Review of Literature

2.1 CANCER AND NANOTECHNOLOGY

The "war on cancer" is now in its fourth decade since the National Cancer Act was passed in 1971. Although, cancer has remained as leading cause of death in people among age less than 85 years and surpassed heart disease as the number one killer (Kawasaki & Player, 2005). There are various methods that are brought to combat the fatal disease including surgery, chemotherapy and radiation therapy but the success rate is often marginal and serious side effects are still common (Beeta, 2007). Despite, much progress made in understanding the environmental causes and cellular and molecular biological basis for this dreaded disease, it is still difficult to precisely understand the difference between the cancer cell and its normal counterpart (Kawasaki & Player, 2005). A key issue of drugs used in cancer chemotherapy is the nonspecific toxicity of drug against normal cell as well as tumor cells, limits the dose of the anticancer drugs to be administered to patients (Kim & Lee, 2001). In addition, presently available anticancer drug in market are mostly hydrophobic in nature and requires various surfactants like polyethylene glycol, tween-80, span, Cremophore®EL and ethanol as co-solvent for their solubilization. It is reported that Cremophor® EL causes side effects of hypersentivity, nephrotoxicity and neurotoxicity as well as effects on endothelial and vascular muscles causing vasodilatation, labored breathing, lethargy and hypotension (Weiss et al., 1990). Limitations in cancer treatment are a result of current challenges seen in cancer therapies today, including lack of early disease detection, nonspecific systemic distribution, inadequate drug concentrations reaching the tumor, and inability to monitor therapeutic responses (Beeta, 2007). Hence, the ultimate goal of cancer therapy is to increase the survival time and quality of life of the patient and can be achieved by design of novel drug delivery systems which have site specificity with decrease in the serious side effects that cancer patients often experience (Danhier et al., 2009).

Nanotechnology has the potential to offer solutions to these current obstacles seen in cancer therapies, because of its unique size (1-100 nm) and large surface to volume ratios. Nanoparticles may have properties of self-assembly, stability, specificity, drug encapsulation and biocompatibility as a result of their material composition (Grodzinski et al., 2006). Numerous investigations have shown that both tissue and cell distribution profiles of anticancer drugs can be controlled by their entrapment in

submicronic colloidal systems (Alexis et al., 2008). The most common examples of these nanoscale delivery vehicles (also referred to as nanocarriers) include polymeric nanoparticles, dendrimers, block copolymeric micelles, nanoshells, liposomes and magnetic nanoparticles (Ferrari, 2005). Loading of anticancer drug in such a carrier improves the therapeutic index by increasing drug efficacy, reduced toxicity and renders controlled release over a period of time. Moreover, it also solves the problems related with drug solubility and stability with scope for potentially effective new chemical substances which floundered due to lack of suitable formulations and halted during preclinical or clinical development (Forrest et al., 2006a, b).

A leaky microvasculature and the lack of intact lymphatic system results in to enhanced permeation and retention (EPR) effect and "passive" cancer targeting through the accumulation of nanocarriers in the tumor at a higher concentration that is present in the plasma and in other tissues (Maeda et al, 2000; Damon et al., 2007). More recently, the breakthrough potential of cancer nanotechnology is becoming increasingly recognized with several examples of first generation nanocarriers approved by the FDA for therapeutic (Abraxane®, Doxil®, DaunoXome® and diagnostic (Feridex®) applications (Alexis et al., 2008). However, the use of nanocarrier for passive tumor targeting partly serves the purpose of tumor targeting. These nanocarriers which adhere to cell surfaces without intracellular translocalization may not give additional benefit by retention effect and release drug to extracellular space. In such situation, if the target cells are resistant to anticancer agents by P-gp efflux mechanism, it will greatly limit the bioavaibility in cells (Bae, 2009).

Thus, cell internalization of nanocarriers is essential for effective drug delivery besides enhanced permeation and retention (EPR) effect. This may help in clearing the carriers from the road, deliver drug intracellulary, avoid P-gp efflux function, and thus kill the cells (Bae, 2009). These can be achieved with nanocarriers modified with surface functionalized ligands such as antibodies, aptamers, peptides, or small molecules that recognize tumor-specific or tumor-associated antigens in the tumor microenvironment. The application of nanotechnology to cancer therapy, including the development of "smart" nanoparticles, is indeed an exciting and promising area of investigation.

