CARBON NANO-DOTS AND MOLECULARLY IMPRINTED POLYMERS LOADED WITH BIOACTIVE MOLECULES FOR ANTI-CANCER DRUG DELIVERY

PREETHA GANGULY

DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI
MARCH 2026

Carbon Nano-Dots and Molecularly Imprinted Polymers Loaded with Bioactive Molecules for Anti-cancer Drug Delivery

by

Preetha Ganguly

Department of Biochemical Engineering and Biotechnology

Submitted

In fulfilment of the requirement of doctor of philosophy

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

MARCH 2026

Dedicated to

My parents Mrs. Subhra Ganguly and Mr. Pradip Kumar Ganguly

CERTIFICATE

This is to certify that this thesis entitled "**Carbon Nano-Dots and Molecularly Imprinted Polymers Loaded with Bioactive Molecules for Anti-cancer Drug Delivery**" submitted by **Ms. Preetha Ganguly** to the Indian Institute of Technology Delhi for the award of the degree of Doctor of Philosophy in Biochemical Engineering and Biotechnology, is a record of the authentic research work carried out by her under my guidance and supervision. She has fulfilled all the requirements for the submission of this thesis, which, to the best of my knowledge, has reached the required standards. The results presented in this thesis have not been submitted in part or fully to any other university or institute for the award of any other degree or diploma.

Professor Prashant Mishra

Department of Biochemical Engineering and Biotechnology

Indian Institute of Technology Delhi

Hauz Khas-110016

New Delhi, India

ACKNOWLEDGEMENTS

I would like to express my deepest and most sincere gratitude to my supervisor, Professor Prashant Mishra, for his invaluable guidance, constant encouragement, and unwavering support throughout my doctoral journey. His scientific vision, rigorous approach to research, and insistence on excellence have profoundly shaped my thinking and research skills. I am especially grateful for his trust in my abilities and for allowing me the academic freedom to explore challenging ideas, which greatly contributed to my intellectual and professional growth. His mentorship has been instrumental in transforming challenges into learning opportunities, and it has been a privilege to pursue my PhD under his supervision.

I would also like to sincerely thank the members of my Student Research Committee (SRC)—Professor Ritu Kulshreshtha, Professor Preeti Srivastava, and Professor Samresh Das—for their valuable suggestions, constructive criticism, and guidance at various stages of my research. Their insightful feedback helped refine the direction of my work and strengthened the scientific depth of this thesis.

I am grateful to the Indian Institute of Technology (IIT) for providing a stimulating academic environment, excellent research infrastructure, and institutional support that made this work possible. I would also like to gratefully acknowledge the Prime Minister's Research Fellowship (PMRF) for the financial support provided during my doctoral studies, which enabled me to focus on my research with dedication and independence.

I would like to extend my heartfelt thanks to Professor Shilpi Sharma for being a constant source of encouragement and emotional support during my PhD journey. Her reassurance and guidance during difficult phases played an important role in helping me stay motivated and focused. I also express my gratitude to my laboratory colleagues, collaborators, and peers for their academic interactions and support throughout the course of this work.

This journey would not have been possible without the unconditional love, sacrifices, and unwavering belief of my parents, Mr. Pradeep Kumar Ganguly and Mrs. Subhra Ganguly. They have been my strongest pillars of strength, offering endless encouragement, patience, and emotional support throughout this demanding journey. Their faith in me gave me the courage to persevere through every challenge, and every achievement of mine stands as a reflection of their sacrifices and blessings. I am also deeply thankful to my family members, especially my Bua, Mrs. Parvati Chakroborty, and my Mama, Mr. Debashish Chatterjee, for their constant

encouragement, emotional support, and belief in my abilities. Their presence, guidance, and reassurance provided me with strength and stability during critical phases of my PhD journey.

Preetha Ganguly

ABSTRACT

Background:

Breast cancer remains one of the leading causes of cancer-related mortality among women globally, despite major advances in diagnostic and therapeutic strategies. Conventional treatment modalities, including chemotherapy, radiotherapy, and surgery, are constrained by systemic toxicity, multidrug resistance, limited tumor selectivity, poor penetration into solid tumors, and the lack of real-time monitoring of therapeutic response. Although nanotechnology has emerged as a promising approach to address these challenges, many existing nanotherapeutic systems continue to suffer from inadequate tumor accumulation, biological instability, immunogenicity, high cost, and batch-to-batch variability. These persistent limitations highlight the urgent need for multifunctional, selective, and clinically translatable nanomedicine platforms. The aim of this thesis was to rationally design and systematically evaluate a progressive nanomedicine framework that integrates therapeutic and diagnostic functionalities to overcome multidrug resistance, enhance tumor penetration, and enable precise, effective, and scalable breast cancer treatment.

Methods:

This thesis presents an evolution-driven nanomedicine strategy progressing from cost-effective passive nanocarriers to advanced, tumor-responsive, and precision-targeted theranostic systems. Carbon-based nanomaterials, Janus nanomotors, nanozymes, and molecularly imprinted polymers (nanoMIPs) were strategically engineered to enhance intracellular drug accumulation, improve tumor penetration, and integrate therapeutic and diagnostic functions. Biomass-derived carbon nanodots (CDs) were synthesised and optimised using response surface methodology and co-loaded with curcumin and quercetin. These nanocarriers were characterised using UV–Vis spectroscopy, XRD, FTIR, and zeta potential analysis, and their stability was assessed across a wide pH range and over extended time periods. Biological efficacy was evaluated using two-dimensional breast cancer cell lines and three-dimensional tumor spheroid models. To overcome the limitations of diffusion-controlled delivery, platinum–mesoporous silica Janus nanomotors capped with carbon nanodots and gated by redox-responsive di-selenide linkages were synthesised to achieve active propulsion, deep tumor penetration, and controlled drug release. Subsequently, a dual-gated iron-doped carbon nanodot nanozyme conjugated with an indole-3-acetic acid prodrug and functionalised with folic acid was developed to combine catalytic activity with receptor-mediated targeting.

