UNIVERSITI PUTRA MALAYSIA

FOLIC ACID CONJUGATED CHITOSAN-BASED Mn(2+)-DOPED ZnS QUANTUM DOT FOR BREAST CANCER CELL IMAGING AND TARGETED DRUG DELIVERY

IBRAHIM BIRMA BWATANGLANG

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By

IBRAHIM BIRMA BWATANGLANG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

March 2017

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DEDICATION

I dedicate the entire work to my late father

Abstract of thesis presented to the Senate of Universiti Putra Malysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

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March 2017

Chairman : Professor Nor Azah Yusof, PhD
Faculty : Science

For the past few decades, many acquisitions were developed to unraveled cancer chemistry by designing smarter nanomaterials that can selectively target cancer cells, respond to its microenvironment and possibly support non-invasive diagnosis. However, despites the encouraging achievements in line with this concept *in vitro*, the use of these theranostics nanomaterials *in vivo* remain an unfinished business. Based on this account, a nanocomposite for targeted delivery and imaging application was developed. The rational was implemented by exploring chitosan-biopolymer based system mediated by folic acid-conjugation with affinity towards folate receptors expressed by cancer cells. The folic acid conjugated chitosan-based system was further equipped with a fluorescence imaging contrast agent (Mn:ZnS) to deliver 5-Fluororaucil anti-cancer drugs selectively into tumor-microenvironment. On the basis of these strategies, four sequential wet chemistry methods was adopted to prepare the 5-FU@FACS-Mn:ZnS nanocomposite. The as-prepared nanocomposite shows an average particle size distribution of 8.42 ± 1.79 nm and emit orange-red fluorescence at ~600nm. The strategy for the preparation involves physicochemical optimization of the nanocomposites for controlled drug release, tumor targeting specificity and bioavailability. The result was accomplished by testing and optimizing the physical properties of the materials using Fourier transform infrared, ultraviolet-visible spectroscopy, thermogravimetric analysis/differential scanning calorimetry, transmission electron microscopy, field emission scanning electron microscopy/energy dispersive X-ray, X-ray diffraction, X-ray fluorescence, X-ray photoelectron spectroscopy, fluorescence microscopy and dynamic light scattering instrumentations. The *in vitro* result showed that the as-synthesized 5-FU@FACS-Mn:ZnS nanocomposite when compared to the pure 5-FU anti-cancer drugs induced high level of apoptosis, selectivity and allowed fluorescence imaging in the cancer cell (MCF-7 and MDA-MB231) lines. This was evident based on the MTT cell proliferation assay, the arrest in the cell cycle and the quadrant-pattern from Annexin assay respectively. In addition to the superior anti-cancer effects demonstrated by the 5-FU@FACS-Mn:ZnS *in vitro*, the nanocomposite was able to reduce the tumor

burden by inhibiting the tumor growth by 51 % compared to 42% induced by the pure 5-FU drugs and effectively suppress the expression of pro-inflammatory NO and MDA activity levels in the 4T1 induced mice *in vivo*. Furthermore, the as-synthesized 5-FU@FACS-Mn:ZnS nanocomposite in comparison to the 5-FU drugs has significantly allowed the stimulation of arsenal T-cells agents (CD3+/CD4+, CD3+/CD8+) , natural killer cells (NK 1.1/CD3+) and the anti-inflammatory cytokines (IL-2, IFN- γ), thus inhibiting cancer progression/metastasis as evident in the clonogenic assay of the lungs section. Furthermore, in comparison to the pure 5-FU drugs, the 5-FU@FACS-Mn:ZnS has markedly decreased the pathological alterations caused by the 4T1 cell lines in the liver, spleen, kidney and the lungs of the cancer induced mice. These superior anti-tumor efficacy and anti-metastasis induced by the 5-FU@FACS-Mn:ZnS nanocomposite compared to the pure 5-FU drug is due to the enhanced selectivity of the folic acid conjugation towards the folate receptors expressing cancer cells, thus mediating enhanced cellular uptake of the folate-5-FU loaded conjugate into the tumor cells as evident from the tissue-biodistribution results in the 4T1 induced mice.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

