# Chapter 1

# Introduction

## 1.1 BACKGROUND AND LITERATURE REVIEW

The efficacy of a medicine depends on how effectively its pharmacologically active ingredient can be employed to combat or manage a disease. It has been observed, that the efficacy of these ingredients is very much depended on their modes of administration. This is because the mode of administration affects various crucial factors like absorption, distribution, pharmacokinetics, metabolism, toxicity, excretion and the timing through which therapeutic effect is sustained[1]. These revelations have led to the conclusion that improvising the modes of drug delivery using rationally designed delivery systems is as important as the development of new drug entities.

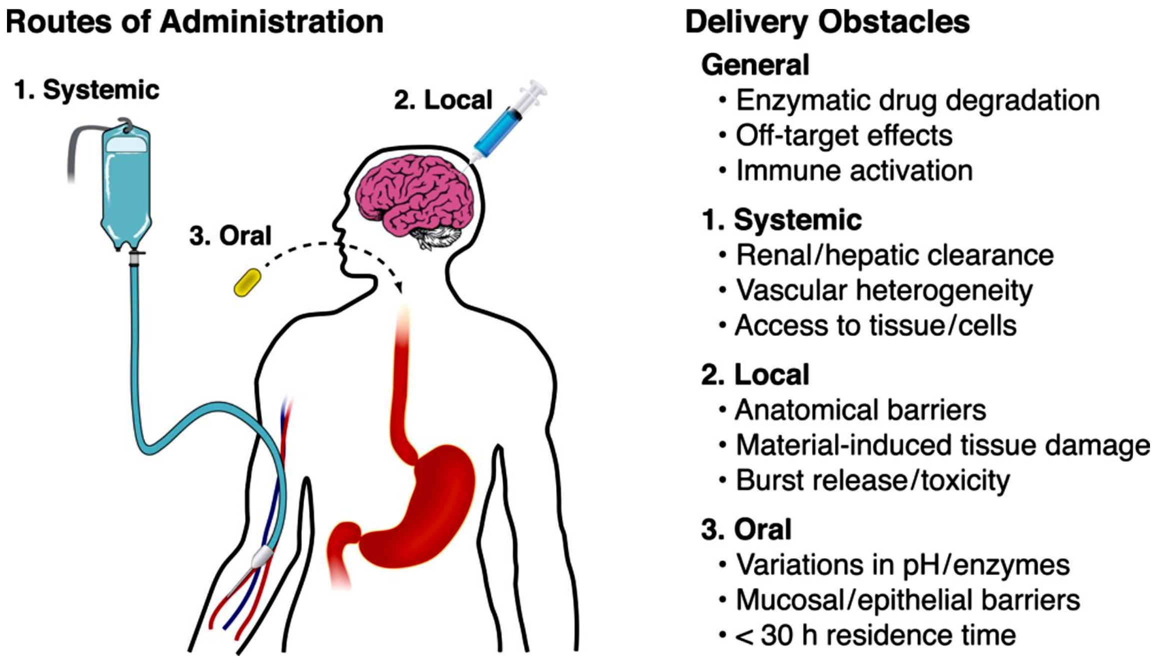

**Figure 1.1 Schematic showing difficulties of delivery. This suggests the designing criteria will vary with administration route. For instance, in case of systemic delivery care should be taken to avoid drug clearance by the reticulo-endothelial system and should be able to enter the desired tissue. (Adapted with permission from Langer et. al., *J. Am. Chem. Soc.* 2016)**[1]

Conventionally speaking drug delivery systems are associated with administration of drug at the site of malady in appropriate concentration to exert the desired therapeutic effect. However easier said than done, this process is much more complicated as bottleneck lies in the prediction of release rates of drug as well as their stability and targeting the specific cells/tissues.

Overcoming these limitations has been the focus of the researchers working in this field by devising various strategies to produce efficacious drug delivery systems (DDS). The DDS are now synthesized in such a way that the formulations are stable in-vivo and the drug release is made more effective by controlling the release thus localizing their effects[2]. The DDS are designed to suit not only the physicochemical features of the drug but also to intended administration mode (as shown in **figure 1.1**)[1].

### 1.1.1 DRUG DELIVERY SYSTEMS AND CANCER THERAPY

Amongst various diseases that need interventions via employing a drug delivery system for its management, cancer holds a specific priority. This is due to a constant surge in cancer cases globally despite availability of various facets for its management[3]. Although there has been a drop in the cancer death rate over the past 2 decades but statistics also indicate that deaths caused by lung cancer alone were above 20 % in the year 2018. Moreover the other form of cancers like prostate, breast, colon and rectum cancers have caused deaths that cannot be ignored [4]. The main stray in an effective and consistent therapy can be attributed to the following characteristics of the disease[5]:

1. A prompt cellular proliferation.
2. Various mechanisms triggering its malignancy viz protein dysfunction or alteration of gene expression.

In the light of this fact, drug delivery systems can be efficacious over conventional treatment strategies as they can be designed to cause the reversal of the disease by employing tumor specific mechanisms. The cancer management can further be catalyzed by employing systems for a preclinical monitoring of the disease along with clinical intervention. For the design of such systems it becomes very crucial to understand the tumor microenvironment.

### 1.1.2UNDERSTANDING THE TUMOR MICROENVIRONMENT

The tumor microenvironment (TME) is basically a complex tissue that is made up of several cell types. These cell types are usually vascular endothelial cells, fibroblasts associated with cancer, cancer stem cells (CSCs), immune cells and pericytes. They also include non-cellular components like the extracellular matrix (ECM) and some extra-cellular secreted molecules. The components of TME have been shown by schematic representation in **figure 1.2**. As per the theory of seed and soil proposed by Stephen Paget, tumors are known to undergo metastasis to another site in the body though an environment similar to the original tumor[6]. Thus it is crucial to understand the nature and composition of TME to help devise appropriate therapeutic strategy for effective tumor regression.

