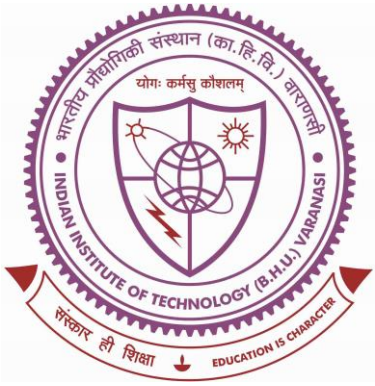
Natural and Synthetic based Polymeric Nanoparticles for Drug Delivery



Thesis submitted in partial fulfilment

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Doctor of Philosophy

by

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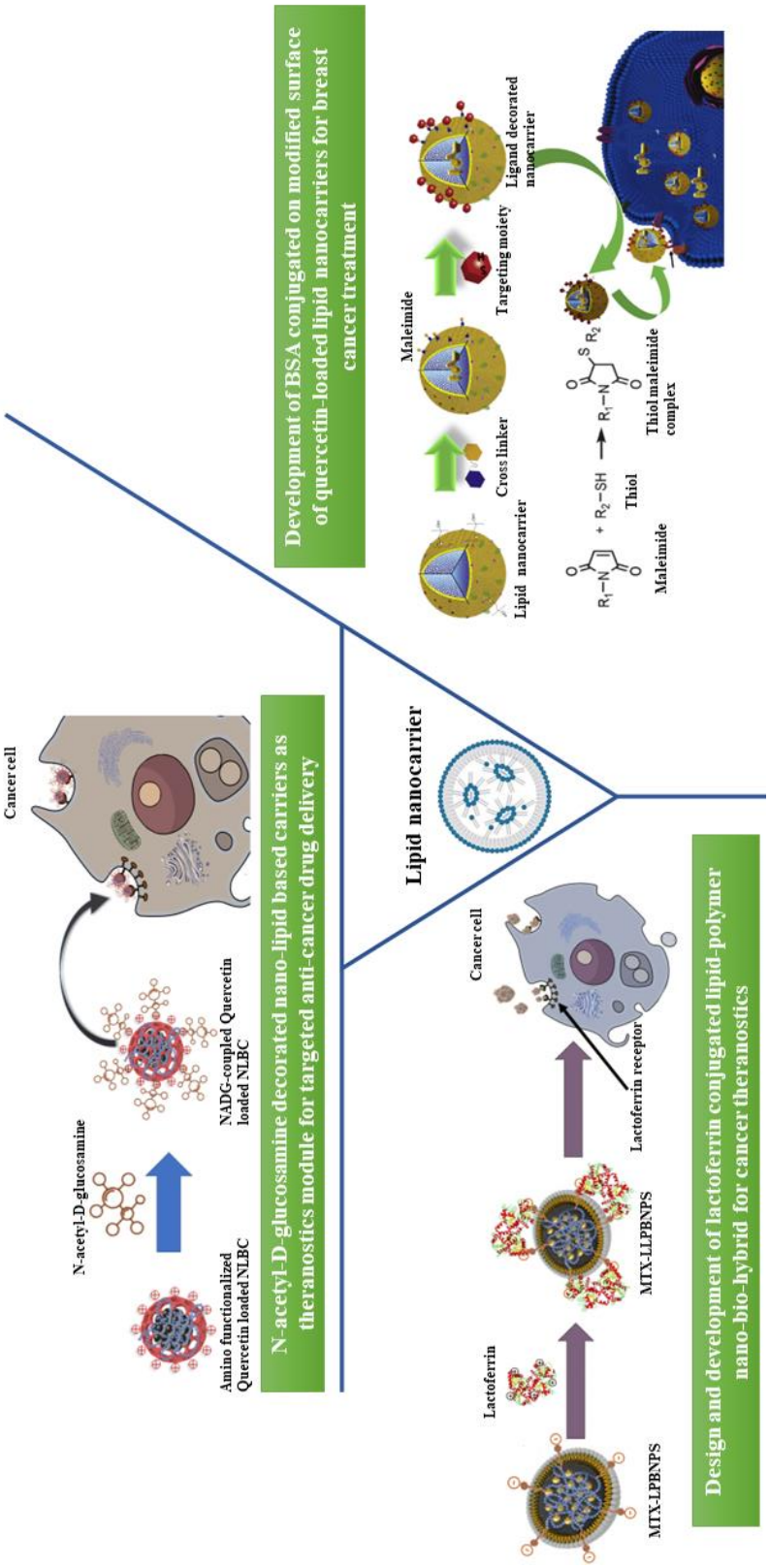
Chapter V

Summary and Future Work



More than two hundred different types of cancer affect the human population socially, emotionally, and financially worldwide. Numerous natural and chemically synthesized anti-cancer medicines have been screened, but solubility and toxicity are the major concerns. These constraints can be conquered using various ligand-nanocarrier conjugates that are well explored in this thesis. In addition, this thesis has elaborated that perforated vascular systems facilitate the passive targeting of nanocarriers in Chapter I. In recent decades, many types of surface receptors that overexpress on the tumor cells have been discovered. Their interactions with target ligands have been demonstrated, leading to the active targeting of nanocarriers. Also, various theranostics agents along with lipid nanocarrier have been well discussed in the tabular form and have shown promising results in drug delivery and detection of target cells, without causing any harm to the healthy cells. Also, the fundamental concept of NLBCs as a suitable carrier has been proven and well documented. The various types of lipid-nanocarriers, such as liposomes, niosomes, SLNs, NLCs, and LPHNs that are being used in developing nanomedicines and their mechanisms of formulation have been discussed. Moreover, various techniques with step-by-step synthesis processes of the NLBCs are well discussed and presented. Apart from these, we have also included the nanocarrier stability and drawbacks, and challenges in the scale-up of lipid nanomedicine manufacturing from laboratories to industries.