ASID FOLIK-TERKONJUGASI DENGAN KITOSAN-BERDASARKAN SISTEM KUANTUM DOT UNTUK PENGIMEJAN DAN TERAPI SEL KANSER

Oleh

IBRAHIM BIRMA BWATANGLANG

Mac 2017

Pengerusi : Profesor Nor Azah Yusof, PhD
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Untuk beberapa dekad yang lalu, banyak kajian telah dibangunkan untuk menyelesaikan kimia berkaitan dengan kanser dengan mereka bentuk bahan nano lebih pintar boleh mensasarkan sel-sel kanser secara terpilih, bertindak balas terhadap persekitaran mikro dan menyokong diagnosis bukan invasif. Walaupun pencapaian memberangsangkan selaras dengan konsep *in vitro*, penggunaan teranostik bahan nano *in vivo* kekal sebagai penyelidikan yang belum selesai. Berdasarkan ini, komposit nano teranostik dibangunkan untuk kegunaan penghantaran dan aplikasi pengimejan. Kajian dilaksanakan dengan meneroka rangsangan sistem berasaskan kitosan-biopolimer difungsikan dengan konjugasi folik asid berinteraksi dengan reseptor folat yang diekspresi oleh sel-sel kanser. Sistem berasaskan FACS telah dilengkapi dengan ejen pengimejan pendarfluor kontras (Mn:ZnS) untuk menyampaikan ubat antikanser terpilih 5-fluororaucil ke dalam persekitaran mikro tumor. Berdasarkan strategi ini, empat kaedah kimia telah diterima pakai untuk menyediakan 5-FU @ FACS-Mn: ZnS komposit nano. Strategi ini melibatkan pengoptimuman fizikokimia daripada komposit nano 5-FU @ FACS-Mn: ZnS untuk melepaskan ubat secara terkawal, menyasarkan tumor secara spesifik dan kesediaan bio. Keputusan ini telah dicapai dengan menguji dan mengoptimumkan sifat fizikal bahan menggunakan spektroskopi inframerah, spektroskopi lembayung-cahaya nampak, kaedah analisis terma/pengimbasan pembezaan kalorimeter, transmisi mikroskop elektron, mikroskop elektron imbasan dan sinar X tenaga serakan, pembelauan sinar X, sinar X berpendarfluor, sinar X spektroskopi fotoelektron, mikroskop pendarfluor dan penyerakan cahaya dinamik instrumentasi. Hasil *in vitro* menunjukkan bahawa 5-FU @ FACS-Mn: ZnS komposit nano tulen mendorong apoptosis pada tahap tinggi, pemilihan yang ketara dan membenarkan pengimejan pendarfluor dalam sel kanser (MCF-7 dan MDA-MB231) berbanding 5-FU ubat anti-kanser tulen. Ini terbukti berdasarkan ujian MTT, penangkapan dalam kitar sel dan corak kuadran Annexin. Selain kesan anti-kanser yang lebih tinggi ditunjukkan oleh 5-FU @ FACS-Mn: ZnS *in vitro*, komposit nano dapat mengurangkan beban tumor dengan menghalang pertumbuhan tumor sebanyak 51% berbanding dengan 42%

disebabkan oleh 5-FU tulen dan berkesan mengurangkan ekspresi tahap aktiviti NO dan MDA dalam tikus 4T1 *in vivo*. Tambahan pula, komposit nano 5-FU @ FACS-Mn: ZnS yang berkaitan dengan 5-FU membolehkan rangsangan ejen senjata T-sel (CD3 + / CD4 +, CD3 + / CD8 +), sel-sel pembunuh semulajadi (NK 1.1 / CD3 +) dan sitokin anti-radang (IL-2, IFN- γ) dan dengan itu mengawal selia aktiviti sitotoksik T-sel dan sel-sel NK untuk bertindak agresif ke arah menghalang perkembangan kanser / metastasis seperti yang terbukti dalam cerakin clonogenic dan histologi paru-paru seksyen. Tambahan pula, jika dibandingkan dengan ubat 5-FU tulen, 5-FU @ FACS-Mn: ZnS dengan ketara dapat mengurangkan perubahan patologi yang disebabkan oleh 4T1 di bahagian sel di dalam hati, limpa, buah pinggang, paru-paru dan tumor tikus kanser dicabar. Ini merupakan kesan anti-tumor yang unggul dan anti-metastasis disebabkan oleh 5-FU @ FACS-Mn: ZnS NPS berbanding ubat 5-FU tulen adalah kerana selektiviti yang disebabkan oleh konjugasi FA berinteraksi dengan FR yang diekspresikan oleh sel-sel kanser, sekali gus mempertingkatkan pengambilan sel konjugat folat-5-FU ke dalam sel tumor seperti yang terbukti dari taburan bio tisu dalam tikus yan dirangsang dengan sel 4T.