Finally, ligand-free HER3-specific nanoMIPs were fabricated using solid-phase imprinting and co-loaded with curcumin and doxorubicin to achieve synthetic molecular recognition and theranostic capability.

Results and Discussion:

Co-loaded carbon nanodots exhibited excellent physicochemical stability for up to 120 h under both acidic and basic conditions and significantly enhanced the stability and bioavailability of curcumin. *In vitro* studies using MCF-7 breast cancer cells demonstrated strong antiproliferative and antimigration effects, with markedly increased apoptosis, reactive oxygen species (ROS) generation, nuclear degeneration, and tumor inhibition compared to single-drug-loaded systems. These effects were mediated through effective blockage of multidrug resistance via downregulation of membrane-bound P-glycoprotein, suppression of BIRC gene expression, and upregulation of the tumor suppressor p53. Enhanced penetration and therapeutic efficacy were confirmed in three-dimensional tumor spheroid models, although the reliance on passive targeting highlighted limitations associated with tumor heterogeneity. Janus nanomotors demonstrated active motion-driven delivery, leading to superior cellular uptake, deep spheroid penetration, and potent cytotoxicity in both MCF-7 and MDA-MB-231 breast cancer cell lines. These systems significantly enhanced apoptosis, ROS production, and tumor cell killing *in vitro* and effectively restricted tumor growth *in vivo*, with treated animals exhibiting a marked reduction in tumor volume compared to controls. While highly effective, these platforms primarily relied on tumor microenvironment cues, underscoring the need for more precise molecular recognition strategies. The dual-gated FeCDs@IAA@Folic Acid nanozyme exhibited intrinsic peroxidase-like activity and selective anticancer efficacy, achieving low IC50 values in hormone receptor-positive and triple-negative breast cancer cells while sparing normal epithelial cells. This nanocarrier induced excessive ROS production, mitochondrial depolarisation, apoptosis, and G2/M cell cycle arrest, and significantly inhibited cancer cell migration. Three-dimensional spheroid studies confirmed deep tumor penetration and efficient tumor eradication; however, dependence on biological ligands raised concerns related to immunogenicity, receptor heterogeneity, and translational robustness. To address these challenges, HER3-specific nanoMIPs were engineered as ligand-free synthetic plastic antibodies capable of precise molecular recognition. Co-loaded doxorubicin-curcumin nanoMIPs effectively blocked P-glycoprotein-mediated drug efflux, resulting in enhanced intracellular drug accumulation and superior cytotoxicity. These nanoMIPs induced apoptosis, ROS generation, and nuclear degeneration in breast cancer cells, while their intrinsic

fluorescence enabled point-of-care detection of HER3-expressing cells. Evaluation in three-dimensional tumor spheroid models demonstrated efficient penetration, high specificity, and robust therapeutic efficacy, supporting the clinical relevance of this scalable and cost-effective approach.

Conclusion:

This thesis establishes a comprehensive and clinically translatable nanomedicine framework for breast cancer treatment and biosensing. By systematically integrating passive delivery, active propulsion, redox-responsive gating, catalytic nanozyme activity, receptor-mediated targeting, and synthetic molecular recognition, this work addresses critical limitations of conventional therapies and existing nanoplatforms. The findings provide significant insights into multifunctional nanotherapeutic design and offer promising pathways toward safer, more effective, and affordable precision nanomedicine for breast cancer.

सारांश (ABSTRACT)

पृष्ठभूमि (Background):

स्तन कैंसर वैश्विक स्तर पर महिलाओं में कैंसर से संबंधित मृत्यु के प्रमुख कारणों में से एक बना हुआ है, यद्यपि निदान और उपचार रणनीतियों में महत्वपूर्ण प्रगति हुई है। पारंपरिक उपचार विधियाँ, जिनमें कीमोथेरेपी, रेडियोथेरेपी और सर्जरी शामिल हैं, प्रणालीगत विषाक्तता, बहु-दवा प्रतिरोध, सीमित ट्यूमर चयनात्मकता, ठोस ट्यूमर में कमजोर प्रवेश, तथा उपचारात्मक प्रतिक्रिया की वास्तविक समय में निगरानी के अभाव जैसी समस्याओं से बाधित हैं। यद्यपि नैनोटेक्नोलॉजी इन चुनौतियों को दूर करने के लिए एक आशाजनक दृष्टिकोण के रूप में उभरी है, फिर भी कई मौजूदा नैनो-चिकित्सीय प्रणालियाँ अपर्याप्त ट्यूमर संचयन, जैविक अस्थिरता, प्रतिरक्षाजनकता, उच्च लागत और बैच-से-बैच परिवर्तनशीलता से ग्रस्त हैं। ये निरंतर सीमाएँ बहु-कार्यात्मक, चयनात्मक और नैदानिक रूप से अनुवाद योग्य नैनोमेडिसिन प्लेटफार्मों की तात्कालिक आवश्यकता को रेखांकित करती हैं। इस शोध का उद्देश्य एक प्रगतिशील नैनोमेडिसिन फ्रेमवर्क का तर्कसंगत डिज़ाइन और व्यवस्थित मूल्यांकन करना था, जो चिकित्सीय और निदानात्मक कार्यक्षमताओं को एकीकृत करके बहु-दवा प्रतिरोध को दूर करे, ट्यूमर प्रवेश को बढ़ाए, और सटीक, प्रभावी तथा स्केलेबल स्तन कैंसर उपचार सक्षम करे।

विधियाँ (Methods):

