Chapter 1

1.1 Cancer

Cancer is a malignant disease where uncontrolled growth of cell due to mutation in their genetic material occurs and results in mortality of patients. However tremendous research in this area has been done and surgical intervention is the potentially curative method for early stage cancer. Conventional chemotherapy or radiation therapy is the most useful approaches for advanced treatment stages. However these treatments have shortcomings due to non selectivity between normal and cancerous cell and the severe toxicity caused by these chemotherapeutics. Shorter half life and widespread distribution of these drugs into non selected organs and tissues leads to more drug intake which is uneconomical and impose severe toxicity. The systemic administration of these drugs cause severe side effects to other normal tissues and cells which limits the maximum allowable dose of the drug. Under such situations targeted delivery of anti cancerous drugs to the tumor site is the most important requirement in chemotherapy.[1]

1.2 Traditional methods of treatment and their drawbacks

1.2.1 Surgery

Surgical resections generally involve a careful excision of cancerous tissue/lump followed by treatment with chemo or radiotherapy, thus eliminating cancer completely. Surgery is the most promising treatment of cancer, where a process known as staging is involved which unveils how far the cancer has spread. In breast cancers the surgery is done either for

removal of whole breast known as mastectomy, when a lump is removed it is known as lumpectomy, or removal of about quarter of breast tissue known as quadrantectomy.

1.2.2 Radiation therapy

Here in this technique high energy x rays or other types of radiations are used to kill the cancer cells and also inhibit their growth. Principally radiation therapy which is used commonly is of two kinds, which are external radiation therapy and internal radiation therapy. Mechanism of external radiation therapy involves outside radiation from a source towards the body to send the radiation to the cancer cell. Whereas in internal radiation therapy, radioactive materials are incorporated in the needles, seeds, catheters or wires are placed inside the body close to the cancerous area.

1.2.3 Chemotherapy

Chemotherapy is the most conventional technique to restrict the proliferation of cancer cells involving the process of killing or forbidding their division. Chemotherapy refers to the treatment with drugs or chemicals for destroying cancer cells. In chemotherapy the chemotherapeutics travels throughout the body to reach the targeted site. Chemotherapeutic drugs are either used alone or with the combination of other drugs for treatment.

1.3 Barriers and side effects in chemotherapy

Solubility of the chemotherapeutic plays a critical role, it must be soluble in blood when administered intravenously or given orally for better absorption. Since most of the chemotherapeutics are hydrophobic and insoluble in aqueous solution therefore results in poor therapeutic effects. Again most of the anticancerous drugs are identified as foreign particles by the macrophages and can be digested or engulfed by them resulting in poor

treatment. Due to non selective nature of chemotherapeutics they affect normal healthy cell which is the major reason for higher mortality rate in cancer patients. Side effects caused include blood related side effects, hair loss (alopecia), nausea and vomiting.

1.4 Drug delivery in cancer (Need of controlled drug delivery)

Development of effective treatment for diseases has been the prime concern of the human race for years. Understanding the human body and the function of various components as well as the living cells has led to the development of various medications both natural and synthetic to fight against diseases, disorders and mal functioning of the body components. Cancer is the prime reason for high mortality rates in many countries. The term cancer signifies uncontrolled cell growth and multiplication. Such cells have outright replication potential, circumvent apoptosis, and stimulate metastasis. Control drug delivery technology is the progressively growing field of science uniting people from different fields like chemistry, materials science and chemical engineering focusing on health care sector**. [2]** Control drug delivery systems provide better efficiency, minimized toxic effect, and better patient compliance. The aim of all control release devices is to enhance the efficiency of drug delivery, which improves the therapeutic efficacy by minimizing the adverse toxic effects and minimizing the amount of drug to be taken during treatment. [3] Since past few years emergence of controlled drug release systems have gained tremendous influence in pharmaceutics. Due to extensive research efforts it has come out as one of the important multidisciplinary research sector. Research in this area has become increasingly important due to the advantages over conventional dosage forms like efficacy, safety, cost effective and improved patient compliance after long term actions of the therapeutics. In general the

control drug release system is an entity that delivers the drug at specific site at predetermined rate for prolonged period of time (***Figure 1.1)***.

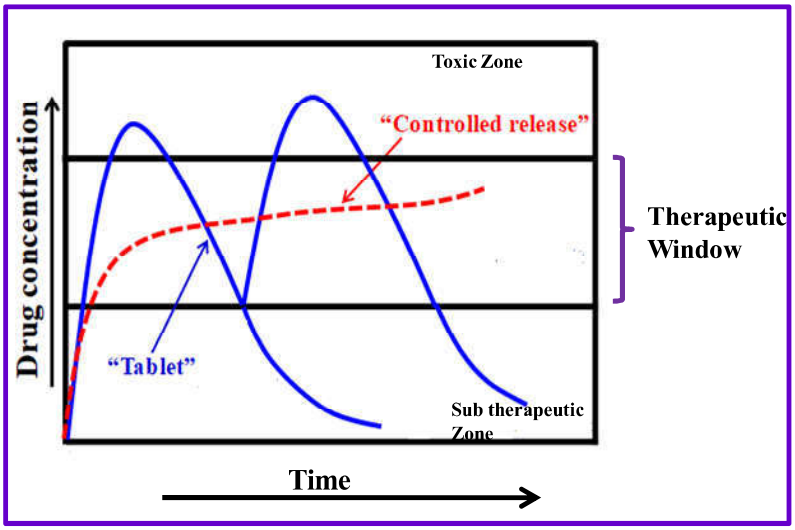

Figure 1.1: *A schematic presentation of controlled release systems over traditional release.*

Controlled drug release systems can be constructed from either polymers or osmotic pumps.[4] Their fabrication is done in such a way that they regulate the entry of drug within body and release them continuously in planned pattern for a particular duration of time, either systematically or to a targeted site. Designing of rate and duration of drug release is done to attain the desired concentration at the targeted site**.** A sustained drug release system is designed for maintaining constant level of drug in patient's blood stream by releasing the drug over prolonged time interval, which enhances the therapeutic efficacy of drug. The numerous advantages of control drug delivery systems such as reduced adverse effects and toxicity caused**,** controlled rate and site specific release, reduced drug dose**,** prolonged and consistent therapeutic effect, and improved patient compliance.

Control release system (CRS) engineering is stimulating because it requires a physical entity where the required amount of curative could be placed, protection of therapeutic from breakdown before release and likely release the therapeutic over the course of time.[5] Materials required for CRS must be biomaterial, among these are biocompatibility, processibility and sufficient mechanical strength.[4] There are basically three general mechanisms for drug release from polymer matrix or lipid based systems, diffusion of the drug from or through the systems, cleavage or degradation of the system by enzymatic or chemical reaction, Solvent accumulation either through osmosis or swelling of the system. Polymer based drug delivery systems has enormous impact in therapies, where the drug is entrapped in the polymer matrix and then can be injected or implanted in the body. The systems earlier used to be non biodegradable (membrane controlled diffusion) like silicone rubber, which could release the drugs for prolonged time. However this approach does not permit the release of ionic species or drugs with higher molecular weight with Mn above 400 because they are unable to diffuse through such polymers.[6] For addressing this issue drugs were physically embedded in polymers at higher concentration enough to create interconnecting pores through which slower diffusion of drug is made possible (a matrix type of systems).