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I certify that a Thesis Examination Committee has met on 27 March 2017 to conduct the final examination of Ibrahim Birma Bwatanglang on his thesis entitled "Folic Acid Conjugated Chitosan-Based Mn(2+)- Doped ZnS Quantum Dot for Breast Cancer Cell Imaging and Targeted Drug Delivery" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

5-FU	5-Fluororaucil
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CB	Conduction Band
CS	Chitosan
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic acid
DSC	Differential Scanning Calorimetry
EDX	Energy Dispersive X-ray
EPR	Enhanced Permeability and Retention
FA	Folic acid
FACS	Folic acid conjugated Chitosan
FACS-Mn:ZnS	Folic acid conjugated chitosan manganese doped zinc sulphide quantum dots
5-FU@FACS-Mn:ZnS	5-Fluororaucil loaded Folic acid conjugated chitosan manganese doped zinc sulphide
FA-FRs	Folic acid Folate Receptor Mediation
FDA	Food and Drug Administration
FESEM	Field Emission Scanning Electron Microscopy
FRs	Folate Receptors
FTIR	Fourier Transform Infrared
H & E	Hematoxylin and Eosin
HRTEM	High-Resolution Transmission Electron Microscopy
IR	Infrared

Mn:ZnS	Manganese doped ZnS
MTT	3-(4-5-dimethylthiazol-2-yl)-2,5-dophenyltetrazolium bromide
nm	Nanometer
NPs	Nanoparticles
PBS	Phosphate Buffer Saline
QDs	Quantum dots
ROS	Reactive Oxygen Species
SQDs	Semiconductors
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric Analysis
THF	Tetrahydrofolate
TPP	Tripolyphosphate
TS	Thymidylate Synthase
UV-vis	Ultra-violet-visible
VB	Valence Band
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction
XRF	X-ray Fluorescence

CHAPTER I

INTRODUCTION

1.1 Background

Globally today, millions in resources and unquantifiable amount in human energy has being expended and channeled into research and development in the fight against all classes of malignant diseases. And despite these efforts, cancer remain one of the major causes of mortality due to its ability to evade treatments (Bray *et al.*, 2013; Ferlay *et al*., 2013; Misra *et al.*, 2010). The unique feature of cancer cells that makes its deadly is characterized based on their tumor-clonality; which is a systemic step towards the development of tumor from a single cell, into a multistep and finally transforming into a malignant tissues through a progressive series of cells alteration (Cooper, 2000). The cause of these cells abnormalities is a complex event that usually involved many causal factors (environmental related risk factors) which are in one way or the other causally interrelated to other salient factors (eating habits and life-style related risk factors) (Gago-Dominguez *et al*., 2016; Kenfield *et al*., 2016).

In 2012, the statistics of cancer reported cases globally were estimated at 14.1 million new cases and 8.2 million cancer-related deaths (Bray *et al*., 2013; Ferlay *et al*., 2013). Among the commonly diagnosed cancers worldwide, breast cancer is single out largely as a gender biased malignancy. Historically speaking, breast cancer anciently referred to as a humoral disease was discovered way back 3500 years ago in Egypt (Akram and Siddiqui, 2012; Papavramidis, and Demetriou, 2010) and up to date remains the most common form of cancer in women worldwide, with nearly 1.7 million new cases diagnosed and about 521,900 deaths cases recorded in 2012 (Torre *et al*., 2015). Statistically, among the 14.1 million reported new cancer cases in 2012, breast cancer accounts for over 25% of all cancer related cases and 15% of all cancer deaths recorded among female population (Torre *et al*., 2015).

Though, significant progress has being achieved in cancer diagnostic, about 30% of patients with early-stage cancer have recurrent metastatic-related cases (Jamal *et al*., 2003). Therefore, cancer treatment, not only should be design to oblate cancer cells, but also should possess properties that can disrupt or inhibit possible metastatic processes (Zijl *et al.*, 2011). Cancer cell metastasis is typically responsible for more than 90% of cancer-related death, exerting clonality through tissue invasion, extravasation, migration, angiogenesis and circulation (Fidler, 2000; Finger and Giaccia, 2010).