यह शोध एक विकास-आधारित नैनोमेडिसिन रणनीति प्रस्तुत करता है, जो लागत-प्रभावी निष्क्रिय नैनोकैरियर्स से उन्नत, ट्यूमर-प्रतिक्रियाशील और सटीक-लक्षित थेरानोस्टिक प्रणालियों तक प्रगति करता है। कार्बन-आधारित नैनोमैटेरियल्स, Janus नैनोमोटर्स, नैनोज़ाइम्स और मॉलिक्यूलरली इम्प्रिंटेड पॉलिमर्स (nanoMIPs) को रणनीतिक रूप से इस प्रकार अभिकल्पित किया गया कि वे कोशिकीय दवा संचयन को बढ़ाएँ, ट्यूमर में गहरी पैठ सुनिश्चित करें, तथा चिकित्सीय और निदानात्मक कार्यों को एकीकृत करें। बायोमास-आधारित कार्बन नैनोडॉट्स (CDs) का संश्लेषण और अनुकूलन रिस्पॉन्स सरफेस मेथडोलॉजी द्वारा किया गया तथा उनमें करक्यूमिन और क्वेरसेटिन को सह-लोड किया गया। इन नैनोकैरियर्स का विश्लेषण UV–Vis स्पेक्ट्रोस्कोपी, XRD, FTIR और ज़ेटा पोटेंशियल द्वारा किया गया, तथा उनकी स्थिरता को विभिन्न pH रेंज और लंबे समय तक परखा गया। जैविक प्रभावशीलता का मूल्यांकन द्वि-आयामी स्तन कैंसर कोशिका लाइनों तथा त्रि-आयामी ट्यूमर स्फेरॉइड मॉडल्स में किया गया। प्रसार-नियंत्रित डिलीवरी की सीमाओं को दूर करने हेतु, प्लेटिनम–मेसोपोरस सिलिका Janus

नैनोमोटर्स, जो कार्बन नैनोडॉट्स से कैप्ड थे और रेडॉक्स-प्रतिक्रियाशील डाइ-सेलेनाइड लिंकेज से गेटेड थे, का संश्लेषण किया गया ताकि सक्रिय गति, गहरी ट्यूमर पैठ और नियंत्रित दवा विमोचन प्राप्त हो सके। इसके बाद, एक ड्यूल-गेटेड आयरन-डोप्ड कार्बन नैनोडॉट नैनोज़ाइम, जिसे इंडोल-3-एसेटिक एसिड प्रोड्रग से संयुग्मित और फोलिक एसिड से फंक्शनलाइज़ किया गया, विकसित किया गया ताकि कैटेलिटिक गतिविधि और रिसेप्टर-मध्यस्थ लक्षित डिलीवरी को संयोजित किया जा सके। अंततः, लिगैंड-रहित HER3-विशिष्ट nanoMIPs को सॉलिड-फेज इम्प्रिंटिंग द्वारा निर्मित किया गया और करक्यूमिन तथा डॉक्सोरूबिसिन के साथ सह-लोड किया गया, जिससे सिंथेटिक मॉलिक्यूलर पहचान और थेरानोस्टिक क्षमता प्राप्त हो सके।

परिणाम एवं चर्चा (Results and Discussion):

सह-लोडेड कार्बन नैनोडॉट्स ने अम्लीय और क्षारीय दोनों परिस्थितियों में 120 घंटे तक उत्कृष्ट भौतिक-रासायनिक स्थिरता प्रदर्शित की तथा करक्यूमिन की स्थिरता और जैवउपलब्धता को उल्लेखनीय रूप से बढ़ाया। MCF-7 स्तन कैंसर कोशिकाओं पर इन विट्रो अध्ययन ने मजबूत एंटी-प्रोलिफेरेटिव और एंटी-माइग्रेशन प्रभाव दर्शाए, जिनमें एपोप्टोसिस, रिएक्टिव ऑक्सीजन स्पीशीज़ (ROS) उत्पादन, न्यूक्लियर डीजेनरेशन और ट्यूमर अवरोध में उल्लेखनीय वृद्धि हुई, जो सिंगल-ड्रग सिस्टम की तुलना में अधिक थी। ये प्रभाव P-ग्लाइकोप्रोटीन के डाउनरेगुलेशन, BIRC जीन अभिव्यक्ति के दमन और ट्यूमर सप्रेसर p53 के अपरेगुलेशन के माध्यम से बहु-दवा प्रतिरोध को प्रभावी रूप से अवरुद्ध करने के कारण उत्पन्न हुए। त्रि-आयामी ट्यूमर स्फेरॉइड मॉडल्स में बेहतर पैठ और चिकित्सीय प्रभावशीलता की पुष्टि हुई, हालांकि निष्क्रिय लक्षितरण पर निर्भरता ने ट्यूमर विषमता से संबंधित सीमाओं को उजागर किया। Janus नैनोमोटर्स ने सक्रिय गति-आधारित डिलीवरी प्रदर्शित की, जिससे बेहतर कोशिकीय अवशोषण, गहरी स्फेरॉइड पैठ और MCF-7 तथा MDA-MB-231 दोनों कोशिका लाइनों में उच्च साइटोटॉक्सिसिटी प्राप्त हुई। इन प्रणालियों ने इन विट्रो में एपोप्टोसिस, ROS उत्पादन और ट्यूमर कोशिका विनाश को बढ़ाया तथा इन विवो में ट्यूमर वृद्धि को प्रभावी रूप से सीमित किया, जहाँ उपचारित जानवरों में नियंत्रण समूह की तुलना में ट्यूमर आयतन में स्पष्ट कमी देखी गई। हालांकि अत्यधिक प्रभावी होने के बावजूद, ये प्लेटफॉर्म मुख्यतः ट्यूमर माइक्रोएनवायरनमेंट संकेतों पर निर्भर थे, जो अधिक सटीक मॉलिक्यूलर पहचान रणनीतियों की आवश्यकता को दर्शाता है। ड्यूल-गेटेड FeCDs@IAA@Folic Acid नैनोज़ाइम ने अंतर्निहित पेरोक्सिडेज़-समान गतिविधि और चयनात्मक एंटीकैंसर प्रभावशीलता प्रदर्शित की, जिसमें हार्मोन-रिसेप्टर पॉजिटिव और ट्रिपल-नेगेटिव स्तन कैंसर कोशिकाओं में कम IC_{50} मान प्राप्त हुए, जबकि

