ABSTRACT

One of the leading causes of cancer-related mortality among women globally is breast cancer, despite significant advances in diagnosis and therapy. Conventional treatment strategies such as chemotherapy, radiotherapy, and surgery continue to suffer from critical limitations, including systemic toxicity, multidrug resistance, poor tumor selectivity, limited penetration into solid tumors, and inadequate real-time monitoring of therapeutic response. These challenges necessitate the development of next-generation therapeutic platforms that are not only effective but also selective, multifunctional, and clinically translatable. Nanotechnology has emerged as a powerful tool to address these limitations; however, many nanotherapeutics still face issues related to poor tumor accumulation, biological instability, high cost, immunogenicity, and batch-to-batch variability.

In this thesis, a progressive and rationally designed nanomedicine framework is developed, advancing from simple, cost-effective passive nanocarriers to highly advanced, tumor-responsive, and precision-targeted theranostic platforms for breast cancer treatment and biosensing. The central theme of this work is the strategic use of carbon-based nanomaterials, Janus nanomotors, nanozymes, and molecularly imprinted polymers to overcome multidrug resistance, enhance intracellular drug accumulation, improve tumor penetration, and integrate therapeutic and diagnostic functions within a single system.

Initially, in this study, we have synthesised biomass-derived carbon nanodots, and their formulation was optimised using response surface methodology (RSM). The optimised CDs were then co-loaded with curcumin (Cur) and quercetin (Quer) (co-loaded carbon nanodots) as model natural bioactive compounds to evaluate their synergistic therapeutic effect on breast cancer. The stability of the synthesised CDs was tested at various pH values (4–8.5) and over time, revealing excellent stability for up to 120 h even under acidic or basic conditions. UV–vis spectroscopy, XRD, Zeta potential, and FTIR confirmed the loading of these bioactive molecules on carbon nanodots. It has also been found that loading of curcumin and quercetin on carbon nanodots increases the stability and bioavailability of curcumin. Antiproliferative and antimetastatic properties of the co-loaded carbon nanodots were further studied on the MCF-7 breast cancer cell line. The data represent that these co-loaded carbon nanodots exhibit strong cytotoxicity and antimigration effects against the MCF-7. Notably, co-loaded carbon nanodots significantly enhanced the apoptosis, ROS production, nucleus degeneration, and tumor inhibition *in vitro* in comparison to single bioactive-loaded carbon nanodots. The

enhancement occurs because the co-loaded carbon nanodots block multidrug resistance and the metastasis of cancer cells by downregulating the expression of membrane-bound P-glycoprotein (P-gp), resulting in the accumulation of curcumin in the cancer cells. Additionally, they also downregulate the BIRC gene expression, which is the biomarker for triple-negative breast cancer cells, whereas they enhance the expression of the p53 gene. To mimic the *in vivo* tumor microenvironment and to better understand the penetration efficacy of the co-loaded CDs, we also evaluated them in 3D spheroid models of breast cancer. While this system offered a simple and cost-effective approach utilising passive tumor accumulation, the inherent heterogeneity and patient-dependent variability of passive targeting highlighted the need for more advanced delivery strategies.

To overcome the limitations of diffusion-controlled delivery and shallow tumor penetration, the work was advanced toward the design of self-propelled, multifunctional Janus nanomotors. Platinum-mesoporous silica Janus nanomotors capped with carbon nanodots and gated via redox-responsive di-selenide linkages were synthesised to achieve active motion, deep tumor penetration, and on-demand drug release. The results showed that the Janus nanomotors exhibited strong cytotoxicity and antimigration effects in both the cancer cell lines (MCF-7 and MDA-MB-231). Notably, Janus nanomotors effectively enhanced the apoptosis and ROS production, nucleus degeneration, and tumor inhibition *in vitro*. Janus nanomotors also showed good uptake, penetration, and killing efficiency of cancer cells in 3D spheroids of MCF7 cells. Furthermore, tumor growth in mice was significantly restricted in the treatment group with a mean tumor volume of 220 mm^3 compared to 725 mm^3 in the control group, respectively. Despite their high tumor specificity and therapeutic efficacy, these systems relied predominantly on tumor microenvironment cues rather than molecular recognition, motivating the transition toward receptor-mediated and catalytic targeting strategies.