1.2 Cancer-Based Therapeutics

Conventionally, one of the commonest response strategy toward advanced malignancy is surgery followed by series of other conventional approaches such as radiation therapy and regimental chemotherapy (Nounou *et al*., 2015).

Chemotherapy is a regimental approach following the use of cytotoxic drugs to systemically kill cancer cells. For the past seven decades, more than 175 anti-tumor drugs hit the market out of which 65% are derived from nature (Mišković *et al.*, 2013). Chemotherapeutic drugs are mostly categories based on their ability to target cells in a particular phase of the cell growth cycle and can either induced cytotoxic effect to actively dividing cells or cells in the proliferating and resting phases (Barbour and Engemann, 2015). Most antimetabolites based drugs enter the cells kinetics by killing proliferating cells during a specific part or parts of the cells cycle or enters the s-phase by inhibiting DNA synthesis or the M-phase by inhibiting the formation and alignment of chromosomes (Barbour and Engemann, 2015). However, as typical of cytotoxic drugs, they exert their killing spree irrespective of cell cycle stages (proliferating or resting) and without recourse to tumor and normal cells (Barbour and Engemann, 2015). Antimetabolites exert similar chemistry to naturally occurring metabolites within the body system, but rather than taking part in the essential metabolic activity, chooses a different pathway by disrupting the normal essential metabolic activities in a toxic way (Hertz and Rae, 2015).

There are basically classified into three analogues form; antifolate (proguanil, pyrimethamine and trimethoprim), pyrimidine and purin analogues. The antifolate analogues work as an inhibitor of dihydrofolate reductase, a co-enzyme that catalyzes the conversion of folic acid (FA) to tetrahydrofolate (THF). Deficiency in THF impaired the normal activities of the folate coenzymes thereby disrupting both deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) synthesis (Hertz and Rae, 2015). 5-fluorouracil (5-FU) are popular pyrimidine derivatives and in their converted 5-fluoro-2′-deoxyuridine-5′-phosphate form readily bind to thymidylate synthases (TS) an essential coenzyme needed for DNA/RNA replication and in the process inhibit their synthesis (Hertz and Rae, 2015; Mišković *et al*., 2013). While the purine analogue works by inhibiting nucleotide biosynthesis by direct incorporation into DNA (Hertz and Rae, 2015; Mišković *et al*., 2013).

Up to date, cancer therapy using antineoplastic drugs assumed a quantitative success rather than qualitative due to its non-specific killing spree on both normal and cancerous cells (Mišković *et al*., 2013). From the pharmacokinetics study reported by some researchers (Chen and Li, 2006; Duan *et al*., 2012; Guimarães *et al*., 2015; Ma *et al*., 2014), the administration of free anti-cancer drugs lacks the inherent ability to selectively target only the cancer cells and grossly exerts strong side effects on healthy tissues. In addition, free cytotoxic drugs suffer premature clearance from circulation owing to their low molecular weight and precipitate readily in aqueous media. Furthermore, as a results of possible degradation *in vivo*, patients are usually subjected to schedule administrations to meets the required therapeutic dosage which more or less could induces drug resistance (Longley *et al.*, 2003).

To deal with the aforementioned limitations in the use of cytotoxic drugs for cancer therapy, scientist over the years proactively combine the basic fundamental properties of nanomaterials and biomolecules with the existing anti-cancer drug to conveniently administer chemotherapeutics with certain degree of efficiency (Bharali and Mousa, 2010; Dhankhar *et al*., 2010; Wang and Thanou, 2010). The incorporation of

engineered nanomaterials in the management of cancer has significantly extended research in clinical oncology as most formulation demonstrated sufficient solubility in aqueous media, encouragingly reduces the associated adverse cytotoxic effects of the free anti-cancer drugs and interestingly allows systemic drug release and accumulation to be achieved (Guimarães *et al*., 2015; Kim *et al*., 2010; Ma *et al*., 2014; Wang and Thanou, 2010; Yuan *et al.*, 2010). For example, the administration of free 5-FU induces some associated side effects ranging from neural to hematological and gastrointestinal disorders. Other side effects reported are myelosuppression and dermatological adverse side effects (Longley *et al*., 2003), but of greater concern is the development of resistance by the tumor cells toward 5-FU showing only ~10% response rate for the treatment of colorectal cancer with slight improvement to about ~45% response rate in combination form with other anti-cancer drugs (Arias, 2008). And similarly, report indicated that prolonged exposure to 5-FU induces thymidine synthase overexpression, that ends up inducing 5-FU resistance in cancer cells; thus limiting its application in cancer treatment (Vinod *et al*., 2013). Therefore, one-way to overcome the associated tumor-resistance to 5-FU is by exploring targeted delivery approaches. Engineered nanoparticles (NPs) with selective targeting characteristics are reported to be an effective approach towards improving the anti-cancer properties and selective bioavailability of 5-FU (Blanco *et al*., 2011; Ma *et al*., 2014; Ngernyuang *et al*., 2016).