1.5 Mechanism of drug release from polymers

Drug carriers are basically categorized on the basis of the mechanism administering the drug release including release controlled by diffusion, release controlled by chemical means, Solvent activated, Modulated release systems. Each type of mechanism mentioned above can present different drug release pattern depending upon the mode of mechanism, the type of polymer and drug used. The main goal of control release is to preserve the

optimum drug amount within the therapeutic window i.e. between minimum effective amount or minimum toxic amount. The amount of drug release from a vehicle is generally governed by several factors such as drugs, polymers, additives, their composition and the interactions among the components. The release mechanism is categorized as physical mechanism and chemical mechanisms. Physical mechanism involves several aspects as drug diffusion from polymer, dissolution/degradation of polymer governing the release rate, osmotic pressure for controlled release and ion exchange for ionized drugs. The chemical mechanism involves breaking of chemical bonds between drug and carrier either by enzymatic or chemical cleavage. Physical mechanism are beneficial over chemical since drug release kinetics can be governed by the carrier system itself while in chemical mechanisms the chemical modification of drug is required for successful tagging it with carrier which results new entities called prodrugs.[7]

1.5.1 Diffusion Controlled release

In diffusion controlled release drug diffuses through the polymer followed by degradation of polymer. The simplest example of diffusion is where drug is entrapped in the core/reservoir and from which it will diffuse. Diffusion controlled systems are of two types reservoir devices and matrix devices. In reservoir systems there is a hollow inner core where drug is suspended and surrounded by the polymer membrane. Overall release is governed by diffusion of drug through membrane which is also rate determining step. In diffusion controlled release occurs in reservoir type systems where the drug is dispersed or dissolved in polymer reservoir and the diffusion is driven by the concentration difference across the membrane. In matrix type systems there is no membrane or layer and drug is

released without any controlling barrier and initial fast release occurs and then the release rate decreases as the diffusion distance increases. [7]

1.5.2 Solvent Controlled release

Release through solvent activation basically occurs by osmosis controlled release and swelling controlled release.[8] Release through osmosis mechanism is observed where the semi-permeable membrane shields the drug carrier allowing the flow of water inside and this mechanism follows zero order release kinetics.[9] Swelling controlled release occurs in case on hydrophilic polymeric carriers water diffuses in the carrier which results in swelling of the polymer. The release rate depends on the diffusion of water and relaxation of polymer chains. Swelling controlled release basically occurs from three dimensional polymeric materials such as hydrogels, where the crosslink density governs the release behavior.[10]

1.5.3 Degradation controlled release

Drug entrapped in environment friendly polymers preferably polyesters, polyamides, polyaminoacids and polysaccharides is released via hydrolytic/enzymatic cleavage of ester or amide bonds in their backbone.[11] Matrices of polymers containing poly(lactic-co-glycolic acid) (PLGA), poly lactic acid (PLA), and poly caprolactone(PCL) usually undergo bulk degradation while polyanhydrides and polyesters undergo degradation from surface to core since the polymer degrades before the water diffuses. [12] Degradation rate of polymer depends upon its molecular weight, functional groups, monomers attached and the crystalline nature which affects the release kinetics.[13] Cleavage of polymer-drug connection through enzyme causes release of drug and it is the frequency of cleavage

which governs the release behavior. Also target specific delivery could be achieved if enzyme is concentrated in targeted tissue.[14]

1.5.4 Stimuli Controlled release

Release of drug from stimuli responsive carriers is resolved by the internal and external stimuli like temperature, pH, concentration, and magnetic field.[14] Due to feasibility of stimuli localization these carriers are employed for specific delivery in solid tumors where the pH is acidic. The main advantage of pH sensitive carriers is the release difference caused between intracellular and extracellular release while for themosensitive delivery vehicle the release is governed by the thermally induced phase transition in polymers. [15]

1.6 Drug delivery systems

So far numerous strategies for carrying drug to its required site are developed by enhancing the potency of the chemotherapeutic and reducing its adverse effects. The carriers generally employed embraces hydrogels, dendrimers, micelles, liposomes, polymer matrix and polymeric nanoparticles ***(Figure 1.2***).

1.6.1 Liposomes

Liposomes are usually composed of one or more lipid bilayer and are generally spherical in shape. They are employed for delivery of both lipophilic and hydrophilic drugs in which former are incorporated in the lipid bilayer while the later is in the aqueous solubilised inner core. PEGylated liposome with DOX i.e. doxil has been approved by the US Food and Drug administration in 1995. Incorporating PEG on the surface of liposomes increases the circulation half life, thus taking benefit of EPR effect.[16] Apart from PEG several other hydrophilic polymers like poly(N-vinyl pyrrolidone) (PVP), poly(vinylalcohol)

(PVA), polyoxazoline (Pox), hyperbanched polyglycerol, or zwitterionic have also been utilized. [17, 18]

1.6.2 Dendrimers

Dendrimers are highly branched structures having 3D architecture with higher surface functionality and versatility. Since their introduction in 1980s they have been promising polymeric materials due to their unparalleled properties like uniform size, aqueous solubility, nanoscale size, low polydispersity, and well defined molecular weight distribution. The inner central cavity of dendrimers is the site for specific encapsulation of guest molecules predominantly drug while the outer periphery with multifunctional groups for conjugation with bioactive agents. Owing to their special structural features dendrimers have attracted very much for drug delivery application. [19] Most commonly used dendrimers in drug delivery include polyamidoamines PAMAM, poly(L-lysine), polyesters PGLA-OH, polypropylimines (PPI) as well as citric acid-carbohydrate based ones.

1.6.3 Hydrogels

Hydrogels are 3D networks comprising of cross-linked hydrophilic polymeric chains. Owing to their biocompatibility, easy fabrication, variable composition, and advantageous physical characteristics have led to their tremendous applications. The prime aim of hydrogel based technology is preparation of injectable hydrogels, where aqueous mixture of gel precursors are mixed with other biopolymers or bioactive agents and administered using syringe at the intended area. The benefit of injectable hydrogels lies in their highly moldable property (adapt the required shape), when applied *in-vivo* results in faster recovery with smaller scar size and less pain caused to the patients, possess high capacity and efficient encapsulation of drug or gene for delivery. In situ gelation of injectable

hydrogel could effectively preserve the enclosed drugs inside the tumor and incisively liberates the drugs into tumorous cells which have led to their application in localized drug delivery for tumor treatment.[20, 21] drug delivery through hydrogels could be attained in different ways like orally, rectal, ocular, epidermal and subcutaneously [22]. Cyclodextrin and PEG modified with gold nanocrystals forming supramolecular hydrogels demonstrated pH dependent release of drug arising from host guest interaction of Doxorubicin (DOX) with Cyclodextrin. These DOX loaded microgels displayed systematic antitumor effect towards HeLa cells as compared to pure microgels.[23, 24]

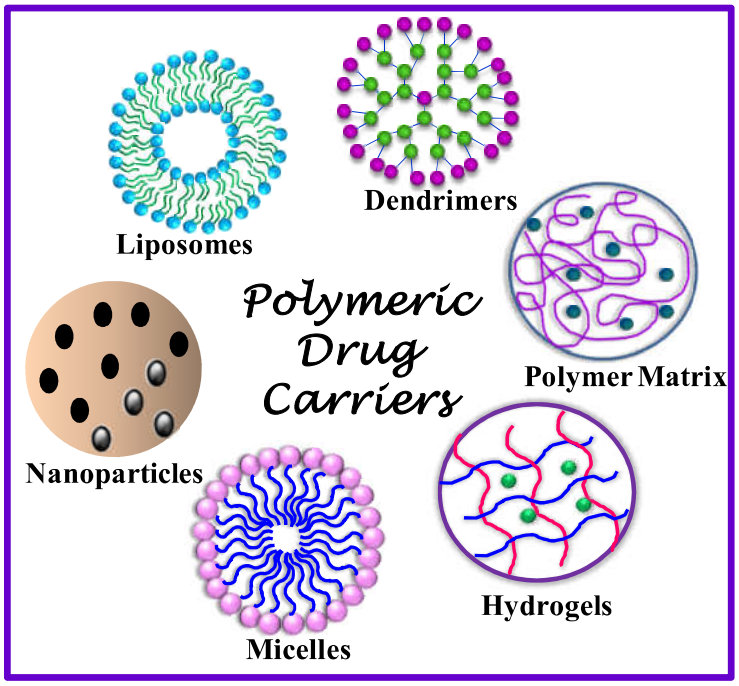

Figure 1.2: *Different polymeric carriers for controlled drug delivery.*

1.6.4 Micelle as a drug carrier

Micelles are generally formed by the self assembly of the amphiphilic block copolymers in an aqueous solution forming hydrophobic core and hydrophilic shell of spherical or