Subsequently, we developed a novel dual-gated nanocarrier based on iron-doped carbon nanodots (Fe-CDs) conjugated with the indole-3-acetic acid (IAA) prodrug and further functionalized via di-selenide (Se–Se) linkages to folic acid. This unique dual-gating strategy combines (i) redox-responsive di-selenide cleavage in the tumor microenvironment and (ii) folate receptor-mediated targeting, ensuring selective prodrug activation and enhanced cancer cell killing. The FeCDs@IAA@Folic Acid (nanocarrier) was systematically characterised using FTIR, XRD, and TEM, and its intrinsic peroxidase-like catalytic activity was confirmed. Biological evaluations demonstrated potent anticancer efficacy, with IC_{50} values of 5.12 μg/mL for MCF-7 (ER^+) and 13.4 μg/mL for MDA-MB-231 (triple-negative) breast cancer cells, while

sparing normal MCF-10A cells. Mechanistic studies revealed that the nanocarrier induced ROS overproduction, mitochondrial depolarisation, apoptosis, and G2/M cell cycle arrest, leading to effective cell death. Live/dead staining with Calcein AM further validated its cytotoxicity. In addition, the FeCDs@IAA@Folic Acid nanocarrier significantly inhibited cancer cell migration in scratch and transwell assays, underscoring its potential to prevent metastasis. Importantly, 3D spheroid culture studies confirmed deep penetration and efficient tumor spheroid eradication. While highly effective, the dependence on biological ligands introduced concerns to find better alternative strategies, related to immunogenicity, cost, receptor heterogeneity, and competitive binding in complex biological environments.

To address this, the final phase of this work focused on the development of ligand-free, synthetic receptor-mimicking platforms using molecularly imprinted polymers (nanoMIPs). HER3-specific, double-layered nanoMIPs were engineered as plastic antibodies capable of precise molecular recognition without relying on biological ligands. A solid phase imprinting approach was utilised to synthesise the nanoMIPs. The dual-functional nanoMIPs were co-loaded with curcumin (a natural bioactive compound) and doxorubicin (a chemotherapeutic agent) (Dox-Cur-nanoMIPs). The curcumin blocked the P-gp channel, resulting in the accumulation of doxorubicin inside the cancer cells, improving the cancer cells' killing efficiency. *In vitro* studies on MCF-7 breast cancer cells demonstrated that the nanoMIPs effectively induce apoptosis, promote ROS generation, and cause nuclear degeneration. Moreover, the inherent fluorescence of the drug-loaded nanoMIPs allows point-of-care detection of HER3-expressing cells, thereby integrating therapeutic and diagnostic capabilities in a single platform. To mimic the tumor microenvironment, the penetration and efficacy of the Dox-Cur-nanoMIPs were also evaluated in 3D spheroid models of breast cancer. This model demonstrated efficient penetration, high specificity, robust therapeutic action, and clinical relevance of this antibody-free, cost-effective, and scalable approach.

Overall, this thesis presents a comprehensive and evolution-driven strategy for breast cancer nanomedicine, systematically addressing the shortcomings of conventional therapies and existing nanoplatforms. By integrating passive delivery, active propulsion, redox-responsive gating, catalytic nanozyme activity, receptor targeting, and synthetic molecular recognition, this work establishes a versatile and clinically translatable framework for precision cancer therapy and biosensing. The findings contribute significant insights into multifunctional nanotherapeutic design and offer promising pathways toward safer, more effective, and affordable treatments for breast cancer.