her under my supervision and guidance. She has fulfilled the requirements for the submission of this thesis, which to the best our knowledge has reached the required standard.

The results contained in this thesis have not been submitted in part or full to any other university for the award of any other degree or diploma.

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ABSTRACT

Background

Early and accurate detection of prostate cancer remains a major clinical challenge due to the limitations of existing diagnostic modalities such as prostate-specific antigen (PSA) screening and invasive tissue biopsy, which often suffer from poor specificity and sampling bias. Gene-specific DNA methylation has emerged as a robust and early molecular signature of prostate cancer progression, offering significant promise for non-invasive and early diagnosis. However, conventional methylation detection techniques rely on chemical conversion, amplification, or labelling steps that compromise DNA integrity, limit multiplexing, and hinder translation to point-of-care platforms. This thesis aims to design and develop nucleic acid-based biosensing systems capable of direct, label-free detection of methylated DNA associated with prostate cancer, integrating molecular epigenetics with nanoelectronics to enhance diagnostic accuracy and clinical relevance while contributing to the innovation economy.

Material and Methods:

Methylation signatures of prostate cancer- associated genes were interrogated using a combination of biochemical discrimination strategies and nanomaterial-enabled transduction platforms. A Nucleic Acid-Based Radial Flow Assay (NABRFA) was initially developed to detect methylation-

dependent DNA recognition using methylation-sensitive restriction digestion, enabling visual differentiation between methylated and unmethylated DNA without chemical conversion. Subsequently, WS,-based field-effect transistor (FET) biosensors were fabricated and biofunctionalized with gold nanoparticle-conjugated thiolated DNA probes for sensitive and selective electrical detection of methylated DNA targets. To achieve multiplexed detection, a WSe,-FET array was developed, integrating methyl-CpG binding proteins (MBPs) as biological recognition elements for simultaneous profiling of multiple gene-specific methylation signatures directly from genomic DNA samples.

Results and Discussion

Electrical measurements demonstrated distinct conductance modulation upon hybridization with methylated DNA, confirming that the WS -FET platform effectively transduces methylation-dependent molecular interactions into measurable signals. The WSe2-FETarray enabled simultaneous, label-free, and gene-specific detection, with MBPs discriminating methylated CpG regions in double-stranded DNA while preserving native methylation patterns. Studies using prostate cancer cell lines (LNCaP, PC-3) and a normal control (WI-38) revealed differential electrical responses consistent with known methylation hierarchies, demonstrating high specificity, reproducibility, and ultrasensitivity at femtogram levels. Validation through fluorescence-based MBP assays and MSRE-qPCR confirmed that the observed signals reflect effects. Collectively, these results establish a robust platform capable of early, minimally invasive

detection of prostate cancer epigenetic signatures, with direct relevance to patient care and translational diagnostic

Conclusion

This thesis establishes nucleic acid-based, nanomaterial-integrated biosensing platforms as a versatile, scalable, and clinically relevant approach for prostate cancer detection. By combining surface chemistry, molecular recognition, and nanoelectronic transduction, the developed systems provide label-free, multiplexed, and ultrahigh-sensitivity detection of promoter methylation. These platforms offer a clear pathway toward early diagnosis, improved patient care, and point-of-care liquid biopsy applications, representing a significant contribution to precision oncology and the innovation economy. The work lays the foundation for next-generation epigenetic diagnostics with strong potential for translational and commercial impact.