globular shape. Hydrophobic drugs are stored in the hydrophobic core, while the hydrophilic shell provides the stability to the hydrophobic core and hydrophobic drug and making the particles of appropriate size for i.v administration. Incorporation of drug into polymeric micelle is done through physical, chemical or electrostatic interactions.[24] Simultaneous delivery of drugs through micelles for improved tumor treatment is reported. Polymeric micelle system based on amphiphilic block copolymer poly(2-methyl-2-oxazoline-block-2-butyl-2-oxazoline-block-2-methyl-2-oxazoline)(P(MeOx-b-BuOx-b-MeOx) is loaded with paclitaxel (PTX) and alkylated cisplatin prodrug for combination therapy of ovarian and breast cancer.[25] DOX is connected though hydrazone linkage with an amphiphilc hyperbranched block copolymer, composed of hyperbranched polyester Boltron H40 core, comprising of poly(aspartate) as hydrophobic part and PEG as outer part. Cleavage of hydrazone linkages between poly(L-aspartate) and DOX in acidic environment facilitated its release.[26]

1.6.5 Polymeric nanoparticles

Polymeric nanoparticles are the particles forming capsules or solid spheres. The drug is integrated in the polymer web and the delivery is facilitated by either drug diffusion of polymer degradation. Many natural polymers such as dextrin, chitosan, and albumin and synthetic polymers e.g poly (lactic acid) (PLA), poly(ε-caprolactone) (PCL), poly(lactic-co-glycolic acid), N-(2-hydroxypropyl)-methacrylamide copolymer (HPMA) have been utilized for nanoparticle preparation including different methods like coecervation, precipitation, emulsification, and layer by layer methods. Polymeric nanoparticles have been widely explored as drug carrier from last few decades.[27] Conjugation of DOX with dextran and its subsequent encapsulation in hydrogel using microemulsion technique

reduces its cytotoxicity and enhances its therapeutic efficiency for tumor treatment[28], also multifunctional PLGA nanoparticles containing taxol showed chemotherapeutic effect and near infrared photothermal demolition of cancerous cells both *in vitro* and *in vivo*. [29]

1.7 Bio polymers for controlled drug release

1.7.1 Chitosan

Chitosan is a linear biopolymer acquired from deacetylation of chitin structural component in the exoskeleton of arthropods or in the cell walls of fungi and yeasts. Due to its high biocompatibility, biodegradability, anti bacterial property and blood coagulation property it is widely used polymer in biomedical applications. [30] Chitosan also exhibits other numerous features due to which it is used as a drug delivery vehicle for nasal[31], ocular[32, 33],and buccal drug delivery [34]. Chitosan is also used in other application like gene delivery[35], cell culture[36] , tissue engineering [37], as a sensor in biology [38]and scaffold generation [39]. Chitosan and its derivatives possess ability of forming nanoparticles and can be used in sustained and targeted release of therapeutic agents, among them cross linked Chitosan [40] and its derivatives having hydrophobic part on the polymer backbone have tremendous applications. N-alkyl-O-Sulfate formed nanomicelles due to self assembly and presented slow release of paclitaxel a potent anticancerous drug[41].

1.7.2 Dextrin

It is a low molecular weight saccharide polymer, manufactured from acidic/enzymatic hydrolysis of starch or glycogen. It is exclusively composed of α(1- 4) linked D-glucose residues. Dextrin is widely used functional biomaterials with desirable properties and chemical structure, low cost, commercial availability, non toxicity and its biodegradability.

Modification of dextrin by grafting with various functional groups in presence of cross linkers leads to formation of polymetic hydrogels with improved functional properties. Introduction of synthetic polymers like poly methyl acrylate, polyvinyl acrylate, poly acryl amide and so on onto dextrin has been done for applications in different field. Dextrin grafted poly(HEMA) hydrogels via free radical polymerization technique has been prepared for controlled release. The prepared hydrogels were biocompatible and showed effective cell proliferation. Further exhibited excellent tendency as an efficient drug vehicle for controlled and sustained release of ornidazole in colonic region.[42] Stimulus responsive and chemically connected dextrin/poly acrylic acid hydrogels using N,N′-methylene bis(acrylamide) (MBA) crosslinker have been designed for sustained release of ciprofloxacin. Prepared hydrogels exhibited swelling and deswelling behavior and higher cell proliferation towards human mesenchymal stem cells. *In-vitro* release of two model drugs ornidazole and ciprofloxacin from hydrogels presented sustained drug release till 3 months.[43]

1.7.3 Cellulose

Cellulose is a biocompatible polysaccharide utilized as drug delivery vehicle in biomedical field. It is a linear polymer composed of α 1-4 β-D-glucopyranoside linkages, this configuration having intramolecular hydrogen bonding forms a rigid structure. Chemical modification of cellulose has been done for developing new biomaterials like methyl cellulose (MC), hydroxyl propyl methyl cellulose (HPMC), hydroxyl ethyl cellulose (HEC) and carboxymethylcellulose (CMC) with innovative physical and chemical properties.[44] Formation of injectable gels by mixing any of cellulose derivatives with natural polymers can be done e.g hyaluronic acid and methyl cellulose or starch and carboxymethyl cellulose

[45]. Biodegradable microspores formed by the reaction between and carboxylated chitosan for doxorubicin delivery is reported. These microspheres have porous structure more favorable for doxorubicin loading and were biocompatible to human umbilical endothelial cells. Also the hemocompatibility and its degradation with lysozyme made it useful as a drug delivery system for wide range of applications.[46]

1.7.4 Pullulan

Pullulan is a water soluble, neutral and non toxic polysaccharide, consisting of α 1-6 maltotriose residues, which consist of three glucose units connected to each other by α 1-4 glycosidic bond.[47] Pullulan is the most suitable material for tissue engineering due to its benign, biologically degradable and bioabsorbable nature. Cholesteryl pullulan (CHP) derivates are effective drug delivery carriers for cancer cell targeting, further conjugating folic acid with CHP hydrogels for loading DOX has been done[48]. In one of the investigations modification of Pullulan with maelic anhydride to obtain maleilated pullulan (MP) with vinyl carboxylic acid groups followed by chemical conjugation of DOX with MP and finally bound with folic acid to produce FA-MP-DOX. [49]

1.7.5 Hyaluronan

HA is a bioactive system and can bind to the CD44 receptors which are over expressed in tumors or other inflammatory tissues[50]. Therefore it is has been explored as an active targeting agent for *in vitro* drug delivery for improved efficacy. HA based micelles have been used for cancer treatment with different anticancerous drug e.g DOX[51], PTX[52], siRNA[53], and curcumin[54]. HA polymer has been used as tumor targeting agent, drug and gene carrier and imaging agent to CD44-overexpressing cells. HA-Paclitaxel conjugates have shown enhanced survival rate of mice bearing ovarian cancer[55], bladder

cancer[56], breast cancer with metastasis[57] and squamous carcinoma of head and neck[58, 59]. Encapsulation of DOX in HA-grafted liposomes have shown enhanced tumor reduction as compared to PEGylated liposome loaded with doxil in human xenograft mouse tumor models.[60] [61]