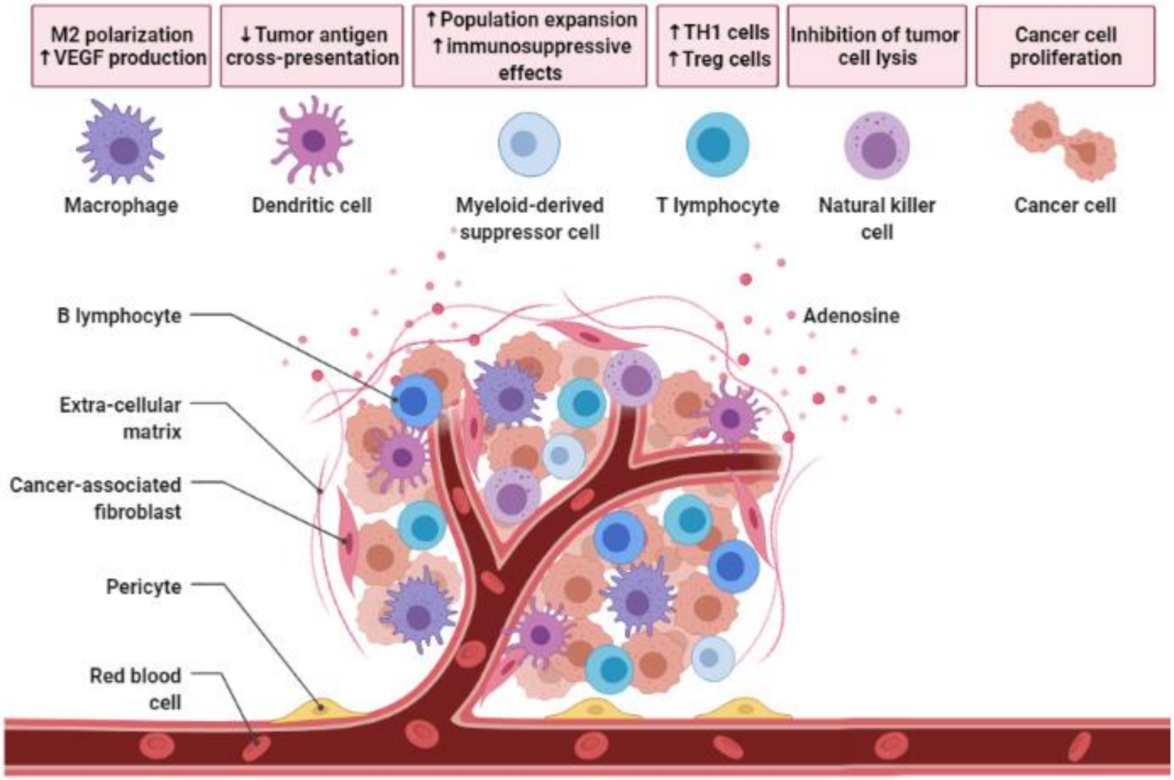

**Figure 1.2: Schematic representation of the tumor microenvironment (TME) (Created using Biorender)**

**Table 1.1: Summary of the constituents present in tumor microenvironment**

| Component | Characteristics | Significance/Indications in cancer progression | Difference as compared to normal cells |
|---|---|---|---|
| **Tumor Endothelial Cells**[7] | Hierarchical branching pattern of blood vessels is not observed making the tumor area unorganized. | Hemorrhage and plasma leakage from leaky vasculature.<br>Restriction of oxygen supply of oxygen to cancer cells inducing hypoxia | Does not form regular monolayers.<br>Possess irregular shapes and sizes. |
| **Cancer Associated Fibroblasts**[8] | It is the major source of cells producing collagen | Promotes the immunosuppressive environment.<br>Imparts drug resistance to cancer cells | They are active and can proliferate robustly. |
| **Cancer Stem Cells**[9] | Possess great potential of cell division and thus can repopulate the tumor area | Recurrence of tumors at original site or some other area post successful treatment | - |
| **Tumor Associated Immune Cells**[10] | They are immune cells that infiltrate the tumor area. Also termed tumor-infiltrating lymphocytes (TIL)<br>T cells<br>B cells | Suppresses the immune response and promotes immune escape of tumor | - |

| | | | |
|---|---|---|---|
| | Natural Killer (NK) cells. | | |
| **Extracellular Matrix**[11] | Acts as structural and biochemical support for cancer cells | Enhances tumor progression by angiogenesis.<br>Resists cell death<br>Activates invasion<br>Causes metastasis | Alteration in the basic composition<br>Post translation modification of ECM proteins |
| **Pericytes**[12] | Cells embedded within the vascular basement membrane | **Low coverage of pericytes**: Associated with triggering metastasis<br>**High Coverage of pericytes**: Indicates presence of aggressive cancer form | - |

The detailed insight of the structural constitution of TME (as shown in **table 1.1)** leads to the conclusion that various properties can be exploited to devise therapeutic strategies for effective cancer therapy. For instance the vascular irregularities can allow the passage of nanoparticles. The major loophole of in sufficient drug delivery at the location of tumor can thus be overcome by application of nanoparticles[13]. This can be achieved in the following ways:

1. Passive targeting of the nanosized drug carriers by the virtue of enhanced permeability and retention effect[14].
2. Active targeting by conjugating/encapsulating targeting agents with nanocarriers[15].
3. Utilization of stimuli responsive nanomaterials for modulation of TME's pathophysiology via its unique properties viz. leaky blood vessels, acidosis, hypoxia etc[16].

### 1.1.3 NANOPARTICLES AND CANCER THERAPY

The revelation of the fact that, nanoparticles have the ability to penetrate the leaky vasculature had led to the exploration of the employment of nanotechnology for drug delivery applications[17]. Nanotechnology has played an essential part in this field as a result of its reach in resolving the following mentioned pressing issues[17]:

1. **An improvement in the delivery of drug having poor water-solubility and thus poor bioavailability.**
2. **The potential of delivering drugs to specifically to cancerous cells/organs in a targeted fashion.**
3. **The transport of drugs across tight epithelial and endothelial barriers via transcytosis.**
4. **The delivery of macromolecular drugs to site of action at the cellular level.**
5. **The ability of combinatorial therapy via co-delivery of more than one drug or therapeutic modalities.**
6. **The functionalization of delivery system with probes capable of imaging allowing visualization of drug delivery site.**
7. **A real-time readability of the therapeutic efficacy *in vivo.***

These features make the nanocarriers promising candidates for both diagnostic and therapeutic interventions especially for disease like cancer. Various nanomaterials with varying compositions, shapes, sizes, and functionalities have been developed and shown to have application as therapeutic agents and theranostic[18–20].