Though most of the nano-based cancer therapeutics for example Doxil™ and Abraxan™ approved by FDA might have reduced some of the toxicity concerns commonly associated with free anti-cancer drug, the critical questions that requires answer are meeting the specific and selective targeted drug delivery to tumor cells exclusively with limited side effects to healthy cells. For that, the choice for targeted delivery approaches using engineered nanomaterials become inevitable. Therefore, engineered nanomaterials consisting of nature derived materials come handy in these noble efforts. The use of nature derived polymeric NPs such as chitosan (CS) has made a considerable debut as a material of choice in the formulation of theranostics nanocomposites. The interest in the use of CS as a pharmaceutical excipient is not limited to the positive appealing properties such as biocompatibility, biodegradability and muco-adhesiveness; but also because of its ability to be fully involved in the entire therapeutic chemistry. This unique signature serves as a good transportation system, protecting encapsulated molecules from physiological matrix species, thus allowing control delivery to be achieved *in vivo* with well-regulated systemic toxicity.

It's a clinical fact that, most of the current anti-cancer drugs rarely dissolve in aqueous solution, readily suffers premature clearance and hence making it nearly impossible to achieve the required therapeutic dosage. And of greater concerns, they can easily trigger cytotoxic effects that could further spur more cellular damage (Bharali and Mousa, 2010; Walko and McLeod, 2009). Thus, the formulation of nontoxic, water soluble, biocompatible and a highly specific targeted drug delivery probe remains an essential crux for most research work. Talking of targeted delivery, ligands such as folic acid, antibody, peptides, protein, aptamer, etc., are reported to demonstrate an active role in specific targeting of cancer cells (Garcia-Bennett *et al.*, 2011). This specific targeting ability is exerted through the molecular-cellular interactions between the ligands and some specific receptors that are uniquely expressed in large

number on the surface of the tumor cells compared to normal cell lines (Basal *et al*., 2009; Garcia-Bennett *et al.*, 2011). These ligand-receptors interactions provides researchers with an acceptable blue-print toward engineering a targeted drug delivery probe mediated by cell receptor pathways (Deng *et al*., 2009; McGuire, 2003; Zhang *et al*., 2011). Functionalization of therapeutics with strong ligands-cell receptors chemistry conjugated unto a biodegradable polymer-based system could support specific targeting of cancer cells with sufficient systemic drugs release and acceptable cargo residence time (Bansal *et al*., 2011).

In an effort to formulate such an integrated nanotherapeutic system which can diagnose and deliver targeted therapy simultaneously, researchers over the years brought into a single construct, fluorescent emitting contrast agents, a receptor targeting ligands and an anti-cancer drug all stabilized within a biopolymer to suit both diagnostic and therapeutic functions simultaneously (Choi *et al*., 2012). For that, engineered quantum dots (QDs) based system have being reported to serve as an efficient fluorescence actors towards non-invasive imaging and assessment of drug bioavailability *in vitro* (Bharali and Mousa, 2010; Mathew *et al*., 2010). Quantum dots possessed excellent characteristics owing to its high degree of intense luminescence efficiencies at room temperature, resistance to photo-bleaching, broad excitation and narrow emission bands. These aforementioned properties makes QDs a unique contrast agent for optical imaging applications (Costa-Fernández *et al*., 2006; Labiadh *et al*., 2013). These intrinsic properties have opened a new chapter for advance molecular and cellular imaging of diseases. A cited example shows that QDs homed to tumor sites either through passive or guided by active targeting ligands allows real time imaging and tracking of receptors molecule on the surface of living cells with improved sensitivity and resolution. (Gao *et al*., 2004). Targeted imaging using QDs based system were successfully achieved *in vitro* in combination with a specific tumor receptor ligand (Bhattacharya *et al*., 2011; Bhattacharya *et al*., 2007; Chen *et al*., 2013; Gaspar *et al*., 2015), and similarly allowed *in vitro* delivery of therapeutic agents stabilized within a biopolymer materials (Aswathy *et al*., 2012; Mathew *et al*., 2010). Thus, the choice of a NPs with the above-mentioned characteristics offers a great advantage in the formulation of cancer theranostics system which includes among many: (i) To attenuate the toxic side effects of the free anti-cancer drugs; (ii) To facilitate tumor targeting efficacy following ligands-receptor binding; (iii) To aid fluorescence imaging of the cancer cells; (iv) and finally, to support prolong and systemic drug release and bioavailability. Therefore, suffice to say that, targeted drug delivery using nano-based formulations not only will provides remedy for the conventional invasive surgery and radiation therapy, it might also revolutionize early detection and prognosis of variety of cancer. Thus, the concept behind this project was deduced by applying a chemistry which involves the loading of anti-cancer drugs into encapsulated CS-quantum dots system with FA-conjugation as schematically represented in Fig-1.1