1.7.6 Alginates

Alginate is linear anionic heteropolysaccharide composed of β-D mannuronic acids repeating units. Alginates are usually extricated from brown sea weeds and are known for their non toxic nature proved from pre-clinical studies. Owing to its fabulous versatility it has been widely used in drug delivery applications.[62] Abe et al reported a form of hydroxyapatite-alginate composite beads for paclitaxel local delivery for treatment of metastatic spine cancer in rat. The significant therapeutic of paclitaxel loaded beads after intra-tumoral injections showed disease free survival compared to controls treated with drug free beads.[63] Bouhadir et al prepared a hydrogel composed of oxidized sodium alginate cross-linked with adipic dihydrazine for local and sustained delivery of chemotherapeutics. The prepared formulation can successfully encapsulate and release various therapeutic agents like methotrexate, doxorubicin and mitoxantrone either individually or simultaneously.[64]

1.7.7 Gelatin

Gelatin is a biologically acceptable, environment friendly, and multifaceted polymer derived from natural resources. It is water soluble polypeptide acquired from acidic, alkaline or enzymatic hydrolysis of collagen protein the main component of skin, bones and connective tissues of animals.[65] Due to its properties as a natural biomaterial it has been extensively used as a drug carrier in biomedical field. Encapsulation of DOX in

amphiphilic gelatin-iron oxide /calcium phosphate shell nanoparticle has been done for highly pH responsive drug release from these carriers. [66] Hydrogels of gelatin with varying concentration of polyvinyl alcohol and cisplatin an anticancerous drug have been reported as slow drug delivery systems. Hydrogels with low dose of drug were efficient in inhibiting tumor growth as compared to conventional treatment of high dose of free drug.[67]

1.8 Strategies for polymer modification

1.8.1 Grafting a versatile method for polymer grafting

Graft copolymerization is the most attractive and versatile method for polymer modification and an important means of imparting various functional groups to a polymer. By grafting one can amalgamate the top most properties of two polymers into a single unit. In general polymers with required properties can be prepared by graft copolymerization, by changing some parameters like types of polymer, degree of polymerization, the graft density and the graft length. Two major types of grafting are well known 1) grafting with single monomer 2) grafting with mixture of two or more monomers. Mosaic grafting has attained importance for binary monomer grafting. Side by side grafting of two monomers to obtain essential properties which are required is done.[68] Graft copolymers are basically polymers with modified physical and chemical properties comprising of two polymer components, the second is the indiscriminately distributed branches, which are linked with the first component functioning as the backbone. Three different strategies used for grafting include grafting through, grafting from and grafting to approach presented in ***Figure 1.3*** [69].

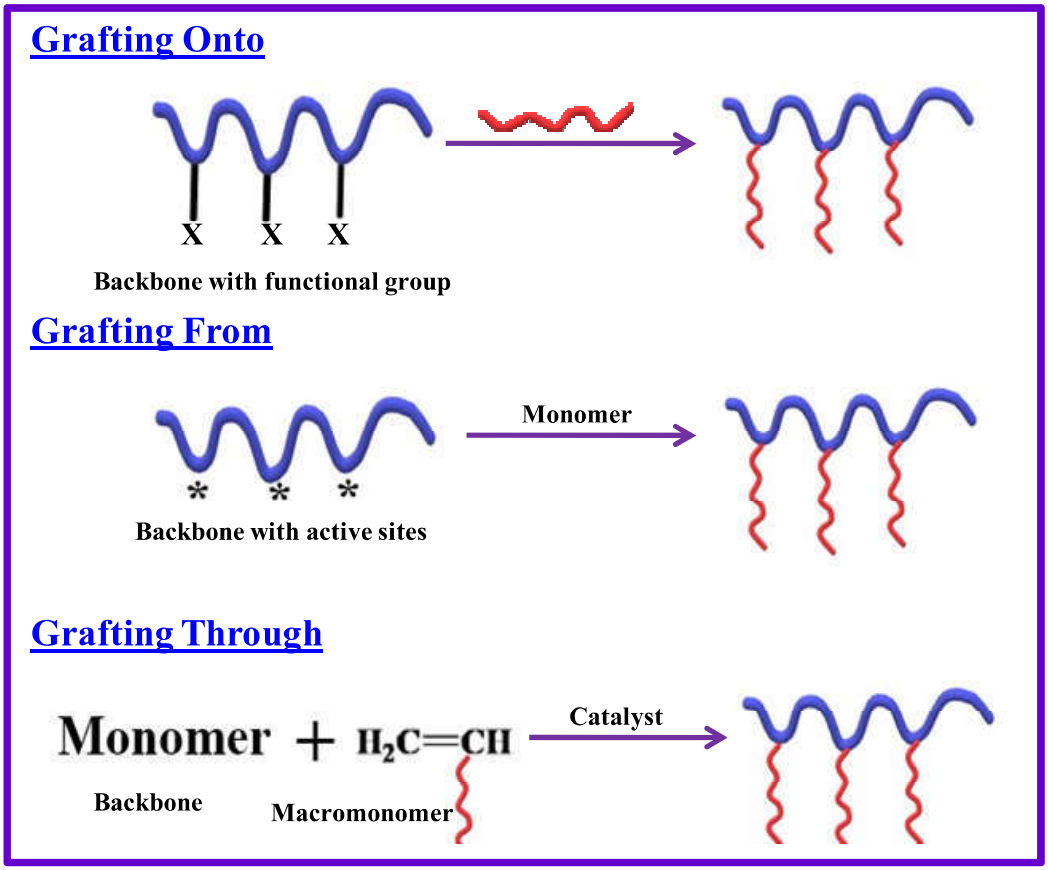

Figure 1.3: *Schematic representation of different grafting methods.*

In the grafting to approach the functional end groups present on previously prepared polymer are connected to functional groups situated on the polymeric backbone. Its schematic presentation is displayed in (***Figure 1.3)***. In the grafting from approach the growth of polymer chain occurs by the initiation sites present of main polymer chains and is shown in (***Figure 1.3)***. In the grafting through approach copolymerization of a macromonomer usually vinyl macromonomer with comonomer having lower molecular weight is done (***Figure 1.3)***. Among the three approaches discussed above grafting from is the most commonly used method drawing its advantage by yielding densely grafted due to the ease of the reactive groups present at the end of growing polymer chain. [70]

1.8.2 Atom transfer radical polymerization

ATRP involves radical formation that can multiply and reversibly becomes inoperative forming inactive species. Formation of radical occurs by the activation of organic initiator catalyzed by transition metal catalyst by blocking the halide present at the end of chain. ATRP is the suitable method for obtaining polymers of low molecular weight and low polydispersity index. Mechanism of ATRP is presented in (***Figure 1.4)***. With ATRP both functionality and architecture can be mingled and multifunctional polymers with various composition and shapes like block, star and hyperbranched polymers can be attained. [71] Polyacrylic acid was grafted at the ends of pluronic P 85 via atom transfer radical polymerization forming a pH responsive block copolymer, PAA-b-P85-b-PAA (P85PAA). The main purpose behind designing such polymers was to attain the high binding capacity of DOX which is a potential anti cancerous drug with high anti tumor activity within the polymeric carrier[72].