The advancements in nanotechnology have helped in the evolution of various strategies for the formulation of nanocarriers for delivery of cancer therapeutics. Nanocarrier formulations have been known to debottleneck various hurdles of conventional cancer therapy viz improvement of drug solubility and its bioavailability, administering a sustained release, targeting the drugs specifically to the tumor location. The nanocarriers can occur in a plethora of formulation type right from bare drug nanocrystals[21–23] to complex multi-functional delivery vehicles[24–26]. Various parameters that are of importance while designing nanocarriers for drug delivery include its morphology, size, type of functionalization, biocompatibility, toxicity, and biodegradability.

Various metallic, inorganic, organic as well as polymeric nanostructures (dendrimers, micelles, and liposomes) have been reported for designing drug carriers[27–29]. The efficiency of the developed nanostructures as drug carriers depends on their size, shape, and various intrinsic biophysical/chemical characteristics. For example, polymeric nanomaterials having diameters in the range of 10-1000 nm are efficient for drug delivery. The fabrication and functionalization of nanocarrier has been done using various natural and synthetic polymers. With the prospect of attaining a highly biocompatible and biodegradable system the most commonly used polymers include synthetic ones like poly-L-lactic acid[30], polyvinyl alcohol, polyethylene glycol, poly(lactic-*co*-glycolic acid), polycaprolactone etc. and various naturally occurring polymers like polysaccharides[31–33]. Such coating or polymeric functionalization provides immunogenicity to the nanocarriers. Nanocarriers can be designed to have a definite release profile for enhanced specificity at the target area. Targeting molecules having affinity for receptors present on the cancer cells can be conjugated onto the surface of nanocarriers taking into account the physiological variables of blood flow and architecture of the target tissue to overcome the barriers for effective delivery.

Polymeric nanoassemblies have also emerged as a potential candidate for biopharmaceutical applications in the recent past. The nanoassemblies/nanoarchitectures are devised from polymers that have the ability to self-assemble due the presence of both hydrophilic and hydrophobic components in the polymer constitution. Such amphiphilic polymers can attain various morphological forms like micelles, rods, worms, cylindrical capsules and vesicular aggregates Such architectures are can have various biomedical applications as carriers for therapeutic and imaging moieties for achieving a local therapeutic effect. The size as well as shape of nanoassemblies has been found to exert an effect on cellular internalization. The prime reason for the rising popularity of nanoassemblies over smaller molecules for drug delivery applications is its long-term stability and superior drug loading capacities. The nanoassemblies can also be rendered stimuli responsiveness as well by designing the amphiphilic polymer via polymerization of suitable monomers.

Recently nanoassemblies have also been used to derive polymeric prodrugs. In this strategy, amphiphilic polymers are designed in such a way that hydrophobic drug is employed as a component of the polymer. The drug is bonded via responses of tumor microenvironment so that its targeted release can be achieved. For instance, Huynh et. al., developed a block copolymer for the release of cisplatin in response to the presence of sodium chloride[34]. Further Shen and co-workers synthesized an esterase-sensitive prodrug of camptothecin using oligoethylene glycol derived micelles[35]. Singh and co-workers have designed DOX prodrug by grafting it to a macromolecular carrier using acid sensitive hydrazone bond[36].

With the advances in therapeutic regimen for cancer therapeutics has improvised the progression of disease intervention from an approach of "one drug for all" to "personalized medication"[37]. This evolution has allowed the researchers to design nanosystems that consider addressing multiple deleterious effects using a single system. For cancer the multiple anomalies considered and exploited to cause better tumor regression are ascribed a) ensuring a scarcity of nutrients is created for cancerous cells and there is a sustained supply of anticancer drugs (b) preventing the development of multidrug-resistant proteins (c) prevention of cellular and (d) suppressing the enhanced expression of various genes causing the up regulation of angiogenesis, tumor invasion and metastases. Stimuli responsive smart polymers have become increasingly important for designing such systems as they are known to provide greater control over drug dosage as well as targeting.

### 1.1.4 STIMULI RESPONSIVE MATERIALS FOR CANCER THERAPY

The term *stimulus* physiologically refers to an internal or external change in environment that is detectable[38]. The living organism/organ possesses an intrinsic ability to respond to external stimulus. This ability is crucial as it aids in sustaining the basic body functioning. This concept can be extrapolated for designing drug carriers due the matter of fact that components of body are made up of macromolecules which aid in controlling the natural processes.

Stimuli responsive drug carriers are those "smart materials" that can respond to the external changes in environment. The carriers can be made sensitive to various stimuli such as pH, light, temperature, magnetic field, enzymes, redox potentials etc (as shown **in figure 1.3**).