सार

पृष्ठभूमि

प्रोस्टेट कैंसर का प्रारंभिक एवं सटीक निदान वर्तमान चिकित्सा विज्ञान की एक महत्वपूर्ण चुनौती है। पारंपरिक निदान विधियाँ, जैसे प्रोस्टेट-स्पेसिफिक एंटीजन (PSA) परीक्षण तथा ऊतक बायोप्सी, कम विशिष्टता एवं सैंपलिंग त्रुटियों जैसी सीमाओं से प्रभावित हैं। जीन-विशिष्ट डीएनए मिथाइलेशन प्रोस्टेट कैंसर का एक प्रारंभिक एवं विश्वसनीय एपिजेनेटिक बायोमार्कर माना जाता है। तथापि, पारंपरिक मिथाइलेशन पहचान तकनीकें रासायनिक परिवर्तन, प्रवर्धन (amplification) तथा लेबलिंग प्रक्रियाओं पर आधारित होती हैं, जिससे डीएनए की अखंडता प्रभावित होती है तथा पॉइंट-ऑफ-केयर अनुप्रयोगों में इनका उपयोग सीमित हो जाता है। इस शोधकार्य का उद्देश्य न्यूक्लिक अम्ल-आधारित जैवसंवेदी प्रणालियों का विकास करना था, जो प्रोस्टेट कैंसर से संबंधित मिथाइलेटेड डीएनए का प्रत्यक्ष, लेबल-रहित एवं संवेदनशील पता लगा सकें।

सामग्री एवं विधियाँ

इस शोध में प्रोस्टेट कैंसर से संबंधित जीनों के मिथाइलेशन संकेतों का विश्लेषण जैवरासायनिक तकनीकों एवं नैनोमटेरियल-आधारित ट्रांसडक्शन प्लेटफॉर्म के माध्यम से किया गया। प्रारंभ में, मिथाइलेशन-सेंसिटिव रेस्ट्रिक्शन डाइजेशन आधारित न्यूक्लिक अम्ल-आधारित रेडियल फ्लो असे (NABRFA) विकसित किया गया, जिसने मिथाइलेटेड एवं अनमिथाइलेटेड डीएनए के बीच दृश्य भेद संभव किया। इसके पश्चात WS_2-आधारित फील्ड-इफेक्ट ट्रांजिस्टर (FET) जैवसंवेदी विकसित किए गए, जिन्हें स्वर्ण नैनोकण-संयुग्मित थायोलेटेड डीएनए प्रोब्स द्वारा क्रियाशील बनाया गया, जिससे मिथाइलेटेड डीएनए का संवेदनशील विद्युत-आधारित पता लगाया जा सके। आगे चलकर, WSe_2-FET ऐरे प्रणाली विकसित की गई, जिसमें मिथाइल-CpG बाइंडिंग प्रोटीन्स (MBPs) का उपयोग कर एक साथ अनेक जीन-विशिष्ट मिथाइलेशन संकेतों की पहचान की गई।

परिणाम एवं चर्चा

विद्युतीय विश्लेषणों में मिथाइलेटेड डीएनए के साथ संकरण के पश्चात चालकता में स्पष्ट परिवर्तन प्राप्त हुआ, जिससे यह सिद्ध हुआ कि WS_2-FET प्लेटफॉर्म मिथाइलेशन-निर्भर आणविक अंतःक्रियाओं को मापन

योग्य संकेतों में परिवर्तित करने में सक्षम है। WSe_2-FET ऐरे ने एक साथ, लेबल-रहित एवं जीन-विशिष्ट पहचान प्रदर्शित की। प्रोस्टेट कैंसर कोशिका रेखाओं (LNCaP, PC-3) तथा सामान्य नियंत्रण (WI-38) के अध्ययन में ज्ञात मिथाइलेशन स्तरों के अनुरूप भिन्न विद्युत प्रतिक्रियाएँ प्राप्त हुईं, जो उच्च विशिष्टता, पुनरुत्पादकता तथा अत्यधिक संवेदनशीलता को दर्शाती हैं। फ्लोरेसेंस-आधारित MBP असे तथा MSRE-qPCR प्रमाणीकरण से यह पुष्टि हुई कि प्राप्त संकेत वास्तविक मिथाइलेशन-निर्भर अंतःक्रियाओं के कारण उत्पन्न हुए।