सामान्य एपिथीलियल कोशिकाएँ सुरक्षित रहीं। इस नैनोकैरियर ने अत्यधिक ROS उत्पादन, माइटोकॉन्ड्रियल डिपोलराइजेशन, एपोप्टोसिस और G2/M सेल साइकिल अरेस्ट को प्रेरित किया तथा कैंसर कोशिका माइग्रेशन को उल्लेखनीय रूप से रोका। त्रि-आयामी स्फेरॉइड अध्ययनों ने गहरी ट्यूमर पैठ और प्रभावी ट्यूमर उन्मूलन की पुष्टि की; हालांकि जैविक लिगैंड्स पर निर्भरता ने प्रतिरक्षाजनकता, रिसेप्टर विषमता और ट्रांसलेशनल मजबूती से संबंधित चिंताओं को जन्म दिया। इन चुनौतियों को दूर करने के लिए, HER3-विशिष्ट nanoMIPs को लिगैंड-रहित सिंथेटिक प्लास्टिक एंटीबॉडी के रूप में अभिकल्पित किया गया, जो सटीक मॉलिक्यूलर पहचान में सक्षम हैं। डॉक्सोरूबिसिन-करक्यूमिन सह-लोडेड nanoMIPs ने P-ग्लाइकोप्रोटीन-मध्यस्थ दवा उत्सर्जन को प्रभावी रूप से अवरुद्ध किया, जिससे कोशिकीय दवा संचयन में वृद्धि और उच्च साइटोटॉक्सिसिटी प्राप्त हुई। इन nanoMIPs ने एपोप्टोसिस, ROS उत्पादन और न्यूक्लियर डीजेनरेशन को प्रेरित किया, जबकि उनकी अंतर्निहित फ्लोरोसेंस ने HER3-अभिव्यक्त कोशिकाओं की पॉइंट-ऑफ-केयर डिटेक्शन को सक्षम बनाया। त्रि-आयामी ट्यूमर स्फेरॉइड मॉडल्स में मूल्यांकन ने प्रभावी पैठ, उच्च विशिष्टता और मजबूत चिकित्सीय प्रभावशीलता प्रदर्शित की, जो इस स्केलेबल और लागत-प्रभावी दृष्टिकोण की नैदानिक प्रासंगिकता को दर्शाता है।

निष्कर्ष (Conclusion):

यह शोध स्तन कैंसर उपचार और बायोसेंसिंग के लिए एक व्यापक और नैदानिक रूप से अनुवाद योग्य नैनोमेडिसिन फ्रेमवर्क स्थापित करता है। निष्क्रिय डिलीवरी, सक्रिय प्रोपल्शन, रेडॉक्स-प्रतिक्रियाशील गेटिंग, कैटेलिटिक नैनोज़ाइम गतिविधि, रिसेप्टर-मध्यस्थ लक्षितरण और सिंथेटिक मॉलिक्यूलर पहचान के व्यवस्थित एकीकरण द्वारा, यह कार्य पारंपरिक उपचारों और मौजूदा नैनोप्लेटफार्मों की महत्वपूर्ण सीमाओं को संबोधित करता है। ये निष्कर्ष बहु-कार्यात्मक नैनोथेरेप्यूटिक डिज़ाइन में महत्वपूर्ण अंतर्दृष्टि प्रदान करते हैं और स्तन कैंसर के लिए अधिक सुरक्षित, प्रभावी और किफायती प्रिसीजन नैनोमेडिसिन की दिशा में आशाजनक मार्ग प्रस्तुत करते हैं।

TABLE OF CONTENTS

Certificate…… i

Acknowledgements ii

Abstract iv

Table of Contents x

List of Figures xvii

List of Tables xxi

List of Symbols xxiii

List of Abbreviations xxiv

Chapter 1 1

Introduction..... 2

Chapter 2 7

Literature Review

2.1 Global Disease Status and Cancer Burden.....**8**

2.2 Breast Cancer Treatment Modalities.....**12**

2.3 Targeted Drug Therapy.....**17**

2.4 Strategies for Targeted Breast Cancer Therapy.....**20**

2.5 Nanoparticle-Based Drug Delivery Systems.....**22**

2.6 Nanobots.....**25**

2.7 Nanozymes.....**36**

2.8 Molecularly Imprinted Polymers (MIPs) **40**

2.9 Traditional Detection and Biosensing in Breast Cancer Cells.....**43**

2.10 Nanoparticles for Breast Cancer Detection.....**47**

2.11 Carbon Nanodots.....**49**

2.12 Common Breast Cancer Cell Lines.....**52**

2.13 Common Drugs Approved for Breast Cancer.....**54**

2.14 Bioactive molecules with Anti-Cancer Activities.....**55**

Chapter 3 58

Carbon Nanodots Loaded with Natural Bioactive Compounds for P-gp Inhibition, Antimetastatic Activity, and Biosensing in 2D and 3D MCF-7 Breast Cancer Cells