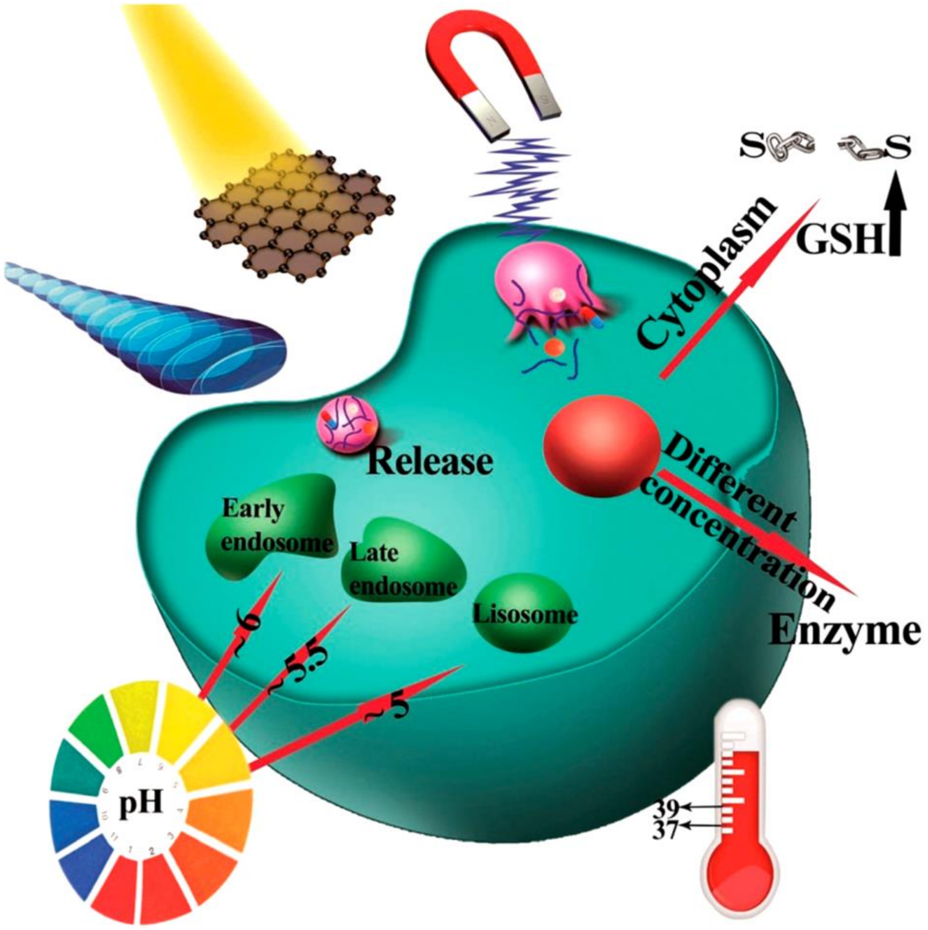

**Figure 1.3: Various stimuli that can be exploited for triggering smart nanocarriers to exhibit a specific and controlled delivery of drugs for cancer therapy. (Adapted with permission from Hamblin et. al., *Chem. Soc. Rev.*, 2016)**[39]

Such systems can be developed by various strategies, for instance by combination of biocompatible biomaterials with appropriately functionalized materials which can self-assemble or form supramolecular complexes. The carrier thus produced is able to transport therapeutic molecules to specific tissues depending on the abnormal physiological conditions that they demonstrate.

This strategy can be employed for a targeted and sustained drug release to improve the specificity of drug delivery systems. Stimuli responsive drug nanocarriers have an upper hand as compared to non-responsive ones as they specifically release drug in the target tissues and exhibit a release profile only upon encounter to physiological events associated to the diseased environment[40]. This approach has been found to have better efficacy for cancer therapy as the treatment of cancer is depended both on the dosage of drug and the release of drug selectively to the tumor site.

The stimuli responsive carriers have to be rationally synthesized in such a way that it can release the loaded drug either by being able to differentiate physiologically between healthy tissues and tumor on the application of an external localized stimulus like light or magnet on at site of malignancy. Various categories of stimuli responsive nanocarriers that have been utilized for cancer therapy include drug nanoconjugates[41,42], liposomes[43], polymersomes[44], micelles[45], liquid crystals[46], niosomes[47] and dendrimers[48]. Different stimuli responsive systems have been discussed below.

### 1.1.4.1 pH RESPONSIVE NANOCARRIERS

The most important feature of pH responsive carriers is that they can exploit pH differences inside the human body. For instance, tumor tissues have hypoxic environment due to "Warburg effect" and hence exhibit low values[49] The distinct regions of cells have varying pH values for instance the lysosomes possess pH 4-5, whereas extracellular region has pH values are in the range of 5.8-7.2. Further the intracellular components have still lower pH values with and endosomes demonstrating pH values 5-6. On the other hand, normal tissues and blood plasma possess pH around 7.4[50]. Further various pH environments are observed even in the gastrointestinal tract; stomach shows a highly acidic environment with a pH of 1.3, pH changes to 4.8-8.2 in small intestine and colon has a pH of 7-8. Such diversities in pH values can be exploited for stimuli responsive drug release which in turn will improve the efficacy of drugs. pH responsive polymers are generally weak bases or weak acids. Charged structures are formed upon protonation or deprotonation from neutral structures which affects the polarity and hence the solubility. They have acidic or basic groups as pendants capable of accepting or donating protons while responding to the pH of the surrounding enviro-----ent and based on this they may be cationic or anionic[4]. Certain polyacids like poly(acrylic acid), poly(methacrylate acid) are well known anionic polymers[51]. On the other hand polymers containing ionizable amine groups in their structure act as cationic ones[52]. pH responsiveness can also be introduced by utilization of acid labile linkages like acetal, ketal, hydrazone etc in the polymer backbone that are easily hydrolysable[39].

### 1.1.4.2 THERMORESPONSIVE NANOCARRIERS

This is one of the most important responses exploited for cancer therapy for the matter of fact that tumors exhibit elevated temperature of 40-42 °C owing to their rapid proliferation and high rate of aerobic glycolysis[53]. Thermoresponsive carriers are developed by tuning the system in such a way that a conformational change is induced in the structure and morphology of the polymers at a transition temperature. In general, the behavior of polymer in solution state can be expressed as a function of concentration and temperature[54]. Two types of behavior are observed: UCST and LCST. In case of UCST cooling causes the separation between single phase and 2 phase region. LCST describes the state wherein transitions between single phase and 2 phase region are observed upon heating. It so happens that the polymer swells below LCST and contracts above this temperature. LCST behavior has been exploited for cancer therapy as they are pertinent to controlled drug release. Various polymers following LCST phenomenon for drug release have been mentioned in **table 3**. Below the LCST the polymer has the ability to form hydrogen bonds with surrounding water molecules which leads to hydration. When the temperature is raised above LCST the water molecules are forced out of the polymer structure into the bulk as it is more favorable. This results in the partial degradation and agglomeration of the polymer chains which also results in conformational changes in the polymer structure. Such transformations are called coil to globule transformations; which can be used for encapsulation or release of drugs as required. Poly(N-isopropylacrylamide) used as thermosensitive hydrogels are exhibits a on demand drug release with specific ON/OFF mechanism. The “ON” condition is exhibited at low temperature and “OFF” condition at high temperature which enables the drug release. Various other thermoresponsive polymers are Poly(N-isopropylmethacrylamide), Poly(N-vinylcaprolactam) and Poly(N,N-diethylacrylamide)[55].