2.2 BLOCK COPOLYMERIC MICELLES IN DRUG DELIVERY

2.2.1 Introduction

Block copolymers are defined as a combination of different polymer segments in a single polymer chain through various polymerization methods, combining the intrinsic properties of each individual block. Depending on how the monomer repeating units are distributed in the copolymer chains, these copolymers may present a variety of macromolecular architectures such as random, alternating, block, and graft copolymers (Kumar et al., 2001). Block copolymers with amphiphilic character, having large solubility difference are known to assemble in an aqueous milieu into polymeric micelles with mesoscopic size range (Kataoka et al., 2001). The capacity of block copolymer micelles to increase the solubility of hydrophobic molecules is due to its hydrophobic core which is sterically stabilized by a hydrophilic corona. The hydrophobic core part serves as a reservoir, in which the drug molecules can be incorporated by means of chemical, physical or electrostatic interactions, depending on their physicochemical properties (Genevieve et al., 2005).

2.2.2 Types of amphiphilic block copolymers

Amphiphilic block copolymers are generally composed of biocompatible, biodegradable hydrophobic polymer blocks such as polyesters or poly (amino acids) covalently bonded to a biocompatible hydrophilic block. The hydrophobic part is generally composed of polymer like polylactic acid, poly (caprolactone), poly(lactic-co-glycolic acid), poly(b-benzyl-L-aspartate) and polybutidine which is covalently linked to hydrophilic block like polyethylene glycol, poly(N-vinyl-2-pyrrolidone), poly(2-ethyl-2-oxazoline) or poly(acrylic acid) (Letchford & Burt, 2007). Block copolymers are classified into several types by sequential arrangement of component segment as representative block copolymers (Figure 2.1). The simplest block copolymer is AB-type block copolymer, which is composed of one segment of A unit of homopolymer with B units of other homopolymer (Figure 2.1 a). In the second type of copolymer, both terminals of B unit is connected at the terminal of A unit, and thus it is referred as an ABA type or tri-block copolymer (Figure 2.1 b). In the third type of block copolymers, A and B segments is connected many times and referred to as multiblock copolymer (Figure 2.1 c). The fourth type of block copolymers is star block copolymers (Figure 2.1 d). In this family of block copolymers, unit A has multi-arm functionality and copolymerizes with the block of B and shows a star-like shape.