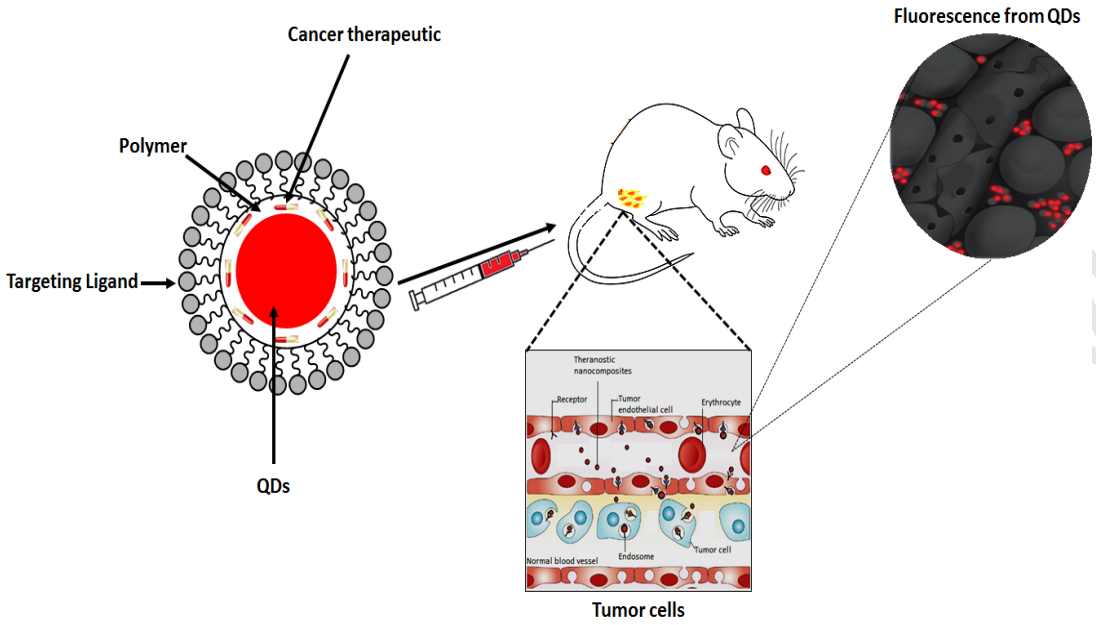

Figure 1.1 : Schematics describing therapy and imaging using targeted cancer-based theranostics NPs

1.3 Problem Statement

The effective use of QDs-based systems for cancer imaging and drug delivery applications remain a subject of concern due to its non-specific cytotoxicity especially in *in vivo* environment. Available data on ZnS QDs toxicity relates largely to *in vitro* settings and widely limited to MTT related assays with some efforts related to its imaging potentials (Aswathy *et al*., 2012; Geszke *et al*., 2011; Malgorzata Geszke-Moritz *et al*., 2013; Mathew *et al*., 2010). Exploration of QDs-based systems in targeted drug delivery applications in addition to its imaging potentials remains attractive; which draws the attention for further *in vitro* study in addition to *in vivo* toxicity evaluation of the QDs-based systems.