### 1.1.4.3 PHOTORESPONSIVE NANOCARRIERS

Photoresponsivity can be used to trigger effect at the desire place with the help of external factors like laser beam. The light irradiation stimulates drug release from a photoresponsive carrier. This response had gained a particular interest of researchers due to its non-invasive nature which is coupled by several other advantages like the precision with which intensity of light can be tuned, the control over duration of light exposure and appropriate beam parameter over the tumor site. Both ultraviolet radiation having wavelength in range of 10–400 nm and visible or near infrared (IR) regions with wavelength in range of 650–900 nm in the light spectrum can trigger release of drug from rationally designed nanocarriers[56]. However UV irradiations are associated with disadvantages of cytotoxicity and poor tissue penetration. The NIR source of irradiation, on the other hand have deeper tissue penetration and is non-toxic. NIR has been employed in a unique form of therapy known as photothermal therapy, wherein the light absorbed by a nanomaterial is converted to heat and causes a localized effect to kill cancer cells. The collective oscillation of free electrons in the conduction band causes light to scatter by localized surface plasmon resonance thus inducing decremented effect on the cancer cells. Low power densities of 0.20 W $cm^2$ have been known to cause a photothermal initiated drug release from nanomaterials like Ag/Au nanoparticles[57], graphene oxide and carbon nanotubes. These irradiations are sufficient to selectively harm the tumor but are safe to normal tissues.

### 1.1.4.4 REDOX RESPONSIVE NANOCARRIERS

Various microenvironments in organisms have varying redox potentials for instance inflammed tissues and healthy tissues possess different redox potentials. Therefore oxidatively degradable arylboronic acid ester based polymers or dialkyl sulfide based polymers find applications in drug delivery to inflammed tissues[58]. Arylboronic acid ester derived polymers undergo oxidative degradation to form phenols and boric acid[59]; whereas dialkylsulphide derived polymers are oxidized to more hydrophilic sulfoxide and sulfones species[60]. Reductively degradable sulfide systems are used particularly due to hypoxic environment of solid tumor.

### 1.1.4.5 ENZYME RESPONSIVE NANOCARRIERS

The highly selective nature of enzymes in catalyzing certain transformations has made enzyme responsiveness an intriguing feature of designing nanomedicine. Certain enzymes are overexpressed in specific tissues with a spike in its concentration during diseased condition which can be exploited to design polymers or nanosystems for a triggered drug release[61].

Enzyme responsive carriers can be designed via two mechanisms of action physical or chemical.

In case of physical action mode, the enzyme responsive carriers undergo a change in its macro-scale structure causing a controlled drug release. However in the chemical action mode, the responsive carriers undergo degradation when exposed to the enzyme causing a drug release. Most of the nanocarriers designed for enzyme responsive drug deliveries are sensitive to oxidoreductases[62] or hydrolases[63].

Some of the stimuli responsive nanocarriers employed for cancer therapy have been summarized in **Table 1.2**.

**Table 1.2: Various stimuli responsive nanocarriers employed for cancer therapy**

| STIMULUS | NANOCARRIER | DRUG | REF |
|---|---|---|---|
| **SOLITARY RESPONSIVE** | | | |
| **pH** | Mesoporous silica- calcium phosphate hybrid nanocarrier | DOX | 64 |
| | Quinoline modified chitosan nanoparticles | Quercetin | 65 |
| | D-mannose methacrylate glycopolymer coated core-shell gold nanoparticles | DOX | 66 |
| | Poly (ethylene glycol)-b-poly (D, L-lactide) and poly (ethylene glycol)-b-poly (L-histidine) block copolymers forming mixed micelles | Paclitaxel | 67 |
| | β-cyclodextrin-poly(lactic acid)-b-poly[(oligo ethylene glycol) methyl ether methacrylates] micelles | DOX | 68 |
| | Glycerol monooleate lipid nanoassemblies | 2-hydroxyoleic acid | 69 |
| **Temperature** | Pluronic F127- chitosan co-polymeric nanoparticles | Curcumin | 70 |
| | Poly(acrylamide-co-acetonitrile)-g-polyethyleneglycol derived micelles | DOX | 71 |
| | Poly(N-isopropylacrylamide) grafted chitosan | Curcumin | 72 |
| | Poly(N-isopropylacrylamide)-Polyethylene gycol derived nanoparticles | Paclitaxel | 73 |
| | poly(N-vinyl caprolactam) microgels | DOX | 74 |

| | | | |
|---|---|---|---|
| | Iron oxide core and a thermoresponsive copolymer shell composed of 2-(2-methoxy)ethyl methacrylate and oligo(ethylene glycol)methacrylate | DOX | 75 |
| **STIMULUS** | **NANOCARRIER** | **DRUG** | **REF** |
| **Light** | Se inserted co-polymer of PEG encapsulated with indocyanine green | DOX | 76 |
| | Hollow mesoporous silica nanoparticles modified with spiropyran-containing light-responsive copolymer | DOX | 77 |
| | chlorin e6 loaded poly(ethylene glycol)-b-poly(ε-caprolactone) | DOX | 78 |
| **Redox Potential** | Heparosan- cystamine-vitamin E succinate micelle | DOX | 79 |
| | disulfide-linked d cysteine-phenylalanine nanoparticles | DOX | 80 |
| | Metal organic frameworks derived from GSH-sensitive organic ligand, 4,4′- dithiobisbenzoic acid (4,4′-DTBA); M(DTBA) (M = Fe, Al or Zr) | Curcumin | 81 |
| | Glycosaminoglycan amphiphiles forming micellar nanoparticles | Nile red | 82 |
| | Cysteine based small-molecule agents | Lapatinib | 83 |
| | 5-methyl-5-propargylxycarbonyl-1,3-dioxane-2-one; polycarbonate-based core-crosslinked nanoparticles | DOX | 84 |
| **Enzyme** | Rotaxane nanovalve containing mesoporous silica nanoparticles | Rhodamine B | 62 |
| | MSNs grafted with phenylboronic acid conjugated human serum albumin | DOX | 85 |
| | Phosphoramidate (PAD) dendrimers | DOX | 86 |