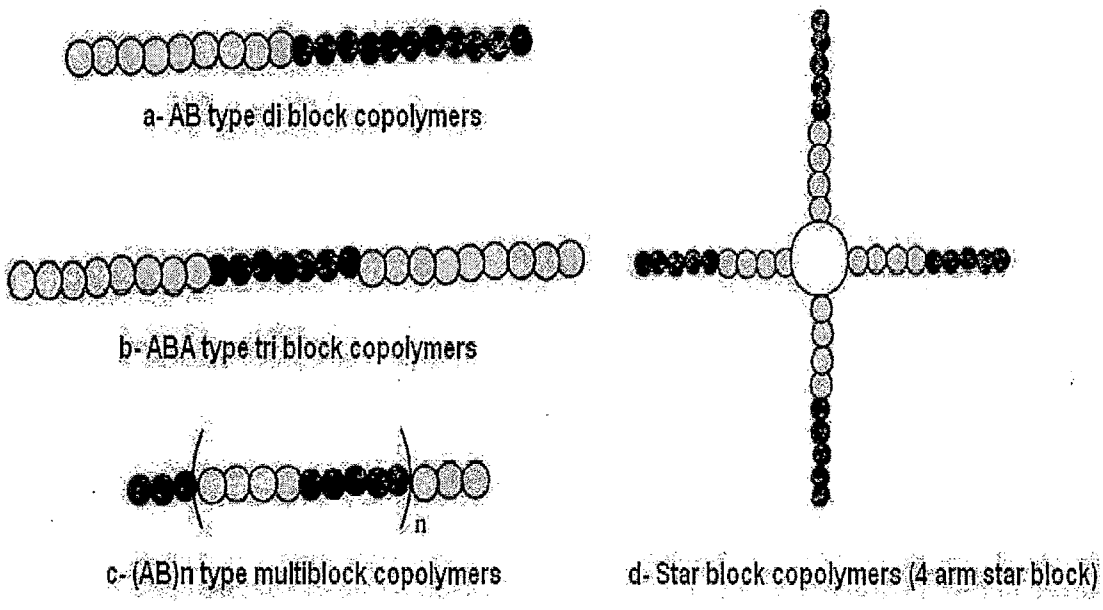


Figure 2.1 Types of block copolymers

To date, numerous block copolymers have been synthesized, not only with a variety of block combinations, but also varying hydrophilic and hydrophobic block lengths. These polymers are obtained by the polymerization of more than one type of monomer, typically one hydrophobic and one hydrophilic, so that the resulting molecule is composed of regions that have opposite affinities for an aqueous solvent. Block copolymers can be polymerized by large number of well known techniques including anionic, cationic, ring-opening, photo, group transfer and Ziegler/Natta polymerization. Among this the most widely used technique is living polymerization, in which the molecular weight of the individual block, the volume ratio and the block arrangement (AB, ABA, BAB) can be adjusted in a desired manner (Kumar et al., 2001).

2.2.3 Formation of block copolymeric micelles

Depending on the physicochemical properties of the block copolymer, two main classes of drug loading procedures can be applied (Allen et al., 1999). The first class, direct dissolution, involves dissolving the block copolymer along with the drug in an

aqueous solvent. This procedure is mostly employed for moderately hydrophobic copolymers, such as poloxamers and may require heating of the aqueous solution to bring about micellization via the dehydration of the core forming segments (Letchford & Burt, 2007; Genevieve et al., 2005).

The second category of drug-loading procedures applies to amphiphilic copolymers which are not readily soluble in water and for which an organic solvent common to both the copolymer and drug (such as dimethylsulfoxide, N,N-dimethylformamide, acetonitrile, tetrahydrofuran, acetone or dimethylacetamide) is needed. The mechanism by which micelle formation is induced depends on the solvent-removal procedure. For water-miscible organic solvents, the copolymer mixture can be dialyzed against water, whereby slow removal of the organic phase triggers micellization (Genevieve et al., 2005). Alternatively, the solution-casting method entails evaporation of the organic phase to yield a polymeric film where polymer-drug interactions are favored. Rehydration of the film with a heated aqueous solvent produces drug-loaded micelles (Lee et al., 2007). Physical entrapment of a hydrophobic drug may be further achieved through an oil-in-water (O/W) emulsion process which involves the use of a non-water-miscible organic solvent (viz. dichloromethane, ethyl acetate). Micelles can also be formed by nanoprecipitation method in case where molecular weight of hydrophobic part exceeds to that of hydrophilic part. This method involves the dissolution of the polymer in an organic, water-miscible solvent, which is then added to the aqueous phase in the presence or absence of a surfactant. Upon addition to the aqueous phase, the organic solvent immediately diffuses out leading to the precipitation of the polymer and formation of nanoparticles (Galindo et al., 2004).

Several factors reported to affect on the loading of drug are type of preparation technique, nature and proportion of organic solvent and affinity between drug and core part. Disparities among micelles prepared by different methods were also encountered by Vangeyte et al. (2004). They demonstrated that, in the case of PEG-b-PCL micelles, the dialysis procedure did not offer adequate size control, whereas stable assemblies with unimodal size distributions were formed by rapid precipitation under stirring (addition of the organic phase containing the copolymer to the aqueous phase or vice versa, followed by dialysis against water).

2.2.4 Stability of micelles

Physical stability is the basic requirement for a micelle drug delivery system to withstand dissociation and premature release of its cargo after entry into the bloodstream. It is well-established that blood proteins are likely to adsorb onto the surface of charged or hydrophobic foreign matter and accelerate its clearance from the blood circulation. Moreover, protein binding may disrupt micelle cohesion as well as trigger premature drug release from the carrier in vivo (Liu et al., 2007). Protein-carrier interactions studies are necessary for evaluation of the targeting potential of a given drug delivery system. Toncheva et al. (2003) applied dynamic light scattering method to track the stability of PEG-b-poly (ortho ester)-b-PEG micelles following incubation at 37 °C in the presence of bovine serum albumin. Their results showed that the impact of serum proteins on the stability of micelles differs significantly according to the nature and composition of the block copolymer. Likewise, interactions between drug and protein molecules can be liable for rapid dissociation of the drug from the vector. While the binding of proteins to PEG-b-poly (5-benzyloxy-trimethylene carbonate) micelles was found to be insignificant, the release of the hydrophobic drug ellipticine was accelerated in the presence of bovine serum albumin (Liu et al., 2005a). It is argued that assessing the partitioning of the drug between the micellar system and the aqueous medium containing protein may allow for a more accurate prediction of the system's fate in vivo.