3.1 Introduction.....59

3.2 Materials and Methods

3.2.1 Materials.....61

3.2.2 Synthesis of Carbon Nanodots (CDs) 62

3.2.2.1 Synthesis of Curcumin-Quercetin Co-loaded CDs (co-loaded CDs) 62

3.2.2.2 Synthesis of Curcumin-loaded CDs (CDs-Cur) 62

3.2.2.3 Synthesis of Quercetin-loaded CDs (CDs-Quer) 63

3.2.3 Response Surface Methodology (RSM) 63

3.2.4 Characterisation of Carbon Nanodots.....64

3.2.4.1 Calculation of Curcumin and Quercetin Encapsulation Efficiency (EE) and Loading Efficiency (LE) 64

3.2.4.2 *In vitro* Drug Release Study and kinetic modelling.....65

3.2.5 Cell Viability Assay.....65

3.2.5.1 Live/Dead Assay.....66

3.2.6 Uptake Study of Co-loaded CDs.....66

3.2.7 Measurement of Intracellular ROS Production.....66

3.2.8 Antioxidant Assays.....67

3.2.9 Flow Cytometry to Determine Apoptosis and Cell Cycle Distribution……..67

3.2.10 Morphological Studies.....68

3.2.11 Transwell Migration Assay.....68

3.2.11.1 Scratch Assay.....68

3.2.12 Gene Expression Analysis by Real-Time PCR.....69

3.2.13 Western Blot Analysis.....69

3.2.14 Improved Penetration and Cell Killing Activity of CDs in Tumor Spheroids.....70

3.2.15 Statistical Analysis.....70

3.3 Results and Discussion

3.3.1 Fabrication and Characterisation of Carbon Nanodots.....70

3.3.2 Response Surface Methodology for Optimising Carbon Nanodots Preparation... .75

3.3.3 Drug Release and Kinetic Study....76
3.3.4 In-vitro Cytotoxicity....78
3.3.5 Uptake Study....80
3.3.6 Pro-apoptosis Effect....82
3.3.7 ROS Production....82
3.3.8 Antioxidants Catalase, SOD and GSH level....83
3.3.9 Cell Cycle Arrest Analysis....85
3.3.10 Morphological Studies of Treated Cells....85
3.3.11 Transwell Assay....87
3.3.11.1Scratch Assay....88
3.3.12 Gene Expression and Western Blot....90
3.3.13 Co-loaded Carbon Nanodots Internalization in 3D Cancer Spheroid and their Efficacy91

3.4 Conclusion....93

Chapter 4 95

Redox-responsive Carbon Nanodot-capped Pt-Mesoporous Janus Nanomotors for delivery of Natural Bioactive Compounds and Simultaneous biosensing *In Vitro* and *In Vivo* Breast Cancer

4.1 Introduction....96

4.2 Materials and Methods

4.2.1 Chemicals....99
4.2.2 Synthesis of Mesoporous Silica Nanoparticles (MSN) and Platinum Nanoparticles (Pt).99
4.2.2.1 Conjugation of Platinum nanoparticle with Mesoporous Silica Nanoparticles (Pt-MSN)100
4.2.2.2 Bioactive Molecules (BM) Encapsulation in Pt-MSN (Pt-MSN@Cue+Quer)..... 100
4.2.2.3 Synthesis of Carbon Nanodots....101
4.2.2.4 Synthesis of Janus Nanomotors (Pt-MSN@Cur+Quer-Se-Se-Carbon Nanodots)…….101
4.2.3 Physico-Chemical Characterisation....101
4.2.4 Peroxidase Like Activity of Janus Nanomotors....101
4.2.4.1 Motion Analysis by MSD Calculation....102
4.2.4.2 Control Release of the Curcumin and Quercetin from Janus Nanomotors)……...102
4.2.5 MTT Assay....102
4.2.5.1 Live/Dead Assay....103

4.2.6 Determination of ROS Production in Cells.....103
4.2.7 Apoptosis.....104
4.2.8 Cell Cycle Analysis.....104
4.2.9 Transwell Migration Assay.....104
4.2.10 Scratch Assay.....105
4.2.11 Comet Assay.....105
4.2.12 Internalization and Localization of Nanoparticles.....106
4.2.13 Bio-imaging of Cells using Janus Nanomotors.....106
4.2.14 Janus nanomotor Internalisation in 3D Cancer Spheroid and their Efficacy).....106
4.2.15 Animal Experiments.....107

4.3 Results and Discussion

4.3.1 Nanomotor Synthesis and Characterisation.....108
4.3.2 Self-Propulsion Analysis and Controlled Drug Release Capabilities of Janus Nanomotors111
4.3.3 *In-vitro* Cytotoxicity.....112
4.3.4 Cellular Uptake and Morphological Changes.....115
4.3.5 Apoptosis, ROS Production and Comet Assay.....118
4.3.6 Cell Cycle Arrest.....122
4.3.7 Evaluation of Anti-Migratory and Anti-Invasive Potential of Janus Nanomotors.....123
4.3.8 Mammosphere (Spheroid) Inhibition Assay.....125
4.3.9 Janus Targeted Delivery of the Drug Showed to Restrict Tumor Growth Alongside Reduced Cytotoxicity *In Vivo*.....126

4.4 Conclusion.....128

Chapter 5 130

A Novel Dual-Gating Strategy Using Ligand Conjugated Iron Doped Carbon Nanodots for Tumor-Selective Prodrug Activation and Breast Cancer Inhibition