निष्कर्ष

यह शोधकार्य न्यूक्लिक अम्ल-आधारित, नैनोमटेरियल-संयोजित जैवसंवेदी प्लेटफॉर्म को प्रोस्टेट कैंसर निदान हेतु एक प्रभावी, संवेदनशील एवं चिकित्सीय रूप से प्रासंगिक दृष्टिकोण के रूप में स्थापित करता है। विकसित प्रणालियाँ प्रमोटर मिथाइलेशन की लेबल-रहित, मल्टीप्लेक्स एवं अति-संवेदनशील पहचान प्रदान करती हैं तथा प्रारंभिक निदान, बेहतर रोगी देखभाल एवं पॉइंट-ऑफ-केयर लिक्विड बायोप्सी अनुप्रयोगों की दिशा में महत्वपूर्ण संभावनाएँ प्रस्तुत करती हैं। यह कार्य भविष्य की एपिजेनेटिक निदान प्रणालियों के विकास की आधारशिला रखता है।

CONTENTS

CERTIFICATE…….....i

ACKNOWLEDGEMENTS……..…….……ii

ABSTRACT…….....v

ABSTRACT (सार)……...viii

CONTENTS…….....x

LIST OF FIGURES……xvii

LIST OF TABLES…….....xx

ABBREVIATIONS……xxii

Chapter 1 Introduction……1

1.1 Introduction……..…2

1.2 Objectives……...10

Chapter 2 Literature Review….....12

2.1 Background…….13

2.1.1 Conventional Diagnostic Approaches……..……13

2.1.2 Molecular Biomarkers in Prostate Cancer…….....15

2.1.2.1 Protein Biomarkers……...….15

2.1.2.2 DNA Biomarkers…….....16

2.1.2.3 RNA Biomarkers……..……16

2.2 Epigenetic Biomarkers in Cancer…….....16

2.2.1 DNA Methylation……17
2.2.2 Histone Modifications (Acetylation, Methylation, Phosphorylation)..... 18
2.2.3 Chromatin Remodeling…… 20
2.2.4 ncRNA-Mediated Regulation…….. 21
2.2.5 Classification of Epigenetic Biomarkers Based on Sample Source…… 21
2.2.5.1 Invasive Epigenetic Biomarkers (Tissue-Based)..... 22
2.2.5.2 Non-Invasive Epigenetic Biomarkers (Liquid Biopsy-Based)..... 22
2.3 Dna Methylation as a Robust Epigenetic Biomarker…….....22
2.3.1 DNA Methlylation and Carcinogenesis……23
2.3.2 Prostate Cancer: Molecular Basis and Diagnostic Challenges…… 30
2.3.3 Diagnostic and Prognostic Potential of Methylated Biomarkers In Pca…….. 33
2.3.4 Liquid Biopsy for Diagnosis Using Epigenetic Biomarkers……... 36
2.3.4.1 Circulating Tumor Cells (CTCs).....36
2.3.4.2 Exosomes…….. 37
2.3.4.3 Circulating Tumour DNA (ctDNA)..... 37
2.4 Conventional Methods for DNA Methylation Analysis…….40
2.4.1 Bisulphite-Based Methods……40
2.4.1.1 Bisulphite Sequencing (BS-Seq)……....40
2.4.1.2 Combined Bisulphite Restriction Analysis (COBRA)…….....41
2.4.2 Restriction Enzyme-Based Methods…….....41
2.4.2.1 MSRE-PCR……....41
2.4.2.2 MSCC……42
2.4.3 Affinity-Based Methods……...42

2.4.3.1 MBD-Seq……...42
2.4.4 Mass Spectrometry-Based Methods……42
2.4.4.1 LC-MS for Global Methylation……..……42
2.4.4.2 MALDI-TOF-MS……43
2.4.5 Methylation-Sensitive Arrays……43
2.4.5.1 Infinium Methylation Arrays……43
2.4.5.2 MassArray with EpiTYPER……43
2.4.6 Electrophoresis and Separation Method ……..……...43
2.4.6.1 RLGS……44
2.4.6.2 HPLC……44
2.4.7 Critical Assessment……...44
2.5 Biosensors-Based Detection Methods……47
2.5.1 Biorecognition Elements……...47
2.5.2 Analytical and Operational Characteristics of Biosensors……..……..……..…50
2.5.3 Role of Nanomaterials in Biosensors ……..…….……...51
2.5.4 Types of Biosensors……..…….....54
2.5.5 Genomic Methods and PCR……..…….…….....55
2.5.6 ELISA……56
2.6 Advances in Nanomaterial-Based Methylation Biosensors…….…..56
2.6.1 Electrochemical Biosensors……...……58
2.6.1.1 Nanocomposite Electrodes…….…...58
2.6.1.2 Direct Electrochemical Oxidation…….……58
2.6.1.2.1 Nanocarbon Films and Carbon Nanotube Composites…….…….59
2.6.1.2.2 Mxene-Based Flexible Sensors……..59
2.6.2 Immuno-Electrochemical Sensors…….....59