There are various strategies employed to increase the physical stability of micelles. Critical micelles concentration (CMC) is one of the parameter which decides the in vivo fate of micelles after intravenous administrations. If the polymer concentration falls below the CMC, micelles dissociation starts and destabilizes the systems. In contrast to low molecular weight surfactants, polymeric micelles exhibit significantly lower CMC values, indicating greater thermodynamic stability. It is noticed that the rate at which the micelles tend to dissociate is related to its composition, physical state and cohesion of the core. Studies carried out by various groups of scientist showed that micellar stability can be increased by increase in the chain length of hydrophobic core part (Bin et al., 2005; Jette et al., 2004). Other groups have focused on modifying the properties of the core forming attempt to enhance their hydrophobicity. For instance, Adams et al. (2003) developed PEG-b-poly(N-hexyl-l-

aspartamide) copolymers containing acyl side chains of varying lengths conjugated to the aspartic acid core segments.

Micelle stability is also strongly related to the physical state of the core-forming polymer, be it amorphous or crystalline. Block copolymer micelles comprised of a hydrophobic block with a glass transition temperature (Tg) exceeding 37 °C are said to have "frozen" cores, i.e. the molecular motions of the chains in the core are constrained, generally accounting for greater kinetic stability upon dilution (Allen et al., 1999). Burt et al. (1999) reported the superior stability of paclitaxel-loaded PEG-b-PDLLA versus PEG-b-P(DLLA-co-CL) micelles, resulting from the reduced fluidity of the PDLLA core (as determined by fluorescence intensity and anisotropy techniques). This finding correlates with the higher Tg of the PDLLA block. Block copolymer micelles can be made up of a crystalline core. Crystallinity contributes to micelle stability and may confer greater drug retention properties by decreasing the rate of diffusion of the drug from the core. Stereocomplex formation using blend of enantiomeric copolymers resulted in to formation of a crystalline triclinic unit cell (Slager & Domb, 2003), which makes the core crystalline in nature. Enhanced stability of the micellar system developed by Kang et al. (2005) was due to strong van der Waals interactions between PLA chains in the stereocomplexes, resulting in a more compact conformation and denser packing of the polymer.

Cross-linking of the shell or core of hydrophobic micelles is yet another promising stratagem to improve the stability of micelles and is often evidenced by a decrease or even the absence of a CMC (Thurmond et al., 1999). Shuai et al. (2004) performed core cross-linking of paclitaxel-loaded PEG-b-PCL micelles by radical polymerization of double bonds introduced into the PCL blocks. These micelles exhibited significantly enhanced stability against dilution with aqueous solvents, i.e. no micelle collapse was detected upon dilution to 1000 times, as determined by dynamic light scattering (DLS) and gel permeation chromatography.

Likewise, strong cohesive forces between the drug and the polymer core segments can also confer physical stability to the system. Lee et al. (2004) demonstrated that PEG-b-PDLLA copolymers with additional carboxylic acid groups could establish hydrogen bonds with the incorporated drug papaverine. These interactions resulted in

a controlled release profile in vitro wherein 90 % of the drug was released within 7 days versus 10 h for micelles without COOH moieties. A drug may otherwise be covalently conjugated to the core-forming polymer through various linkers, which consist mainly of hydrolysable bonds (including esters (Yu et al., 2000), amides (Yoo et al., 2004), or pH-sensitive bonds such as β-thiopropionate (Oishi et al., 2005) and cis-acotinyl (Yoo et al., 2002).

2.2.5 Functionalized block copolymeric micelles

Majority of research work for anticancer drug delivery has been carried out using stealth polymeric micellar systems for passive accumulation inside tumor due to well known enhanced permeability and retention (EPR) effect (Maeda et al., 2000), but they are still cleared by RES system, resulting in short half lives and unwanted micelles deposition in the liver and spleen (Yamamoto et al., 2001). Consequently, insufficient uptake at tumor sites will decrease the therapeutic benefit of the administered drug dose, and non-specific association with healthy tissues can lead to toxic side effects, limiting the maximum dosage that can be safely applied. This limitation prevents drug-loaded micelles from achieving the potential therapeutic effects they might otherwise attain (Mariano et al., 2006). Development of multifunctional micelles, either through conjugation of targeting ligands on the micelle surface or a triggered release mechanism, can lessen these problems by increasing particle/drug exposure to the tumor. Figure 2.2 illustrates the different types of functionality that have been introduced to micelle structures.