| | | | |
|---|---|---|---|
| | G4 PAMAM dendrimer molecules were modified via covalent conjugation of H-arg-gly-asp-D-tyr-cys-SH (RGDyC), Fmoc-arg-gly-asp-cys-SH (Fmoc-RGDC), and PEG chains on the periphery | DOX | 87 |
| | bovine serum albumin and green tea polyphenol derived nanoparticles | DOX | 88 |
| **DUAL RESPONSIVE** | | | |
| **pH and temperature** | Poly(methacrylic acid) and poly(N-isopropylacrylamide) block copolymer coated onto silica nanoparticles | DOX | 89 |
| **pH and redox** | Poly(N-isopropylacrylamide)-ss-acrylic acid (P(NIPAM-ss-AA)) nanogels (s-s: disulfide linkage | DOX | 90 |
| **Temperature and Light** | Silica and Oleic Acid -Coated rare earth oxide NaYF4: Yb3+/Tm3+ Upconversion Nanoparticles | DOX | 91 |
| **pH and Light** | Upconversion nanoparticle-loaded folate-conjugated polymeric lipid vesicles containing photosensitizer Merocyanine 540 | DOX | 92 |
| **STIMULUS** | **NANOCARRIER** | **DRUG** | **REF** |
| **Light and magnetic field** | Porous silicon nanoparticles/ iron oxide/ gold nanocomposites | DOX | 93 |
| **Enzyme and redox** | Cysteine functionalized dopamine coated mesoporous silica nanoparticles containing both peptide and disulfide bonds | DOX | 94 |
| **Redox and light** | Pheophorbide A , a hydrophobic photosensitizer conjugated *via* a redox-sensitive disulfide linkage to alginate | DOX | 95 |

### 1.1.4.6 DUAL RESPONSIVE NANOCARRIERS

The specificity and sensitivity of the carriers can be further enhanced by introducing multiple-responsiveness. This area has attracted the attention of researchers despite being a challenging work. The idea is to incorporate several responsive moieties within single system. Dual responsive systems contain individual polymer sequences of both the responses and are capable of exerting a dual effect in the single system. Introducing dual functionality also provided the advantage of the ability to control polymer phase behavior. For instance in a pH and temperature dual responsive polymer, the chemical groups that render pH responsiveness also assist the modification of hydrophilic-hydrophobic balance which is also useful for tuning the LCST of polymer. For instance pH and temperature dual sensitive capsules were designed by grafting random copolymer brushes of 2-(2-methoxyethoxy) ethyl methacrylate with oligo (ethylene glycol) methacrylate. The pH and temperature changes in the solution containing such capsules were found to affect both loading and release characteristics of the capsules. The attempt of designing materials having multiple-responsiveness is an effort towards mimicking natural body systems. Materials with multi stimuli responsive properties have used to acquire systematic release kinetics.

### 1.1.5 CARBOHYDRATES AS PRECURSORS FOR DESIGNING DRUG CARRIERS

The need for development of sustainable products as drug carriers has also been the purview of researchers developing them. This has led to the advent of developing carbohydrate derived materials. Carbohydrates are promising precursors in this regard due to their chemically well-defined structure, bioavailability, biocompatibility and bulk availability **(figure 1.4)**. Thus the past few decades have seen an uprising trend in the use of carbohydrates for instance starch, cellulose, dextran, chitosan, alginates, cyclodextrin etc. as well as their derivatives for development of drug carriers.