5.1 Introduction.....131

5.2 Materials and Methods

5.2.1 Chemicals.....134
5.2.2 Synthesis of FeCDs (Nanozyme) 135
5.2.2.1 Synthesis of FeCDs@IAA.....135

5.2.2.2 Synthesis of FeCDs@IAA@Folic Acid (Nanocarrier) 135
5.2.3 General Characterisation Methods.....136
5.2.4 Peroxidase-Like Activity of FeCDs@IAA@Folic Acid.....136
5.2.5 MTT Assay.....137
5.2.5.1 Live/Dead Assay.....138
5.2.6 Determination of ROS Production in Breast Cancer Cells.....138
5.2.7 Apoptosis.....138
5.2.8 Cell Cycle Analysis.....139
5.2.9 Mitochondrial Potential Assay.....139
5.2.10 Transwell Migration Assay.....139
5.2.11 Scratch Assay.....139
5.2.12 Internalization and Localization of Nanocarriers.....140
5.2.13 Improved Penetration and Cytotoxic Activity of Nanocarriers in Tumor Spheroids.140
5.2.14 Animal Experiment.....141

5.3 Results and Discussion

5.3.1 Characterisation and Catalytic Properties of FeCDs@IAA@Folic Acid.....141
5.3.2 *In vitro* Antitumor Efficacy.....143
5.3.3 Apoptosis Assay.....147
5.3.4 Effect on Mitochondrial Membrane Potential (MMP) 149
5.3.5 Cellular Uptake and ROS production.....150
5.3.6 Effect on Cell Cycle.....152
5.3.7 Morphological Changes.....153
5.3.8 Anti-Migration Analysis.....154
5.3.9 3D Spheroid.....156
5.3.10 FeCDs@IAA@Folic Acid Targeted Delivery of the Drug Showed to Restrict Tumor Growth Alongside Reduced Cytotoxicity *In Vivo*.....157

5.4 Conclusion.....162

Chapter 6 163

Smart Molecularly Imprinted Polymers-Drug Conjugates for Precision Targeting and Biosensing in 2D and 3D MCF-7 Breast Cancer Cells

6.1 Introduction.....163

6.2 Materials and Methods

6.2.1 Materials**.....165**

6.2.2 Preparation of HER3 Epitope-Modified Glass Beads Using Solid-Phase Synthesis**..166**

6.2.2.1 Synthesis of Single Layer Fluorescent HER3 Specific NanoMIPs**.....166**

6.2.2.2 Synthesis of Double-Layer Fluorescent Drug-Loaded NanoMIPs**.....167**

6.2.3 Size and Concentration Analysis of NanoMIPs**.....168**

6.2.4 Cell Culture**.....168**

6.2.5 Binding Analysis of Fluorescent NanoMIPs to Cell Surface**.....169**

6.2.6 Curcumin and Doxorubicin Encapsulation Efficiency (EE) and Loading Efficiency (LE)**169**

6.2.7 Controlled Release of the Drug Molecules**.....170**

6.2.8 Internalisation of NanoMIPs**.....170**

6.2.9 Cytotoxic Assay**.....170**

6.2.10 Determination of Reactive Oxygen Species (ROS) **.....171**

6.2.11 Cell Cycle Analysis**.....172**

6.2.12 Cellular Impact of the NanoMIPs on 2D and 3D Cell Culture**.....172**

6.3 Results and Discussion

6.3.1 Phase Synthesis and Characterisation of NanoMIPs**.....173**

6.3.2 Selective Binding of Rhodamine B-loaded Single-Layer NanoMIPs to HER3-Expressing cells **…….….174**

6.3.2.1 Binding Efficiency of Double Layered Drug Loaded HER3 Specific NanoMIPs….**..175**

6.3.3 Cellular Uptake Study**.....180**

6.3.4 Drug Release Profile**..... 180**

6.3.5 Cytotoxicity Analysis**.....181**

6.3.6 Cell Cycle Arrest**.....184**

6.3.7 Oxidative Stress Induction and Morphological Changes**.....185**

6.3.8 NanoMIPs Internalisation, Penetration Efficiency in 3D Cancer Spheroid… ..**...186**

6.4 Conclusion.....187

Chapter 7 189

Summary, Conclusion and Future Prospective.....190

Reference 194
Appendix I 222
Appendix I 224
Author Biography 225

LIST OF FIGURES

Figure No.	Figure Legend
Figure 2.1	Proportional distribution of global mortality attributed to major non-communicable diseases.
Figure 2.2	Worldwide distribution of cancer incidence and mortality in 2020 across major cancer types.
Figure 2.3	Global age-standardised incidence and mortality rates of breast cancer in women showing regional variations.
Figure 2.4	Comparison of conventional breast cancer treatments with emerging therapeutic approaches such as nanomedicine and precision therapy.
Figure 2.5	Molecular classification of breast cancer subtypes based on ER, PR, and HER2 receptor status.
Figure 2.6	Schematic illustration of passive (EPR-mediated) and active nanoparticle targeting strategies in cancer therapy.
Figure 2.7	Overview of nanobot technologies in breast cancer, highlighting propulsion mechanisms and applications.
Figure 2.8	Multifunctional applications of Janus nanoparticles are enabled by their asymmetric structure.
Figure 2.9	Nanozyme-mediated catalytic therapy mechanism inducing oxidative stress and tumor cell death.
Figure 2.10	Working principle of nanoparticle-assisted biosensing platforms for cancer diagnostics.
Figure 3.1	Schematic representation of the synthesis of carbon nanodots and co-loaded carbon nanodots.
Figure 3.2	Physicochemical characterisation of carbon nanodots using UV–vis, XRD, FTIR, TEM, TGA, XPS, and zeta potential analyses.
Figure 3.3	Response surface and contour plots showing the influence of synthesis parameters on fluorescence intensity.
Figure 3.4	Stability and pH-responsive drug release profiles of Cur, Quer from co-loaded carbon nanodots.