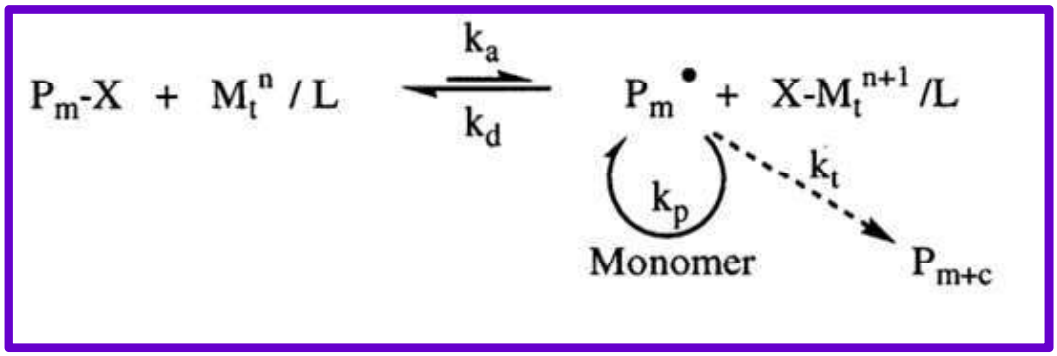

Figure 1.4: *Mechanism of ATRP[71].*

Physical encapsulation of DOX inside pluronic micelle enhanced the release rate of DOX inside solid tumor. DOX release from the polymer drug conjugate was regulated by the

release medium and the polymer composition, release was highly pH responsive. At mild acidic conditions at pH 5 protonation of carboxyl groups occurs which results in faster release of DOX as compared to pH 7.2. The slower release at pH 7.2 is attributed to the strong electrostatic interactions among DOX and PAA segments. Synthesis of poly(ethylene glycol)-b-poly(alkyl acrylateco-t-butyl methacrylate) (PEG-b-P(AlA-co-tBMA)) block copolymers having controlled and variable hydrophobic block lengths was prepared through atom transfer radical polymerization[73]. Carboxylic groups in the pendant form are present in hydrophobic block results in pH dependent aggregations and supramolecular assemblies at acidic pH i.e 4.7. The water insoluble drug progesterone(PRG) was embedded in the supramolecular assemblies by dialysis or O/W emulsion technique and *in vitro* release at pH 1.2 and 7.2 was evaluated. The PRG was released when the pH of the release medium was elevated from 1.2 to 7.2. Therefore such pH sensitive self assemblies can improve the bioavailability of poorly soluble drugs.

1.8.3 Reversible addition-fragmentation chain transfer polymerization

Macromolecules with well defined architectures and low polydispersity index are prepared through reversible addition-fragmentation chain transfer radical polymerization[74]. RAFT polymerization includes three main constituents- a monomer, radical initiator and a chain transfer (RAFT) agent (***Figure 1.5)*** Synthesis of poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) as a potential carrier for nucleic acids is done. RAFT polymerization is used for introducing terminal thiol groups in oligomers of (DMAEMA) using di-functional chain transfer.[75]

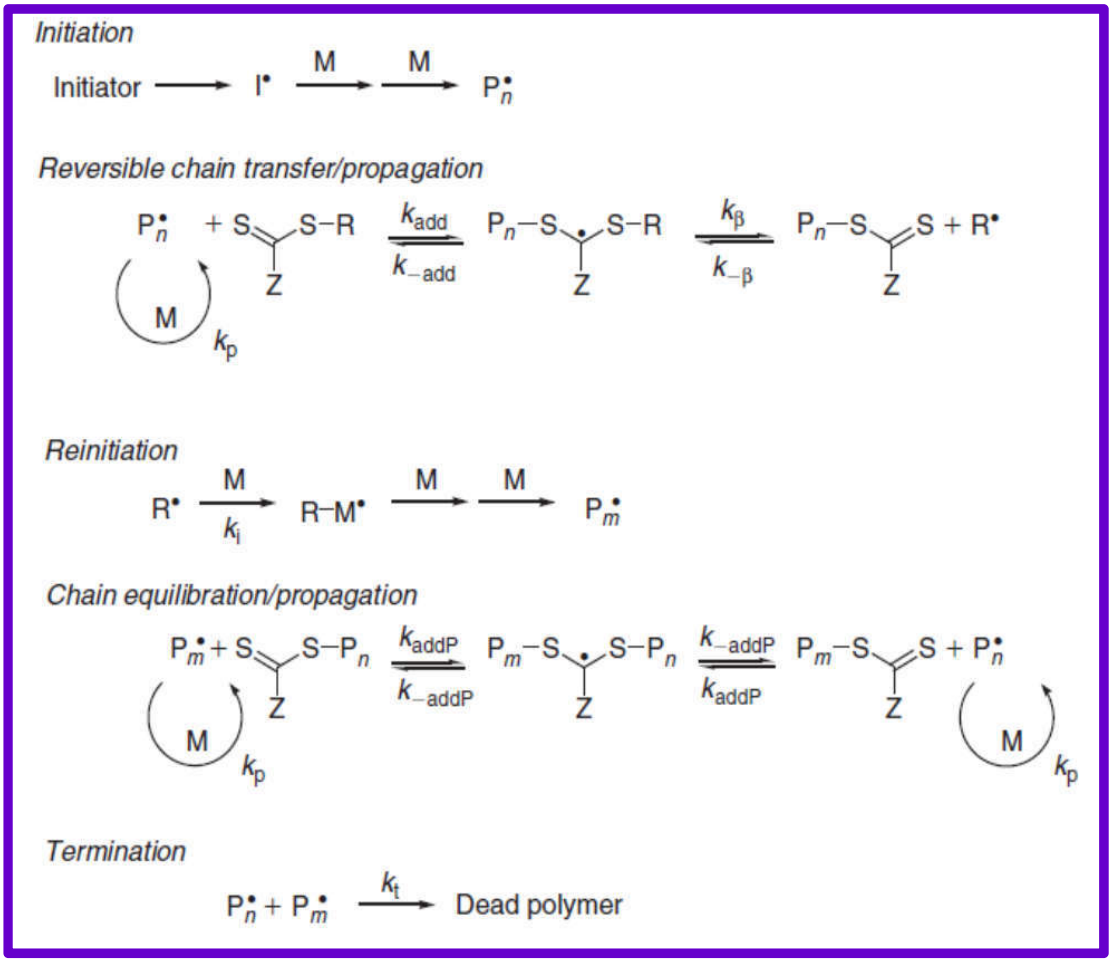

Figure 1.5: *RAFT polymerization mechanism.*

1.9 Modes of delivery and cancer treatment

1.9.1 Transdermal patches

Transdermal patches are painless offer an interface which facilitates the systemic drug administration. Usually the composition of patch includes a protective backing layer that prevents the drug leakage, a reservoir lipophilic in nature, which stores the drug, and the adhesive layer that assist skin contact[76]. Polymeric patch through emulsion electrospinning of block copolymers of methoxy poly(ethylene glycol)-block-poly(lactide-co-glycolide) and dextran emulsion with hydroxycamptothecin (HCPT) in the oil phase and

tea polyphenols (TP) in the aqueous phase has been prepared. Application of this patch to orthotopic hepatoma where the sequential and sustained release of these two drugs inhibited the progression of hepatoma.[77] Microneedles is a micron-sized needle having height of 10-2000 mm and 10-50 mm wider and could penetrate into dermal tissues without any pain. Microneedle delivery systems delivers the drug transdermally by creating pores in the skin through which drug enters and hence improves its bioavailability. So far different kinds of microneedles have been used for cancer treatment.[78] A dissolving needle developed from triblock copolymer in situ generated micelles on their dissolutions after cutaneous applications which facilitated the encapsulation of receptor and delivery of hydrophilic antigens. Hao et al prepared a peculiar system with combination of chemotherapy and photothermal therapy (PTT) via NIR-responsive PEGylated gold nanorods (HNR-PEG) coated with poly(L-lactide) microneedles (GNRPEG@MNs) for enhancing the antitumor efficiency of DTX-loaded MPEG-PDLLA micelles for A431 tumor therapy presented in (***Figure 1.6)*** .