2.6.3 Optical Biosensors……60
2.6.3.1 Colorimetric Sensors with Gold Nanoparticles……...……60
2.6.3.2 FRET Sensors……...60
2.6.3.3 SERS……..….....60
2.6.3.4 SPR Biosensors…….……63
2.6.4 Emerging Advances…….63
2.6.4.1 Paper-Based Sensors…….63
2.6.4.1.1 Lateral Flow Assays…….……..63
2.6.4.1.2 Microfluidic Paper Based Analytical Devices……...64
2.6.4.2 Bio-Field Effect Transistors (Bio-FETs)…….....65
2.6.4.3 Integrated Systems: Lab-on-Chip Devices…….…..65
2.7 Methyl-Binding Proteins and Their Role in Epigenetic Biosensing….....65
2.8 Multiplexed Detection of Gene-Specific Methylation Signatures……66
2.8.1 FET Array-Based Systems for Multi-Gene Detection…….66
Chapter 3. Unravelling PCa Using DNA Methylation of the GSTP1 Gene Promoter Using Paper Based, Low-Cost Nucleic Acid-Based Radial Flow Assay (NABFRA) ……..71
3.1 Introduction…….72
3.2 Materials and Methods……..…….....75
3.2.1 Materials…….....75
3.2.2 Methods……...76
3.2.2.1 Growth Conditions of Cell Lines and Genomic DNA Isolation……..…….....76
3.2.2.2 Synthesis of Gold Nanoparticles……...…….76
3.2.2.3 Surface Functionalization and Characterization of 5'ThG-AuNP……..……...77
3.2.2.4 Quantification of DNA Probe Loading on Gold Nanoparticles……..……..78
3.2.2.5 Colloidal Stability of AuNPs……...…….……78
3.2.2.6 Preparation of Test Spot……..…….…79

3.2.2.7 Capture and Detection of Methylated GSTP1 DNA…….…….80
3.2.2.8 DNA Electrophoresis……80
3.3 Results and Discussion…….....81
3.3.1 DNA Isolation and MSR Digestion……...…….....81
3.3.2 Colloidal Stability and Optical Characterization of 5'ThG-AuNPs……..……...82
3.3.3 Radial Flow Mediated Optical and Nanoscale Signatures of GSTP1 Methylation..90
3.3.4 Different Aspects of Concentric Ring Generation and Pattern-Based Quantitative Analysis and Sensitivity of NABRFA……...92
3.4 Conclusion……100

Chapter 4. WS_2-Based Field Effect Transistor with Gold Nanoparticles-Conjugated Thiolated DNA Probes for Detection of Prostate Cancer-Associated DNA Methylation ……**103**
4.1 Introduction……...104
4.2 Materials and Methods……..109
4.2.1 WS_2 FET Biosensors Fabrication Process……. 109
4.2.2 Sequence Specificity Analysis of the Probe DNA……..109
4.2.3 Preparation of Thiol Probe-AuNP Conjugates…….....110
4.2.4 Deposition of DNA-AuNP Conjugates on WS_2……..……111
4.2.5 Characterization…….111
4.2.6 Methylation Profiling and Sample Preparation for FET Detection……..…….....112
4.2.7 Detection of Methylated DNA from Cell Lines ……..……..112
4.3 Results and Discussion…….....113
4.3.1 Characterization of Heterostructure WS_2-AuNP FET Device…….....113
4.3.2 ssDNA_AuNP@WS_2 Heterostructure Design and Biofunctionalization of the FET Device…….115
4.3.3 Electrical Characterization of Biofunctionalized WS_2-FET ……...…….…117
4.3.4 GSTP1 Methylated DNA Detection…….……..120
4.3.5 Real Time Detection of Target GSTP1 Methylated DNA……...……..…….122
4.4 Conclusion……130