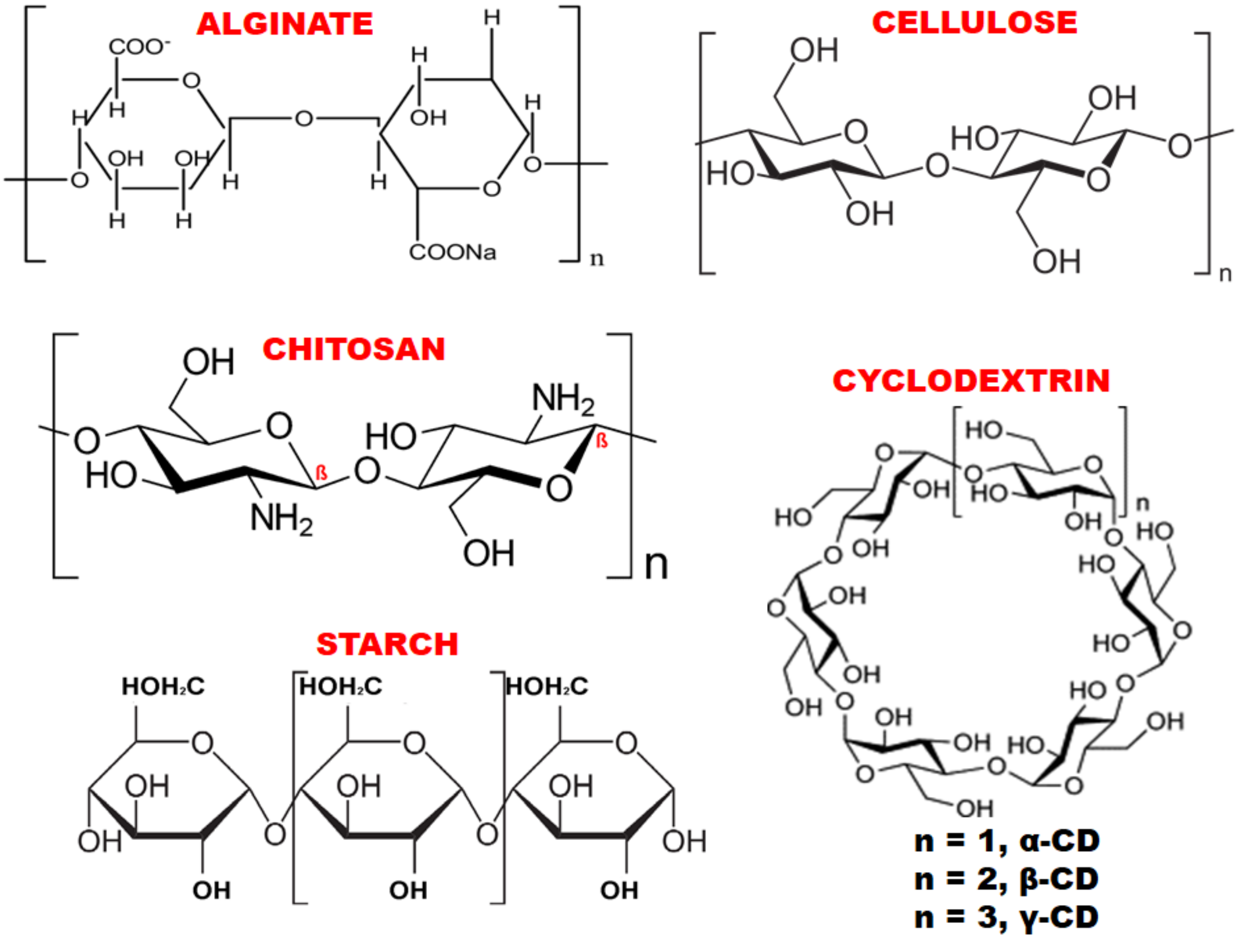

**Figure 1.4: Structures of commonly used carbohydrates for designing of drug carriers**

### 1.1.5.1 Alginate

Alginates are naturally occurring anionic polysaccharide and hydrophilic in nature. They originate from brown seaweed and bacteria. The structural composition comprises of (1–4)-linked β-D-mannuronic acid (M) and α-L-gluconic acid (G) monomers[96].

The anionic nature is pH-dependent, further it has the ability to intermingle with cationic polyelectrolytes and proteoglycans. Thus, they can be employed as delivery systems for cationic drugs and molecules due to formation of simple electrostatic interactions. They are easily modified by chemical transformations into derivatives with suitable structures and properties for drug delivery. On conjugation with other biomaterials via physical or chemical crosslinking, alginates can be tuned to obtain suitable biodegradability, mechanical and gelation property and improved cell affinity. For example alginate-gelatin composite have the capability to form biodegradable hydrogels. Some amino functionalized biomaterials like chitosan can be employed to form Schiff-base crosslinking with oxidized form of alginate[97,98].

### 1.1.5.2 Cellulose

Cellulose is a linear comprising of polymer β-(1, 4)-D-glucopyranose units acquired from the rigid cell wall of plants. The completely equatorial conformation of β-linked glucopyranose residues impart stability to the chair structure of cellulose minimizing its flexibility. Cellulose is a popular carbohydrate for designing drug carriers due to its properties like chirality, biodegradability and presence of multiple hydroxyl groups that can assist chemical modification [99]. Various derivative of cellulose being widely reported for producing controlled release drug delivery systems include carboxymethyl cellulose, hydroxypropyl cellulose, cellulose acetate and cellulose nanocrystals. It is noteworthy that nanocellulose can be easily prepared from cellulose by a facile acid hydrolysis. This process causes the removal of amorphous ingredient of cellulose while maintaining its crystalline ingredient. Surface modification of nanocellulose based nanocomposites has been found to further enhance its compatibility and dispersibility.

### 1.1.5.3 Chitosan

Chitosan (CS) is a natural polymer made of glucosamine and N-acetyl glucosamine units and obtained from de-acetylation of chitin comprising of a net cationic charge. Modification of CS, has been known to form derivatives via the interaction of its amine and hydroxyl groups. The quarternization with carboxyl alkyl, hydroxy alkyl and acyl derivatives has been known to enhance its water solubility at high pH. Various systems of chitosan based nano drug delivery systems like chitosan-alginate 3D porous scaffolds for in vitro gene targeted delivery of tumors[100],Chitosan-dextran aldehyde hydrogels [101] etc have been reported in literature.

### 1.1.5.4 Cyclodextrin

Cyclodextrin (CDs) are macrocyclic oligoglucoside containing six, seven or eight α-D-glucopyranose units connected via α-(1 → 4) linkages and are referred to as α-, β-, and γ-CDs, respectively. An enclosed tubular space allows encapsulation of poorly water soluble drugs by formation of supramolecular inclusion complexes in their hydrophobic cavity. There are various factors that make cyclodextrins best precursors as drug carriers; like protection of included drug molecules from biodegradation, availability of potential sites for modification, low pharmacological activity, low toxicity etc.

β-Cyclodextrin (CD), possess a torus shaped non-toxic cyclic oligosaccharide, with a hydrophobic cavity inside and hydrophilic functional groups on the periphery. The inclusion of the drug molecule with CD improves its bioavailability, solubility and stability. As it is multi hydroxyl compound it can form crosslinked network and the drug molecules can get entrapped in the thus formed polymer matrix. Various derivatives like methylated cyclodextrins , urethane linked cyclodextrins, hydroxypropyl cyclodextrin and epichlorohydrin β-Cyclodextrin (CD) have been known to act as precursors for synthesis of drug delivery systems[102,103,104,105].

### 1.1.5.5 Dextran

Dextran is a polysaccharide comprising of many glucose units and is synthesized from sucrose by bacterial decomposition. The glucose units present in dextran are linked by C-1 → C-6glycosidic bonds. It has multiple hydroxyl groups leading to its excellent water solubility and supporting a facile functionalization. For instance dextran aldehydes can be easily prepared by periodic acid oxidation.