Application of these microneedles containing tumor model antigen to the skin of tumor bearing mice resulted in significant antitumor activity.[79] Recently three dimensional printing technology has emerged as a major innovation in biomedical field especially drug delivery. 3D printing permits the controlled release of drug from the manipulative architecture of construct. 3D printed patches comprised of PCL-PLGA loaded with 5-FU have been investigated *in-vitro* release kinetics and therapeutic efficacy. These 3D printed patches delivered the 5-FU for more than 4 weeks and suppressed the pancreatic cancer without side effects.[80] Sustained delivery of powder through epidermis for safe and sustained release of hydrophilic drugs through the skin is investigated. Powdered drug is

coated onto micro channels followed by their topical application of patches onto micro channels on skin generated by laser to deliver the drug into skin. Interestingly these sEPD enhanced the bioavailability of zidovudine by 100 % as compared to oral delivery. sEPD of anti-programmed death-1 antibody showed more potent anti-tumor efficacy than intraperitoneal injection in B16-F10 melanoma models.[81]

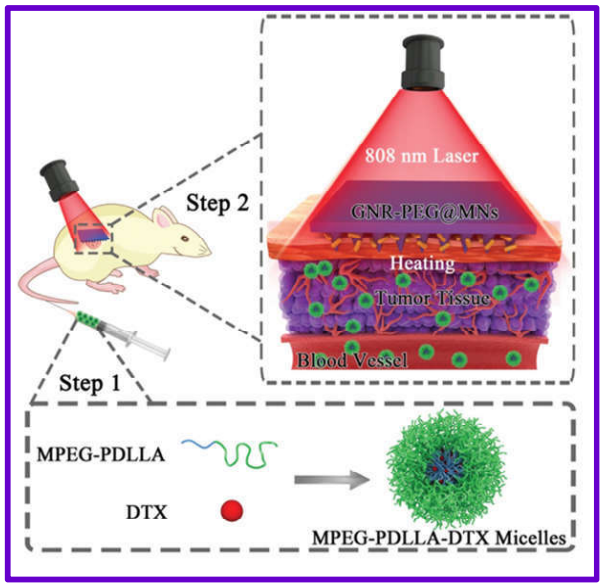

Figure 1.6: *A cooperative system of chemotherapy and photothermal therapy for treatment of A431 tumors by the integration near-infrared responsive GNR-PEG@MNs and MPEG-PDLLA-DTX micelles. (Step 1: Injection of DTX loaded micelles; Step 2: After the injection, pressing of GNR-PEG@MNs at the tumor sites and under 2 W/cm^2 irradiation by 808 nm laser within 5 min).*

1.9.2 Subcutaneous

Subcutaneous route of drug delivery is the most common route used for nanoparticles delivery[82], while conventional vaccines are used clinically are administered via intramuscular route (IM) [83]. Subcutaneous mode of administration significantly enhanced the delivery of nano particles to draining lymph nodes, effective uptake of

naodiscs by antigen-presenting cells, and generated 7-fold higher frequency of neoantigen-specific T cells, compared with the IM route. Significantly, these nanodiscs plus anti-PD-1 and anti-CTLA-4 IgG therapy when done in mice bearing B16-F10 melanoma tumors, showed excellent antitumor effect, resulting in demolition of tumor induced in almost ~60% of animals[84]. Lee et al prepared a triblock copolymer vitamin E functionalized polycarbonate and polyethylene glycol physically cross-linked injectable hydrogels for sustained release of herceptin. The anti tumor specificity and efficacy of herceptin in hydrogels against normal and breast cancer cell line was investigated with different HER2 expression. Herceptin loaded hydrogels were specific towards HER2 overexpressing cancer cell and exhibited toxicity similar to herceptin solution. The biocompatibility and biodegradability of hydrogels is estimated after subcutaneous injection to mice and analyzed by histological examination, showing no inflammatory responses and biodegradability within 6 weeks [85]. Single subcutaneous injection of Herceptin-loaded hydrogel in mouse bearing BT474 tumor showed excellent retention of antibody within tumor. This resulted in higher antitumor activity and tumor size shrank by 77 % at 28^{th} day as compared to intravenous (i.v.) and subcutaneous (s.c.) delivery of herceptin in solution.[85]

1.9.3 Intravenous

Sung et al reported a newly developed low molecular weight benign, environment friendly, amphiphilic diblock copolymer of monomethoxy poly(ethylene glycol)-block-poly(D,L-lactide) (mPEG-PDLLA) for intravenous administration of paclitaxel In melanoma induced mice to increase its efficiency. The results demonstrated the therapeutic potential

biodegradable polymeric micellar system containing paclitaxel against variety of solid tumors and can be clinically used against human solid tumor treatment.[86].

1.9.4 Photodynamic therapy

Photodynamic therapy (PDT) involves radiation for cancer treatment and is known for its efficiency for both curative and palliative treatment. It involves the application of a photo sensitizer and irradiating tumor with specific wavelength usually of NIR region that can perforate extremely in the tissue. Upon irradiation the photosensitizer absorbs light and undergoes photochemical reactions yielding reactive oxygen species (singlet oxygen) which are harmful to cells. [87, 88]. Apart from cell killing PDT even destructs vascular tissues and hinders blood flow restricting nutrients to cancer cells0 [89] PDT has been approved for treatment of various cancers including head and neck tumor, basal cell carcinoma, cervical, bladder and gastric cancer.[90] The main advantages of PDT over conventional chemotherapy is its low systemic toxicity, ability to destroy cancer cells selectively and PDT can be applied either alone or in combination with chemotherapy, surgery, or immunotherapy.[91,92] Subramaniyam et al fabricated polypyrole nanoparticles by utilizing bovine serum albumin-phycocyanin complex and were stable under physiological conditions. Nanoparticles effectively killed MDA-MB-231 cells generating reactive oxygen species through phycocyanin complex upon laser illumination. Also these nanoparticles generated good ultrasound signal under photoacoustic imaging facilitating imaging of treated cells. [93]

1.10 Cyclodextrin in drug delivery and cancer treatment

CDs are cyclic (α-1, 4)-linked oligosaccharides composed of α-D-glucose units. [94] CDs can exist in three forms α, β and γ, are comprised of six, seven and eight α-D-

glucopyranose units, respectively (***Figure 1.7***). They have numerous hydroxyl groups, and are generally water-soluble.[95] Due to higher crystal energy they are strongly bound in crystal state as compared to linear dextrin. CDs have poor aqueous solubility. Moreover, β-CD is less soluble among all, due to the formation of internal hydrogen bonds among secondary hydroxyl groups. This partly explains its lower aqueous solubility than α- and γ-CDs, whose aqueous solubility is 7- and 14-fold as much as that achieved by β-CD, respectively. One of the typical features of CD is its torous shaped structure having hydrophobic interior cavity and hydrophilic exterior cavity.[96-98] One of the important features of CDs is their ability to form inclusion complexes both in solution and solid state, where the guest molecule is enclosed by the hydrophobic cavity of CD. Currently many CD based polymers have been prepared for better inclusion abilities as compared to pure CD. Hosting the drug inside lipophilic cavity of CD as inclusion or non inclusion complexes increases the apparent stability and solubility in formulations of solution or in solid forms for control release. Also, CDs improve the bioavailability of drugs by regulating the release rate or time profile of drug. CD based polymers are explored due to their great versatility, since their molecular weight, different architectures (linear or branched) or with pendant ligands can be varied easily. CD based polymers possesses ability of forming inclusion complexes which may be ameliorated due to cooperative effects. Different formulations of Cyclodextrins that have been used in drug delivery and cancer treatment are mentioned is next section.