Chapter 5. Multi-Analyte Detection of Gene-Specific Methylation Signatures in Prostate Cancer Cell Lines Using Methyl Binding Protein (MBD2) in a Biofunctionalized WS_2……..133

5.1 Introduction……...134

5.2 Materials and Methods…….……137

5.2.1 Biological Sample Preparation……...138

5.2.2 WSe_2 Flake Synthesis and FET Device Fabrication……138

5.2.3 Surface Activation FET Array……138

5.2.4 Biofunctionalization of The FET Array……139

5.2.5 Genomic DNA application and Real-time Methylation Detection Using MBD Proteins...140

5.2.6 Characterization ……..141

5.2.7 MBD-based DOT-Blot Assay …… 142

5.2.7 MSRE qPCR-based Validation ……142

5.3 RESULTS AND DISCUSSION……...144

5.3.1 GPTMS-Assisted Biofunctionalization of WSe_2 FET Array…… 144

5.3.2 Electrical Detection of Gene-Specific DNA Hybridization and Methylation-Specific Signal Enhancement Using MBD Proteins……... 148

5.3.3 Validation of Methylation Selectivity and Quantitative Performance of The Multiplexed WSe_2 FET Biosensor……...152

5.3.4 Validation of Methylation-Dependent Recognition Using Methyl-CpG Binding Proteins And MSRE-qPCR…….…154

5.4 CONCLUSION…… 160

CHAPTER 6. SUMMARY, CONCLUSION AND FUTURE PROSPECTIVES…… 163

6.1 Summary……... 164

Chapter 1: Introduction……. 164

Chapter 2: Literature Review…… 165

Chapter 3: Nucleic Acid Radial Flow Assay-Based Detection of Methylated DNA….166

Chapter 4: WS_2-Based FET Biosensor for Gene-Specific Methylation Detection…….166

Chapter 5: Multiplexed Detection Using Biofunctionalized FET Array Systems……...167

6.2 Conclusion…….168

6.3 Future Perspectives…… ..168

REFERENCES……. 171

APPENDIX……... 214

AUTHOR BIOGRAPHY……226

LIST OF FIGURES

Fig. No.	Figure Title	Page No.
Fig. 2.1	Global cancer burden in 2022 highlighting prostate cancer prevalence among males.	**14**
Fig. 2.2	Schematic representation of the evolution of prostate cancer diagnostics, highlighting the transition from serum prostate-specific antigen (PSA)-based screening toward advanced molecular diagnostics, including gene expression signatures and DNA methylation-based liquid biopsy approaches.	**19**
Fig. 2.3	Schematic representation showing the interplay of genetic and epigenetic events in prostate carcinogenesis of progression from localized disease to metastatic stage, correlated with epigenetic alterations and chromatin organization.	**25**
Fig. 2.4	Diagram illustrating key molecular alterations driving prostate cancer progression and the correlation between gene-specific DNA methylation profiles and disease stages, as defined by Gleason grading and metastatic potential.	**32**
Fig. 2.5	Overview of Epigenetic biomarkers and their diagnostic/prognostic relevance in prostate cancer.	**35**
Fig. 2.6	Schematic illustration of common biorecognition elements, nanomaterials and transducers used in biosensors.	**49**
Fig. 2.7	Overview of biosensor types used in biomedical and epigenetic detection.	**55**
Fig. 2.8	Colorimetric detection of methylated DNA using thiolated AuNP probes.	**61**
Fig. 2.9	Schematics for integration of nanomaterials, MBPs, and FET arrays for multi-gene methylation detection in prostate cancer.	**69**
Fig. 3.1	Schematic representation of the proposed methyl sensitive restriction (MSR) digestion.	**82**
Fig. 3.2	Colloidal Stability and Optical Characterization of 5'ThG-AuNPs.	**86**