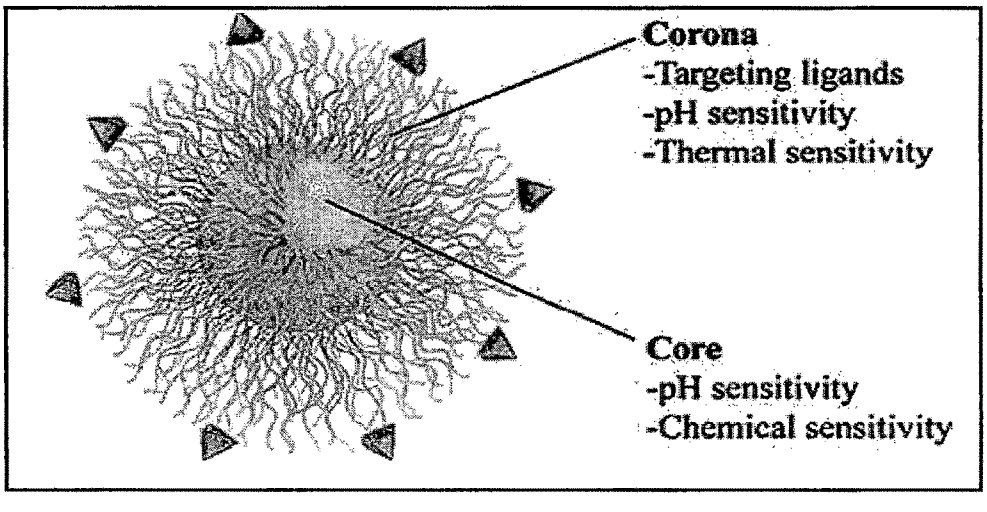


Figure 2.2 Schematic diagrams of functionalized polymer micelles with active targeting to tumors and responsive drug release properties

2.2.5.1 Ligand conjugated micelles

To achieve tumor-selective and cell specific localization of micelles, a pilot molecules (ligands) are required to conjugate at the terminal end of the hydrophilic part so that they may extend outward from the micelle corona and readily encounter and interact with membrane receptors. The principal purpose of attachment of ligand is to modulate the biodistribution of polymeric micelles and induce specific cellular uptake by receptor-mediated endocytosis. This can be particularly useful for the intracellular delivery of drug which is required particularly for tumor treatment. Tumor cells are known to overexpress certain receptors compared to normal cells and the localization of such site-specific receptors has contributed to several advancements in the field of targeted drug delivery. The tethering of a ligand to the outer shell of micelles is most often achieved through the post insertion method or pre-insertion technique. Two different strategies were applied for ligand attachment to the micelle. In one case, the ligand was attached to the copolymer before micelle self assembly (Lee et al., 2007). In the second case, the micelles were assembled first, and the ligand attached afterwards, a method that would ensure that the ligand was attached to chemically available sites on the micelle surface (Nasongkla et al., 2004). The common ligands which were utilized for conjugation of micelles are folic acid, peptides and proteins (cRGD, biotin), antibodies, aptamers and carbohydrates.

i) Folate functionalization

The folate receptor, a 38 kDa glycosyl-phosphatidylinositol-anchored glycoprotein, is one of the most highly researched targets for cancer therapeutics (Sudimack & Lee, 2000; Yoo & Park, 2004). The expression levels of this receptor in tumors have been reported to be 100 to 300 times higher than those observed in normal tissue (Ross et al., 1994). Since folic acid is required for essential cell function, the cargo attached to the ligand is retained within an endocytic vesicle or released into the cytoplasm. Zhao & Yung (2008) prepared folate conjugated PEG-PLGA micelles loaded with drug doxorubicin. In vitro cytotoxicity and cellular uptake studies of these micelles were carried out against three cancer cell lines (KB, MATB III and C6) and normal fibroblast cell line (CCL-110). The cytotoxicity of PLGA–PEG–FOL micelles to cancer cells was found much higher than that of normal fibroblast cells, demonstrating that the folate conjugated micelles has the ability to selectively target to cancer cells. These group also studied the optimum folate amount required for

maximum cellular uptake and observed that for KB cells, the cellular uptake increased with more folate conjugation and peaked at 65% folate content.