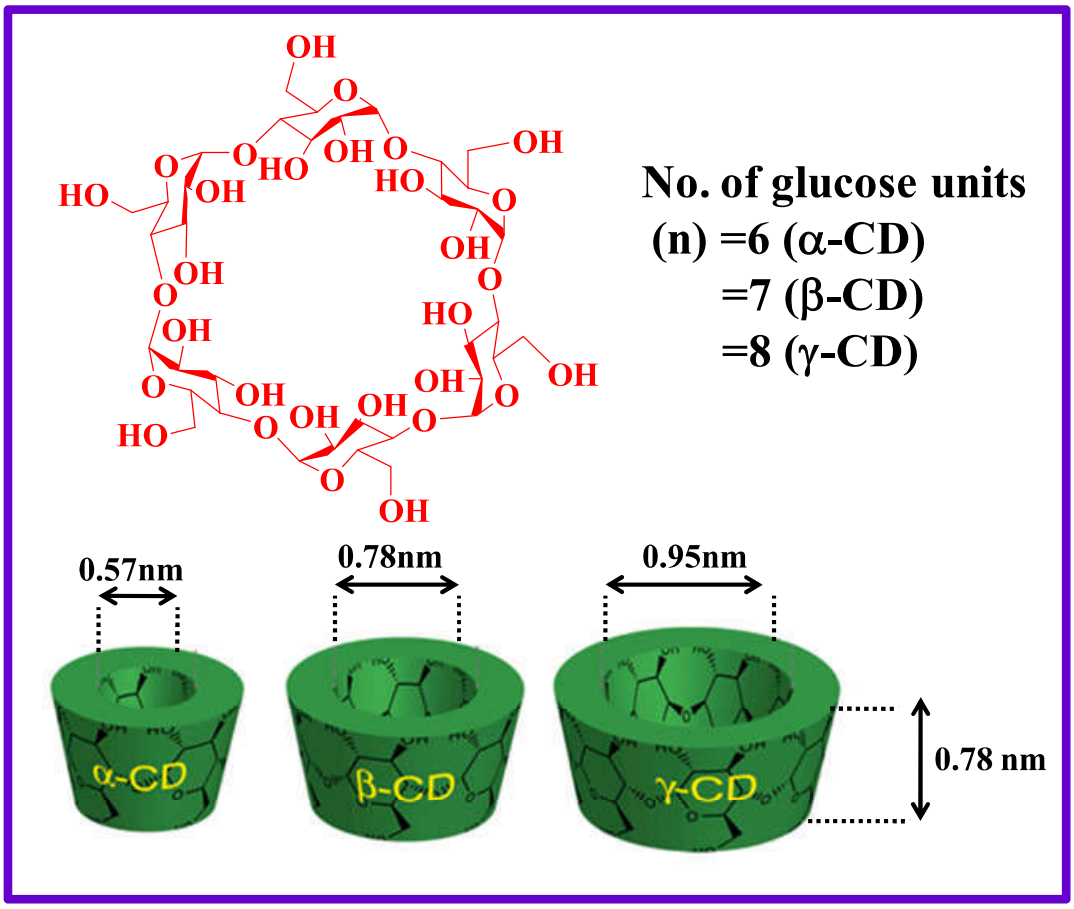

Figure 1.7: *Structures of cyclodextrin. Top- chemical structure of cyclodextrin, Bottom 3D structure of α, β and γ cyclodextrins with their dimensions.*

1.10.1 Graft copolymers

Chitosan-g-CD scaffolds prepared through freeze drying were biodegradable and were found suitable for ketoprofen release in controlled manner. Crosslinking in scaffold controlled the morphology, swelling and drug release profiles. It has been currently observed that binding of chitosan-g-βCD with insulin occurs through electrostatic and host guest interactions forming supramolecular aggregates over different pH range, protecting insulin from digestive enzymes. [99] Hydrophobic modification of CDs has been done for development of sustained release carriers. For such type of carriers alkylated and acylated

derivates, such as heptakis(2,3,6-tri-O-ethyl)-β-CD and heptakis(2,3,6- tri-O-butyryl)-β-CD were synthesized by [100]. CD-containing polymers of different architectures are prepared for obtaining materials with multiple recognition sites to improve the biocompatibility of polymers in medical applications and producing functional materials for control drug release and gene therapy[101]. Davis's and coworkers fabricated and prepared a chain of linear polymers having β-CD in the main chain which are utilized intensively for the controlled drug release and gene therapy applications. For drug release applications β-CD based linear polymers with flanking carboxylic acid groups were prepared through polycondensation of diamino β-CD derivative with difunctionalised PEG co-monomer. These prepared polymers were biocompatible, camptothecin (CPT) was covalently conjugated with CDPs. β-CD based amphiphilic copolymers were prepared for drug delivery, in which drug encapsulation was done by physical encapsulation and covalent conjugation.[102] [103],[104]

1.10.2 Nanoparticles

Preparation of pH responsive nano formulations based of CDs has been reported where, actalation of α cyclodextrin with various acetal types and controlled acetal linkages have been prepared for controlled drug delivery of anticancer drug (PTX). pH responsive hydrolysis and drug release from these nano formulations significantly enhanced antitumor effect against various cancer cells[105]. Fictionalization of surface of mesoporous silica nanoparticles with amino β CD rings with disulfide bonds for drug entrapment (DOX) inside nanoparticles and specific targeting to cancer cells have been studied. PEG was functionalized with admantane unit at one end and with folate group at other end followed by its immobilization on nanoparticle surface by strong CD-AD complexation (***Figure***

1.8a). The drug release from nanoparticle is triggered by acidic endosomal pH followed by disulfide cleavage in high glutathione in cytoplasm further promoting release from vehicle (***Figure 1.8b)***. The better efficacy of drug release from nanoparticles is attributed to combined effect of folate targeting and stimulus triggered release which is very well reflected in cell killing with varying drug concentration as a function of time ***(Figure 1.8c)*** [106].

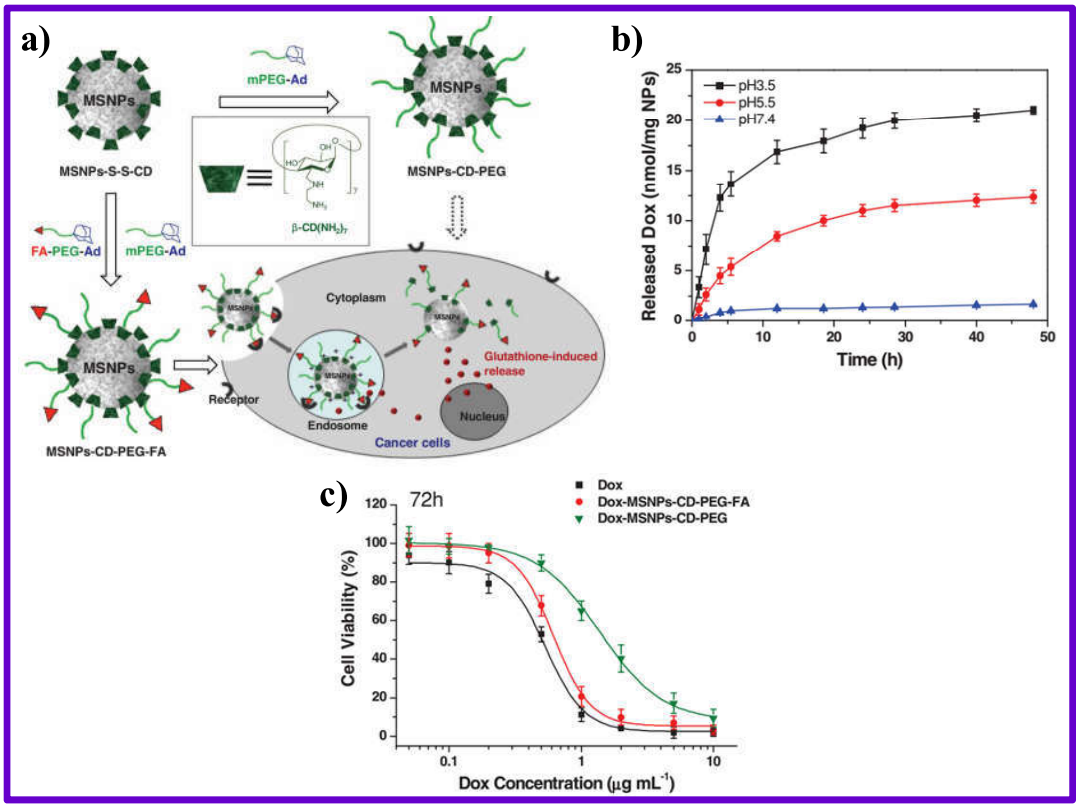

Figure 1.8: *a) Schematic presentation of multifunctional MSNPs-CD-PEG-FA for targeted and controlled drug delivery; b) Release profiles of Dox loaded MSNPs-S-S-CD under different pH environment at 37 °C; c) Cell viability of HeLa cells after incubation with free Dox, Dox-MSNPs-CD-PEG-FA, or Dox-MSNPs-CD-PEG at different Dox doses.*