Fig. 3.3	Schematic representation and aggregation pattern visualization using FESEM and HRTEM	**89**
Fig. 3.4	Correlation of NABRA assay with chromic shift observed by UV-Vis spectroscopy.	**93**
Fig. 3.5	Concentration-dependent nucleic acid based radial flow assay (NABRFA) response for detection of GSTP1 methylated DNA on nylon membranes.	**94**
Fig. 3.6	Quantitative analysis of the NABRFA using image-based gray value measurements	**99**
Fig. 4.1	Schematic representation and characterization of the heterostricture WS_2-AuNP FET device	**114**
Fig. 4.2	Electrical characterization of WS_2 FET.	**120**
Fig. 4.3	Biosensing Characterization of WS_2-FET for different DNA concentration	**125**
Fig. 4.4	Real-time sensing of target methylated DNA	**127**
Fig. 5.1	Schematic illustration of GPTMS-mediated biofunctionalization of the WSe_2 FET surface for covalent immobilization of single-stranded DNA probes.	**144**
Fig. 5.2	Biofunctionalization strategy and morphological validation of the WSe_2 FET device.	**146**
Fig. 5.3	Transfer characteristics (I_{DS}-V_{GS}) of the WSe_2 field-effect transistor recorded at different stages of device functionalization and sensing.	**148**
Fig. 5.4	Transfer characteristics (I_{DS}-V_{GS}) of WSe_2 FET devices functionalized with gene-specific ssDNA probes targeting the promoter regions of GSTP1, RASSF1, and APC following methylation-dependent recognition using methyl-CpG binding proteins (MBDs).	**151**
Fig. 5.5	MBD concentration dependent electrical response for GSTP1, RASSF1 and APC simultaneously.	**153**
Fig. 5.6	Fluorescence-based Dot-Blot assay for validation of methylation-dependent DNA recognition using methyl-CpG binding protein (MBP).	**155**
Fig. 5.7	qPCR validation of gene-specific methylation status before and after MSRE digestion.	**157**
Fig. A1	Preparation of the bidirectional probe conjugated hybridization complex of 5'ThG-AuNP	**214**

Fig. A2	UV-Visible Spectroscopic Validation of Probe–Conjugated AuNPs for GSTP1 DNA Detection	**215**
Fig. A3	Visual Colorimetric Response of Probe-Conjugated AuNPs upon Target DNA Binding	**215**
Fig. A4	Image Processing and Pattern Extraction for NABRFA Analysis.	**217**
Fig. A5	Sequence Alignment of Probe DNA with GSTP1 mRNA Transcript	**218**
Fig. A6	Transfer of WS_2 flakes using scotch tape method.	**220**
Fig. A7	FESEM Characterization and PAGE analysis	**220**
Fig. A8	Differential Interference Contrast (DIC) and Fluorescence Microscopy imaging after staining using DAPI, of the WS_2 flakes	**221**
Fig. A9	The Biosensing Characterisation of WS_2 FET for different DNA concentration	**221**
Fig. A10	Device response for targeted mGDNA concentration	**222**
Fig. A11	Methyl Sensitive Restriction Enzyme Digested Real Time Quantification Polymerase Chain Reaction (MSRE-qPCR) data were compared with Genomic DNA Real Time Polymerase Chain Reaction (gDNA-qPCR) obtained from LNCaP and WI-38 cell lines for the GSTP1 gene and the ACTB gene (B-actin) as a control.	**222**
Fig. A12	Optical and atomic force microscopy (AFM) characterization of the WSe_2 FET device.	**223**