Folic acid was conjugated to PEG-PLGA micelles by covalently conjugating the ligand via its carboxyl group (Yoo & Park, 2004). In vitro cytotoxicity studies of the folate conjugated micelles against KB cells showed little enhancement in cell uptake and cytotoxicity over non-targeted micelles Despite this small increase in invitro cytotoxicity, the targeted micelles showed marked improvement in in-vivo antitumor efficacy with two times decrease in the growth rate compared to non-targeted micelle control.

Park et al. (2005) also conjugated folic acid as a targeting ligand to the hydrophobic end of PEG-PCL block copolymer and prepared micelles loaded with paclitaxel. It is anomalous to conjugate folic acid to the core part of micelles which will not provide an additional benefit compared to non conjugated micelles. These folate conjugated micelles were showed increased in toxicities to two cancer cell lines MCF-7 & HeLa 229 with folate receptor expression, but not in the normal fibroblasts cells.

Recently, Bae et al. (2005) developed micelles with folate conjugate to the end of the PEO shell of PEO-*b*-PBLA and found that these micelles can be an excellent intelligent nanodevice for actively delivering drugs inside the cell via selective protein-binding affinity. Shao-Qiong Liu and colleagues (2007) developed bio-functional micelles with folate conjugation and pH sensitive property. Folate was conjugated to synthesized polymer poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide-co-2-aminoethyl methacrylate)-b-PUA and their micellar formulation was formulated with drug doxorubicin. The cytotoxicity observed for folate conjugated micelles was 2 and 2.5 fold higher against 4T1 and KB cells respectively compared to non conjugated micelles. The in vivo studies showed higher accumulation of folate conjugated micelles compared to free doxorubicin.

ii) Peptide functionalization

cRGD is one of the most studied peptide conjugated to polymeric micelles. Maleimide-terminated poly(ethylene glycol)-poly (ε-caprolactone) (MAL-PEG-PCL)

copolymer was synthesized and conjugated to cRGD ligand after micelle formation loaded with doxorubicin (Nasongkla et al., 2004). Flow cytometry studies show that the percentage of cell uptake increased with increasing cRGD density on the micelle surface. With 5% cRGD surface density, a modest threefold increase of cell uptake was observed, while a more pronounced 30-fold increase was observed by flow cytometry with 76% cRGD attachment. Zeng et al. (2006) investigated the epidermal growth factor peptide (EGF) as ligand for the EGF receptor (EGFR) overexpressing on cancer cells, attached with the polymeric micelles of block copolymer of poly(ethylene glycol)-*b*-poly(δ-valerolactone) PEG-*b*-PVL. The targeted micelles loaded with CM-DiI (hydrophobic fluorescent probe) accumulated intracellularly in EGFR-overexpressing MDA-MB-468 breast cancer cells following a 2 h incubation period, while no detectable cell uptake was observed for the nontargeted micelles. Lee et al. (2007) developed an apoptotic EGF-conjugated micelle of PEO-*b*-PCL block copolymer system and found that these micelles were found to be more potent than free EGF at inhibiting EGFR-overexpressing breast cancer cell growth.

PEO-*b*-PCL block copolymers conjugated with RGD (arginine-glycine-aspartic acid) as a ligand was able to recognize integrins receptors overexpressed on the surface of metastatic B16F10 cancer cell line (Xiong et al., 2007). These RGD conjugated micelles exhibited pronounced cellular uptake compared to unconjugated PEO-*b*-PCL micelles and in that sense are promising ligand targeted carriers for drug delivery to metastatic tumor cells. Sethuraman & Bae (2007) developed TAT based micelles loaded with doxorubicin. The system is based on the complexation of block copolymer of (methacryloyl sulfadimethoxine) (PSD) and PEG (PSD-*b*-PEG) to cationic TAT micelle constituted of poly(L-lactic acid) (PLLA) core and a hydrophilic shell consisting of polyethylene glycol (PEG) conjugated to TAT (TAT micelle). These systems could target tumor areas that provide an acidic profile. The designed micellar system was able to effectively distinguish a small difference in pH and internalize into cells. Interestingly, the TAT functionalization helped the micelles' translocation not only into the cells but also near the nucleus.