Based on this account, this work will utilize low-toxic ZnS quantum dots doped with Mn(2+). In this work, post treatment of the QDs under microwave irradiation will be carried out to improve the dispersity of the colloidal suspension. The direct in-core homogenous temperature gradient is expected to lead to the formation of smaller particles of uniform size and shape. The synthesized QDs will be stabilize using biodegradable chitosan biopolymer system to improve it biocompatibility and attenuate the none-specific cytotoxic effects of the anticancer drugs. The system will be incorporated with FA, a specific folate receptor targeting ligands, widely upregulated in the body and highly needed for DNA synthesis for selective targeting of cancer cells. In this work, rather than the usual EDC-based chemistry (Geszke *et al*., 2011; Mathew *et al*., 2010), anchorage of the FA to the CS will be initiated through electrostatic interactions between the carboxylate groups on FA to the amine group of chitosan. This is to minimized any possible structural changes that may affect the essential ingredients following the preparation (Bhattacharya *et al*., 2007; Castillo *et*

al., 2013; Nakayama *et al*., 2007). The strategy will be utilized to selectively deliver a highly cytotoxic anticancer (5-FU) drugs to cancer cells. Is therefore hypothesizes that, the strategy will render the 5-FU anti-cancer drugs less toxic, improve its plasma half-life and bioavailability and same time stabilize the colloidal QDs contrast agents toward *in vitro* and *in vivo* targeted delivery and imaging applications.

The protocols for the preparation of the 5-FU@FACS-Mn:ZnS nanocomposite is developed with the view to ensure sufficient fluorescence characteristic from the QDs, to also ensure the FA and the anti-cancer drugs retain their characteristic bioactive properties. Therefore, a step-wise synthetic route will be employ in this work. The sequential step-wise methods are to ensure the nanocomposite retains significant part of their properties following each modification. For that, wet chemistry method and room temperature synthesis following electrostatic interaction and non-covalent loading route will be consider throughout the experiment.

The biological safety indices of the as-prepared 5-FU@FACS-Mn:ZnS nanocomposite will be evaluated on normal (MCF-1-A) and cancer breast cell (MCF-7 and MDA-MB231) lines using *in vitro* cell viability assay and apoptosis study. Further *in vivo* sub-chronic toxicity evaluation will be conducted using animal models. The *in vivo* toxicity study will include the activity of both liver and kidney function enzymes, evaluate the level of proinflammatory agent (NO and MDA) in addition to evaluating the level of QDs bioaccumulation in some major organs base on Zn^{2+} ion distribution. Furthermore, the histology of the harvested organs will be evaluated to ascertain possible toxic induce effect.

Finally, the fully characterized 5-FU@FACS-Mn:ZnS nanocomposite will be tested for its anti-cancer efficacy and anti-metastasis effects base on the activity of 4T1 cancer cells inoculated into female Balb/c mice.

While mindful of the toxicity of QDs and its clearance/excretion from the body system, this work is however limited to studying the biodistribution profile and targeting selectivity of the 5-FU@FACS-Mn:ZnS nanocomposite in relation to both *in vivo* toxicity, anti-cancer efficacy and *in vivo* anti-metastasis effects. Detail study into the QDS biodistribution kinetic and clearance/excretion pattern is not covered in this study. Which often require extended period and large number of text subjects with the attending regulations in animal use and utilization act. However, this does not limit the significance and practicability of the scope of this study towards understanding QDs-based system for biomedical application.

1.3.1 Objectives of the Study

I. To synthesize and characterize manganese-doped zinc sulphide (Mn:ZnS) QDs based system embedded in chitosan biopolymer with folic acid conjugation (FACS) following three sequential wet chemistry methods

II. To assess the cyto-biocompatibility of the FACS-Mn:ZnS nanocomposite and to investigate the *in vitro* fluorescent imaging in normal and breast cancer cell lines

III. To study the loading of 5-Fluororaucil (5-FU) on biocompatible FACS-Mn:ZnS nanocomposite and evaluate its control release characteristics

IV. To investigate the suitability and biocompatibility of the 5-FU@FACS-Mn:ZnS nanocomposite *in vitro* and in female Balb/c mice *in vivo*.

V. To investigate the anti-tumor efficacy and folate receptor targeting efficiency of the as-synthesized 5-FU@FACS-Mn:ZnS nanocomposite *in vitro* and in 4T1 induced mice *in vivo*

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