1.10.3 Dendrimers

Anticancerous drugs DOX and CPT and a photensitizer porphyrin have been loaded onto β CD conjugated GO and β CD and DEN conjugated GO exhibited controlled release, better cellular uptake and antitumor activity.[107] PEGylated-α-CD/CyD polyseudorotaxenes systems are novel systems for sustained release of DNA glucronylglucosyl-β-CD (GUG-β-CD) conjugates with dendrimer G2 are known to show superior gene transfer activity and are thus promising material for cell specific and safe delivery of DNA.[108]

1.10.4 Hydrogels

Supramolecular hydrogels developed from inclusion complex of macrocyclic α CD and polyethylene glycol are excellent biomaterial and possessed huge potential for its application in biomedical field. Rheological properties of supramolecular hydrogels were improved by incorporating nucleo bases guanine and cytosine and mixing G-PEG-G /C-PEG-C with α CD solution. *In vitro* DOX release from supramolecular hydrogel showed excellent cytocompatibility.[109] Host guest interactions between supramolecular hydrogel of admantane and β CD have been investigated extensively showing self healing and injectable properties. Dendritic like multifunctional polymer composed of β-CD-PEG conjugate via Michael addition have been prepared. DOX embedded in hydrogel precursor has been prepared by simple mixing of Dendron like CD-PEG with AD-DOX in aqueous medium via host guest interactions followed by their crosslinking with poly[oligo(ethylene glycol) mercaptosuccinate] (POEGMS) via thiol ene click reaction forming injectable DOX loaded hydrogels with higher stability[21]. A novel injectable hydrogel with high drug content loaded in (PTX-mPECT NP/α-CDgel) has been prepared by inclusion complexes of PTX-loaded mPECT (methoxy poly(ethylene glycol)-b-poly(ε-caprolactone-co-1,4,8-

trioxa[4.6]spiro-9-un-decanone)) nanoparticles (PTX-mPECT NPs) and α-cyclodextrin (α-CD). Sustained release of drug was observed and higher cellular uptake resulted in efficient killing of tumor cells thereby a promising candidate for local and sustained drug delivery.[110] Alio et al reported the combined delivery of paclitaxel and curcumin complexed with PCDT Poly(β-cyclodextrin triazine) a new polymeric drug carrier for cancer models: ovarian, lung, prostrate and breast. A highly synergistic interaction between two drugs complexed with PCDT for human ovarian carcinoma and human nosmall lung carcinoma cells was observed. [111]

1.10.5 Nanofibers

Electrospun nanofiber composed of inclusion complex of Naproxem (NAP) with CD incorporated in PCL matrix via electrospinning have been prepared for controlled drug release and cancer treatment. The release of NAP from PCL naoofiber and PCL/NAP-bCD-IC showed higher release due to enhanced solubility of NAP by CD inclusion complex.[112] Chen et al developed silk fibroin based electrospun nanofiber and infused with hydroxyl propylcyclodextrin as delivery vehicle for tamoxifen. The slow and sustained release of TAM about 50 % in two weeks from fiber matrix was observed suggesting an outstanding drug delivery system for breast cancer therapy.[113]

1.11 Scope and objectives of present work

From the above detailed discussion of introduction, it has been found that controlled drug release through polymer formulations have been studies extensively especially biopolymers due to their several advantages offered. Moreover Cyclodextrin based polymers offers more benefits apart from biocompatibility i.e. the host guest inclusion complex with several small molecules and diverse functionalization of CD into different architectures as per

requirement. Through grafting polyurethane on CD with different graft density and graft length plays an important role in drug release which has not been reported in literature. Further, the controlled drug delivery using CD-PU hydrogels/films have not been explored till date for cancer treatment, though huge number of CD based hydrogels been reported for cancer treatment. Therefore the main objective of present work is to prepare different polymeric architectures based on CD/dextrin and polyurethane with different properties by changing the chemistry for regulating the release of drug. Further the objective is to see the efficacy on sustained release in cellular studies as well as *in vivo* studies for better cancer treatment. The main aim of the present work is embraced in following sub-objectives.

1.12 Plan of present work

To accomplish above mentioned objectives, the following plan of work are executed

a) Efficacy of polyurethane graft on cyclodextrin to control drug release for tumor treatment

- Synthesis of CD grafted polyurethane of various graft density with long PU chain for controlled drug release and cancer treatment.
- Confirming the grafting through spectroscopic techniques and investigating the thermal and mechanical properties of prepared graft copolymers.
- *In vitro* drug release study after embedding drug in copolymers and evaluating the efficacy of grafting.
- Analyzing the biocompatibility of copolymers and comparing the cell killing efficiency of pure drug with graft copolymers.

- Demonstration of *in vivo* studies on melanoma mice to study the real efficacy of sustained drug release for melanoma treatment using drug loaded patch of copolymer.
- b) Grafted Cyclodextrin as carrier for control drug delivery and efficient cell killing.
- Synthesis of different graft density of CD-PU copolymers with shorter polyurethane chain.
- Performing different spectroscopic characterization to proof grafting and also studying the thermal and mechanical responses of prepared copolymers.
- *In vitro* drug release study from drug loaded copolymers and interactions between drug and polymer.
- Biocompatibility and cell adhesion studies of developed copolymers.
- *In vitro* cytotoxicity studies from drug loaded copolymers and pure drug as function of time.
- c) Third Generation Cyclodextrin Graft with Polyurethane Embedded in Hydrogel for a Sustained Drug Release: Complete Shrinkage of Melanoma.
- Synthesis of different generation of CD using small spacer and finally grafting with PU forming superstructure.
- Spectroscopic and thermal characterization for proof of grafting and stability of prepared generations.
- *In vitro* release of anti cancerous drug paclitaxel from different generations.
- Demonstration of biocompatibility and cell killing efficiency of pure drug and prepared different generations.

- Preparation of injectable gel from superstructure and its application for *in vivo* melanoma studies.
- Effect of treatment on body weight, survival rate, vital organs and distribution of drug in blood.
- d) Modified biopolymer dextrin grafted polyurethane copolymer hydrogels for sustained drug release and Melanoma treatment
- Grafting of polyurethane onto dextrin and tuning the graft density of PU.
- Evidences for grafting through spectroscopic techniques and studying the thermal and mechanical stability through TGA and UTM.
- *In vitro* drug release from prepared brush polymers and hydrogel.
- Demonstration of biocompatibility, cellular uptake and cell killing efficiency of pure drug and prepared different graft copolymers.
- Application of hydrogel for *in vivo* melanoma studies.
- Effect of treatment on body weight, survival rate, vital organs, distribution of drug in blood and immunostaining of cancer tissues.