Figure 3.5	MTT-based cytotoxicity evaluation of bare and co-loaded carbon nanodots in normal and cancer cells.
Figure 3.6	Flow cytometry-based live/dead analysis of MCF-7 cells treated with different carbon nanodot formulations.
Figure 3.7	Fluorescence microscopy showing multicolour emission and time-dependent cellular internalisation of carbon nanodots.
Figure 3.8	Apoptosis and necrosis analysis of MCF-7 cells using Annexin V-FITC/PI assay.
Figure 3.9	Intracellular ROS generation induced by different carbon nanodot formulations in MCF-7 cells.
Figure 3.10	Antioxidant enzyme activity (CAT, SOD, and GSH/GSSG) in MCF-7 cells after treatment.
Figure 3.11	Cell-cycle distribution of MCF-7 cells following carbon nanodot treatment.
Figure 3.12	Fluorescence microscopy showing cytoskeletal and nuclear morphological changes in treated MCF-7 cells.
Figure 3.13	Transwell migration assay demonstrating the antimigratory effects of carbon nanodots.
Figure 3.14	Wound-healing assay showing inhibition of MCF-7 cell migration by carbon nanodots.
Figure 3.15	Molecular analysis of antimetastatic mechanisms and P-gp protein regulation.
Figure 3.16	Cytotoxic effects of carbon nanodots on 3D MCF-7 tumor spheroids after 48 h treatment.
Figure 3.17	Cytotoxic effects of carbon nanodots on 3D MCF-7 tumor spheroids after 72 h treatment.
Figure 4.1	Schematic illustration of the synthesis process of Janus nanomotors.
Figure 4.2	Structural and physicochemical characterisation of Janus nanomotors using TEM, SAED, FTIR, and XRD.
Figure 4.3	Catalytic activity, propulsion behaviour, and stimulus-responsive drug release of Janus nanomotors.
Figure 4.4	Cytotoxicity, Calcein AM and live/dead analysis of Janus nanomotors in normal and breast cancer cell lines.

Figure 4.5	Microscopic evaluation of cellular morphology and intracellular internalisation of Janus nanomotors.
Figure 4.6	Fluorescence microscopy showing cytoskeletal and nuclear alterations induced by Janus nanomotors.
Figure 4.7	Evaluation of apoptosis, ROS generation, and DNA damage in breast cancer cells treated with Janus nanomotors.
Figure 4.8	Cell-cycle arrest analysis of breast cancer cells following Janus nanomotor treatment.
Figure 4.9	Migration and invasion assays demonstrating the antimigratory effects of Janus nanomotors.
Figure 4.10	Multicellular tumor spheroid response to nanoparticle treatment at different incubation times.
Figure 4.11	*In vivo* therapeutic efficacy and biosafety evaluation of Janus nanomotors.
Figure 5.1	Schematic illustration depicting the stepwise synthesis of the FeCDs@IAA@Folic Acid nanoreactor–nanocarrier.
Figure 5.2	Physicochemical characterisation of FeCDs@IAA@Folic Acid showing FTIR, TEM, SAED, XRD analysis, and evaluation of peroxidase-like nanozyme activity.
Figure 5.3	Dose-dependent cytotoxicity assessment of FeCDs@IAA@Folic Acid in breast cancer (MCF-7, MDA-MB-231) and normal (MCF-10A) cells using MTT assay and Calcein AM staining.
Figure 5.4	Flow cytometry–based live/dead analysis demonstrating enhanced cytotoxicity of FeCDs@IAA@Folic Acid compared to IAA and nanozyme treatments in breast cancer cells.
Figure 5.5	Annexin V–FITC/PI flow cytometric analysis showing significant apoptosis induction by FeCDs@IAA@Folic Acid in MCF-7 and MDA-MB-231 cells.
Figure 5.6	Evaluation of mitochondrial membrane potential in MCF-7 and MDA-MB-231 cells, untreated and treated with IAA, nanozyme and FeCDs@IAA@Folic Acid.
Figure 5.7	Fluorescence microscopy and flow cytometry analysis confirming elevated intracellular ROS generation upon FeCDs@IAA@Folic Acid treatment.
Figure 5.8	Flow cytometric cell-cycle analysis indicating G2/M phase arrest and increased sub-G0/G1 population following FeCDs@IAA@Folic Acid treatment.
Figure 5.9	Cytoskeletal and nuclear morphology analysis revealing actin disruption and nuclear fragmentation induced by FeCDs@IAA@Folic Acid.

Figure 5.10	Transwell invasion assay demonstrating significant inhibition of invasive potential in breast cancer cells treated with FeCDs@IAA@Folic Acid.
Figure 5.11	Scratch wound-healing assay showing strong anti-migratory effects of FeCDs@IAA@Folic Acid in MCF-7 and MDA-MB-231 cells.
Figure 5.12	Evaluation of penetration and therapeutic efficacy in 3D breast cancer spheroids, highlighting enhanced cell death induced by FeCDs@IAA@Folic Acid.
Figure 5.13	*In vivo* therapeutic efficacy and biosafety evaluation of FeCDs@IAA@Folic Acid nanocarrier.
Figure 6.1	HER3 protein structure highlighting the selected epitope and schematic synthesis of Dox–Cur nanoMIPs.
Figure 6.2	Flow cytometric evaluation of receptor-specific binding of single-layer nanoMIPs.
Figure 6.3	TEM and DLS characterisation of single- and multilayer drug-loaded HER3-targeted nanoMIPs.
Figure 6.4	Flow cytometry-based binding analysis of Dox–Cur nanoMIPs in breast cancer cells.
Figure 6.5	Dose-dependent binding efficiency of curcumin- and doxorubicin-loaded nanoMIPs.
Figure 6.6	Fluorescence microscopy showing selective uptake and time-dependent internalisation of nanoMIPs.
Figure 6.7	pH-responsive drug release profile of Dox–Cur nanoMIPs.
Figure 6.8	*In vitro* cytotoxicity and live cell staining analysis (Calcein AM) of nanoMIPs.
Figure 6.9	Live/dead and apoptosis analysis of MCF-7 cells treated with nanoMIPs.
Figure 6.10	Cell-cycle arrest induced by different nanoMIP formulations in MCF-7 cells.
Figure 6.11	ROS generation and morphological alterations in MCF-7 cells following nanoMIP treatment.
Figure 6.12	Penetration and cytotoxic effects of nanoMIPs in 3D breast cancer spheroids.
Figure 7.1	Graphical Summary