### 1.1.5.6 Starch

Starch is a polysaccharide produced by photosynthesis in plants. It exists in two structural forms amylose and amylopectin[106]. Starch can be modified chemically by various methods like cross-linking, grafting, etherification or esterification to prepare derivatives that are suited for drug delivery applications.  For instance derivative like carboxymethyl starch (CMS) is an anionic form of starch that is soluble  in cold water and exhibits excellent hydrophilicity[107,108]. Alkyl ether starch like Hydroxypropyl starch (HPS) and Hydroxyethyl starch (HES) are prepared by etherification of non-ionic forms of starch[109]. HES not only have better solubility as compared to starch but it also highly resistant to hydrolysis. The degree of degradation can be tuned in starch derivatives by controlling the molar mass and degree of hydroxyethylation; such derivatives are useful for designing controlled drug delivery systems.

## 1.2 THESIS AIMS AND HYPOTHESIS

Nanotechnology derived formulations for delivering drugs have emerged as a promising strategy to tame their acute toxicity and improvise their efficacy. Several strategies have been employed in the literature for enhancing the efficacy of drug delivery by employing metallic nanoparticles, magnetic iron oxide, silica nanoparticles, carbon derived nanomaterials like carbon nanotubes and graphene. Development of multidrug resistance and lack of targeted drug delivery are major challenges in cancer management that has attracted the focus of researchers. Literature review suggests that incorporation of biomaterials as precursors and employment of simple robust synthetic processes can aid in developing potent nanocarriers for targeted drug delivery. The circulating half-lives and biodistribution of such targeting carriers depends on their size, surface charge, hydrophilicity as well as on the nature and density of the ligands on their surface. The conjugation of targeting ligands can increase the interaction of the drug delivery system with the cells in the target tissue, thus enhancing cellular uptake by receptor-mediated endocytosis. For non-targeting systems the surface properties like presence of ordered striations of functional groups, shape and size of the carriers have been known to enhance the cellular uptake

With this background, this work aims towards addressing various challenges encountered in cancer therapy viz. non targeted delivery of drugs, development of multi-drug resistance by the cancer cells, toxicity caused by the carriers and poor bioavailability of certain potent drugs. This was done by the synthesis and characterization of stimuli responsive nanocarriers (nanoparticles, nanoconjugates and nanoassemblies) for targeted delivery of a multiple chemotherapeutic drugs specifically towards cancer cells. They were also adorned with additional features for exploring various facets of cancer treatment like magnetic hyperthermia, photothermal therapy and angiogenesis. The work was executed in the following steps.

### 1. Designing surface functionalized multifunctional nanoconjugates

Nanoconjugates are tailored macrostructures harboring covalently-bound biologically active modules like drugs that target specific tissues and cells. They are generally derived by surface functionalization of magnetic nanoparticles, graphene, mesoporous silica, carbon nanotubes, carbon or gold nanoparticles. Out of these, we selected magnetic nanoparticles and carbon nanotubes as nanoplatforms and conjugated with dual stimuli polymers for loading multiple drugs.

Cyclodextrin was selected as a biocompatible macrocyclic host and was covalently conjugated with the nanoplatforms. A novel CD derived pH and temperature responsive polymer was synthesized. The nanocarriers were engineered for exploring multiple facets of cancer therapy like magnetic targeting, photothermal therapy and anti-angiogenesis. The nanocarriers were further adorned with multiple functionalities viz. fluorophore conjugation for imaging the carrier en-route and covalent conjugation of folic acid for targeted drug delivery. Dual drugs were loaded due to amphiphilic nature of cyclodextrin for cocktail of two classes of drugs for reversal of multidrug resistance.

### 2. Development of amphiphilic polymeric nanoassemblies

Due to inherent toxicity associated with nanoplatforms mentioned above the focus of our research shifted to more biocompatible carriers. Polymersomes like vesicular and micellar assemblies, inspired by liposomes are promising drug carriers since they resemble the structure of the cell membrane. Supramolecular interaction using dynamic covalent bonds can help to construct the vesicles/micelles. Self-assembled amphiphilic polymers possess a hydrophilic core and hydrophobic layer which can simultaneously load both water insoluble and soluble type of anticancer drugs for combinatorial therapy. Stimuli-responsive characteristic can be introduced in the nanoassemblies by suitable chemical modifications.

Accordingly, biocompatible, multi stimuli responsive amphiphilic polymers were synthesized by appropriate chemical modifications of dextran as a hydrophilic carbohydrate precursor. Depending on the hydrophobic precursor, the resulting amphiphile self assembles into tubular / vesicular nanoassemblies. The polymers are duly functionalized for triggered release in presence of enzymes and in tumor microenvironment. Due to amphiphilicity, the nanoassemblies can also carry both hydrophilic and hydrophobic drugs and thus employed for cocktail chemotherapy. The effect of shape on drug loading, release, cellular internalization and hence the tumor regression was also analyzed.

### 3. Developing self-therapeutic nanocarriers by pro-drug strategy

The self-therapeutic nanocarriers are pro-drug nanoassemblies formed by self-assembly of rationally synthesized block co-polymers which disassemble eventually releasing the therapeutic in its active form. For the development of this category of nanocarriers, the anticancer potential of curcumin (also a P-gp inhibitor) was utilized in a unique manner. Curcumin was converted into its prodrug form using ester linkage. Further this curcumin pro-drug was stitched into a PEG derived polymer backbone via acid labile acetal linkages and adorned with biotin for targeting purpose. The resulting amphiphile can self-assemble into micelle and is capable of loading DOX. The micelles exhibit hierarchical disassembly in the tumor microenvironment and release the drugs.

All the three class of nanocarriers obtained by various strategies were analyzed for their anticancer drug release potential by preclinical evaluations. In-vitro studies were performed on cancer as well as non-cancer cell lines to demonstrate the non-toxicity of carriers, their internalization, as well as sustained and targeted drug release. The antiangiogenic potential was demonstrated ex-ovo via chorioallantoic membrane assay on chick embryo. The in-vivo studies were performed by induction of hepatocellular carcinoma on nude mice model. The efficacy of drug delivery systems for tumor regression and suppressing the cardiotoxicity of DOX was demonstrated by qualitative as well as quantitative examinations viz. histoarchitechture analysis, the changes in levels of cancer specific biomarkers etc.

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