In Chapter II, we have developed N-acetyl-d-glucosamine (NADG) decorated nano-lipid-based carriers (NLBCs) as a theranostics module for targeted anti-cancer drug delivery. To the best of our knowledge, this investigation is the first attempt to conjugate NADG to poly-L-lysine functionalized lipid surface for targeted drug delivery. The nano-bioconjugate was successfully synthesized using a single emulsion solvent evaporation method. The conjugate NADG was successfully coupled with the surface of the nanocarrier using classical conjugation chemistry. Overall characteristics such as solubility, release kinetic profile, and bioavailability

were significantly improved as compared to the previous study. Release kinetics pattern of NADG-Q-NLBCs required 200 h for 100 % drug release whereas, for another system, 100 % of the drug was released within 160 h (Garg et al., 2016), (Jain et al., 2015). Particle charge and size were found to be 11.22 ± 0.98 mV, and 390 ± 2.44 nm, respectively. The encapsulation efficiency of the system was found to be in the range of 69 ± 1.72%. Cell toxicity of the final bioconjugate showed significant results at lower concentrations of drugs with IC50 of 7.25 µg/mL and flow cytometry depicted early and late apoptosis of MCF-7 cells.

In Chapter III, we have designed the BSA conjugated on a modified surface of quercetin-loaded lipid nanocarriers for breast cancer treatment. To the best of our knowledge, this investigation is the first attempt to conjugate BSA to maleimide functionalized lipid surface for targeted drug delivery. Overall characteristics of BSA-Q-LNs such as solubility, and bioavailability were further improved as compared to NADG-Q-NLBCs. Release kinetic pattern showed more than 200 h for 100 % drug release, which favors the constant and sustained rate of drug release. Particle charge and size were found to be 18.4 ± 2.5 mv and 530.43 ± 4.90 nm, respectively. Encapsulation efficiency was found to be 76 ± 0.5 %. Cell toxicity showed significant results at lower concentrations of drugs with IC50 of 6.90 µg/mL and confocal microscopy demonstrated significant morphological changes in MCF-7 cells.

In Chapter IV, we have developed a lactoferrin conjugated lipid-polymer nano-biohybrid for cancer theranostics. To the best of our knowledge, this work is the first attempt to conjugate lactoferrin to a stearic acid anchored lipid-PCL hybrid system for targeted drug delivery**.** The nano-biohybrid was successfully synthesized using one-step nanoprecipitation technique. Overall characteristics such as stability, solubility, bioavailability, and crystallinity were significantly improved as compared to NADG-Q-NLBCs and BSA-Q-LNs. Release kinetic pattern revealed the restricted delivery of drugs in physiological conditions which may overcome the problem of multiple drug resistance. Particle charge and size were found to be -

20.72 ± 1.23 mV and 650.70 ± 4.90 nm, respectively, which significantly improved the stability of the system. Encapsulation efficiency was found to be in the range of 84.0 ± 3.2 %. Cell toxicity revealed that free drug was more lethal to MCF-cells than formulated NPs in *in vitro* conditions because small molecules can easily travel across the plasma membrane via passive diffusion. However, LLPBNPs significantly facilitated the alteration of cell morphology as compared to the LPBNPs system. Through such hybrid-nano-bioconjugate, the maximum tolerated dose of drugs can be increased for the effective therapy of cancer.

Various cancer stem cells overexpressed certain biomarkers, including CD133, epidermal cell adhesion molecule (EpCAM), and aldehyde dehydrogenase 1(ALDH1). In the case of hepatocarcinoma, colorectal cancer, and gastric cancer, a membrane-bound protein called Eph B2 (receptor tyrosine subfamily) is expressed significantly. Further various interleukins comprising IL-4Rα, IL-1β , and IL-8 are membrane-bound and secretory proteins produced at higher levels in various carcinoma. Several techniques such as XRD, FT-IR, and NMR can be used to determine the grooves, functional groups, and chemical structure of the aforementioned biomarkers. Next generation sequencing may help in finding the sequence of the overexpressed receptors on the melanoma cells. Once the detailed structure of the biomarkers is determined the specific molecules (ligands) against them can be synthesized. Another approach can be antibody engineering, especially monoclonal antibody production, by inducing whole protein, fragment, or sequence of a specific domain of a biomarker of interest. Further, various binding techniques such as fluorescence in situ hybridization, enzyme-linked immunosorbent assay, western blotting, fluorescence spectroscopy may utilize to confirm the binding affinity between ligand and biomarker. The bioinformatics modeling method can also predict the ligand-biomarker interactions and boost the targeting and diagnosis affinity. Moreover, the proposed ligands can be conjugated with our lipid based nano system using various conjugation chemistry for anticancer drug delivery and detection of cancer cells. Metal-

organic frameworks (MOFs) are materials with adjustable porosities, functional surfaces, and bulk conjugated backbones of metal ions and organic linkers. MOFs have been reported to have exchange abilities between the extra framework ions and various external guest species. These MOFs, delivered via lipid nanocarriers, can also sense and diagnose colon cancer, usually showing 7-fold higher copper concentration than normal cells. These approaches may boost the formulations' targeting and diagnosis ability against various carcinoma. We further conclude that various ligands need to be engineered, which may pave the way towards detecting and targeting malignant cells.