LIST OF TABLES

Table No.	Table Legend
Table 2.1	Overview of major classes of anticancer chemotherapeutic agents, their mechanisms of action, representative drugs, therapeutic outcomes, and associated risks.
Table 2.2	Advantages and disadvantages of selected modern therapeutic approaches for breast cancer treatment.
Table 2.3	Summary of major breast cancer biomarkers with their functional roles and corresponding diagnostic, prognostic, and targeted treatment strategies.
Table 2.4	Overview of major nanocarrier- and biomolecule-based delivery systems used in breast cancer therapy, highlighting their advantages and representative applications.
Table 2.5	Summary of chemically powered nanorobot platforms detailing fuel mechanisms, structural design, therapeutic or diagnostic payloads, and biomedical applications.
Table 2.6	Summary of externally powered nanorobot platforms outlining actuation methods, material composition, loaded cargos, and key biomedical applications.
Table 2.7	Summary of biohybrid nanorobot platforms highlighting biological components, propulsion strategies, therapeutic payloads, and biomedical applications.
Table 2.8	Overview of multifunctional Janus nanoparticles employed in imaging, chemotherapy, photothermal therapy, and targeted drug delivery for breast cancer.
Table 2.9	Overview of engineered nanozymes used in cancer therapy, detailing enzyme-mimetic activities and treatment strategies including CDT, PDT, PTT, SDT, immunotherapy, and starvation therapy.
Table 2.10	Summary of molecularly imprinted polymer (MIP)-based therapeutic systems across cancer models, highlighting polymer composition, target templates, functional payloads, and applications.
Table 2.11	Comparison of traditional biosensing techniques used for breast cancer detection, highlighting their principles, advantages, and limitations.
Table 2.12	Comparative evaluation of carbon nanodots with other nanoparticle systems based on physicochemical and biological properties.

Table 2.13	Overview of theranostic carbon-dot systems used in PTT/PDT, emphasizing biocompatibility, therapeutic efficacy, and multimodal cancer treatment outcomes.
Table 2.14	Summary of commonly used breast cancer cell lines, their receptor expression profiles, and molecular subtypes.
Table 2.15	Summary of widely used chemotherapeutic drugs for breast cancer, their mechanisms of action, and major adverse effects.
Table 2.16	Summary of selected bioactive molecules and their molecular mechanisms of action against breast cancer cells.
Table 3.1	Experimental design based on the Box–Behnken model for optimising synthesis parameters of carbon nanodots.
Table 3.2	Correlation coefficients and release exponents of kinetic models describing curcumin and quercetin release from carbon nanodots.
Table 3.3	Molecular docking analysis of quercetin and curcumin with the target protein, showing docking scores, confidence values, and ligand RMSD.
Table 4.1	Quantitative assessment of DNA damage in breast cancer cells using alkaline comet assay following treatment with different nanomaterial formulations.

LIST OF SYMBOLS

θ Theta

μ Micro

º Degree Celsius

~ Approximately

h Hour

g Gram

M Molar

% Percentage

s Seconds

U Units

v/v volume/volume

L Liter

λ Wavelength

LIST OF ABBREVIATIONS

CDs	Carbon Nanodots
P-gp	P-Glycoprotein
EPR	Enhanced Permeability and Retention
TME	Tumor Microenvironment
Cur	Curcumin
Quer	Quercetin
Dox	Doxorubicin
ROS	Reactive Oxygen Species
HER	Human Epidermal Growth Factor Receptor
PI	Propidium Iodide
MIPs	Molecularly Imprinted Polymers
TNBC	Triple Negative Breast Cancer
APTS	3-Aminopropyltriethoxysilane
SIA	Succinimidyl Iodoacetate
NIPAM	N-Isopropylacrylamide
BIS	N,N′-Methylene Bisacrylamide
AA	Acrylic Acid
TBAm	N-tert-Butylacrylamide
NAMPA	Methacrylamide Hydrochloride
APS	Ammonium Persulfate
PBS	Phosphate-Buffered Saline
DAPI	4′,6-Diamidino-2-Phenylindole
FITC	Fluorescein Isothiocyanate
DCFDA	2′,7′-Dichlorodihydrofluorescein Diacetate
FBS	Fetal Bovine Serum
LE	Loading Efficiency
EE	Encapsulation Efficiency
RSM	Response Surface Methodology
MDR	Multidrug Resistance

TGA	Thermogravimetric Analysis
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-ray Diffraction
XPS	X-ray Photoelectron Spectroscopy
PCR	Polymerase Chain Reaction
Pt	Platinum
MSN	Mesoporous Silica Nanoparticle
PVA	Polyvinyl Alcohol
TEOS	Tetraethyl Orthosilicate
MTPMS	3-Mercaptopropyltrimethoxysilane
CTAB	N-Cetyltrimethylammonium Bromide
ABTS	2,2′-Azinobis(3-ethylbenzothiazoline)-6-sulfonic Acid
NHS	N-Hydroxysuccinimide
EDC	1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide Hydrochloride
MSD	Mean Square Displacement
IAA	Indole Acetic Acid
FA	Folic Acid
TEM	Transmission Electron Microscope
UV	Ultraviolet
µM	Micromolar
µL	Microliter
mm	Millimetre
µm	Micrometre
mg	Milligram
mL	Millilitre
mins	Minutes
sec	Seconds
nm	Nanometre
cm	Centimetre