LIST OF TABLES

Table No.	Table Title	Page No.
Table 2.1	Protein-based biomarkers currently used in prostate cancer diagnostics	**26**
Table 2.2	Epigenetic biomarkers reported across multiple cancer types	**27**
Table 2.3.	DNA methylation-based biomarkers specific to prostate cancer	**29**
Table 2.4	Correlation between gene methylation patterns and prostate cancer stage	**34**
Table 2.5.1	Summary of DNA methylation-based epigenetic biomarkers for prostate cancer detectable through liquid biopsy samples	**38**
Table 2.5.2	Representative epigenetic biomarkers in prostate cancer detectable through liquid biopsy	**39**
Table 2.6	Overview of conventional DNA methylation detection methods	**45**
Table 2.7	Key analytical and operational characteristics of a Biosensor.	**50**
Table 2.8	Representative nanomaterial classes used in biosensing applications relevant to epigenetic methylation detection, highlighting signal transduction advantages	**53**
Table 2.9	Overview of nanomaterial-assisted biosensing for methylated DNA detection	**57**
Table 2.10	Characteristic Raman/SERS vibrational modes of DNA bases and their relevance to methylation discrimination	**62**
Table 2.11	Comparison of conventional vs. nanomaterial-based methylation detection approaches	**69**

Table 4.1	Comparative performance of existing methods for DNA methylation detection, including the proposed WS_2-FET biosensor	**128**
Table A1	Statistical Determination of Analytical LOD for NABRFA	**216**
Table A2	BLASTn Alignment Summary	**219**
Table A3	Buffers Used in Various Stages of WS_2-FET Biosensor Workflow	**219**
Table A4	Calculations	**223**
Table A5	MSRE-qPCR-based methylation analysis of PCa-associated genes	**224**
Table A6	Interpretation Scale for ΔCq Values	**225**
Table A7	Interpretation Scale for % Methylation Values	**225**
Table A8	Comparative analytical benchmarking of the developed biosensor	**225**

ABBREVIATIONS

Abb.	*Full forms*
APC	Adenomatous Polyposis Coli
AR	Androgen Receptor
AuNPs	Gold Nanoparticles
BPH	Benign Prostatic Hyperplasia
BS-Seq	Bisulfite Sequencing
cfDNA	Cell-Free DNA
COBRA	Combined Bisulfite Restriction Analysis
COMSOL	Computer-Oriented Multiphysics Simulation
CpG	Cytosine-Phosphate-Guanine Dinucleotide
ctDNA	Circulating Tumor DNA
ddPCR	Droplet Digital Polymerase Chain Reaction
DRE	Digital Rectal Examination
DNMT	DNA Methyltransferase
ECM	Extracellular Matrix
ELISA	Enzyme-Linked Immunosorbent Assay
EMT	Epithelial-to-Mesenchymal Transition

FET	Field-Effect Transistor
FRET	Fluorescence Resonance Energy Transfer
GS	Gleason Score
GSTP1	Glutathione S-Transferase Pi 1
HPLC	High-Performance Liquid Chromatography
LFA	Lateral Flow Assay
LOD	Limit of Detection
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass
-MS	Spectrometry
MBPs	Methyl-CpG Binding Proteins
MBD	Methyl-CpG Binding Domain
MBD-seq	Methylated CpG Binding Domain Sequencing
5-mC	5-Methylcytosine
MSCC	Methyl-Sensitive Cut Counting
MSP	Methylation-Specific PCR
MSRE-PCR	Methylation-Sensitive Restriction Enzyme PCR
MXene	Two-Dimensional Transition Metal Carbide/Nitride
ncRNA	Non-Coding RNA

PCa	*Prostate Cancer*
PCR	Polymerase Chain Reaction
PHI	Prostate Health Index
POC	Point-of-Care
PSA	Prostate-Specific Antigen
qMSP	Quantitative Methylation-Specific PCR
QCM	Quartz Crystal Microbalance
QDs	Quantum Dots
RARB / RARβ2	Retinoic Acid Receptor Beta 2
RASSF1A	Ras Association Domain Family Member 1 A
RFA	Radial Flow Assay
RLGS	Restriction Landmark Genomic Scanning
SAMs	Self-Assembled Monolayers
SEM	Scanning Electron Microscopy
SERS	Surface-Enhanced Raman Scattering
SPR	Surface Plasmon Resonance
TET	Ten-Eleven Translocation Enzyme
TMDs	Transition Metal Dichalcogenides
TRUS	Transrectal Ultrasound
WS_2	Tungsten Disulfide
WSe_2	Tungsten Diselenide