iii) Carbohydrate functionalization

Targeting efficacy of anticancer drugs can also be improved by nanocarrier conjugation with bio-recognizable groups such as carbohydrates. Asialoglycoprotein

receptor (ASGPR) is a membrane lectin receptor that is commonly found in liver cells (Gilbert & Joe, 1982). Carbohydrate molecules such as galactose and mannose are found as specific ligands to this receptor (Jansen et al., 1991; Goto et al., 1994). Young et al. (2005) synthesized galactose-conjugated poly(ethylene glycol)-copoly(g-benzyl L-glutamate) block copolymer (gal-PEG-b- PBLG) micelles loaded with paclitaxel and evaluated against ASGPR expressing cancer cell line. They observed greater uptake of these micelles with a 30% increase in cytotoxicity compared to non-ASGPR expressing cell line SK-Hep01. Wang et al. (2008) developed micelles based on diblock copolymer of poly(ethyl ethylene phosphate) and poly(ε-caprolactone) and surface conjugated with galactosamine for ASGPR targeting of HepG2 cells. Paclitaxel-loaded micelles with galactosamine ligands exhibited comparable activity to free paclitaxel in inhibiting HepG2 cell proliferation, in contrast to the poor inhibition activity of micelles without galactose ligands particularly at lower paclitaxel doses.

iv) Monoclonal antibodies functionalization

Antibodies are the first macromolecular ligands used for targeted delivery (Frank et al., 2008). The use of monoclonal antibodies (mAb) became widespread after the discovery of hybridoma technology. Due to it's inherent immunogenicity, murine monoclonal antibodies were not suitable for clinical applications. Engineering antibody technologies led to the development of chimeric humanized and fully humanized antibodies. Recent advances in protein engineering have led to the development of single chain antibodies, antibody fragments (Fab or scFv) and dibodies (Weiner & Adams, 2000). Antibody fragments show less immune response and can be stabilized with disulfide bond or chemically crosslinked (Presta, 2006).

Recently, brain targeting of PEG-PCL polymerosomes was evaluated using mouse-anti-rat monoclonal antibody OX26 as ligand after conjugation to maleimide group present on polymerosomes (Pang et al., 2008). Coupling of OX-26 to polymerosomes was confirmed by immuno-gold labeling of OX-26 using TEM and X-ray photoelectron spectroscopy test. They found that the optimized number of OX-26 per polymerosome of 34 resulted into significantly enhanced brain delivery of NC- 1900 peptide with ameliorating the scopolamine-induced learning and memory impairments

in a water maze task via i.v. administration. Taiho and his colleagues (2008) has conjugated anti-EGFR antibody to NHS activated PEG-PCL copolymer and developed micellar formulation loaded with doxorubicin. These micelles showed higher cytotoxicity and apoptosis than free doxorubicin as well non conjugated micelles. Further more, presence of anti-EGFR antibody on the doxorubicin micelles increased the internalization and nuclear accumulation of doxorubicin and enhanced the drug induced cell death.

Selective tumor accumulation of paclitaxel encapsulated in PEO–distearyl phosphatidylethanolamine micelles conjugated to tumor-specific antinucleosome antibody 2C5 (''immunomicelles'') was reported by Lukyanov et al. (2003) for the Lewis lung carcinoma mouse model. Torchilin et al. (2003) developed these ligands to target micelles to lung cancer cells. Diacyllipid-PEG-conjugated polymer (PEG-PE) micelles were functionalized with one of two antibodies, either an anti-cancer monoclonal antibody (mAb 2C5), or an anti-myosin mAb 2G4. Both antibodies retained their ability to bind to their substrates after conjugation to micelles. Moreover, the 2C5 antibody targeted micelle was loaded with paclitaxel and induced a fourfold increase in drug accumulation at the tumor after 2 h, with a corresponding increase in anti-tumor efficacy.

v) Aptamer functionalization

Aptamers are DNA or RNA oligonucleotides that can be identified from screening a random library to specific molecular targets (Ellington & Szostak, 1990). These agents are stable in vitro, tumor specific, generally considered non-immunogenic and provide a new targeting platform for micellar drug delivery applications. Frank et al. (2008) used an RNA aptamer for the prostate specific membrane antigen (PSMA) to target PEG-PLGA nanoparticles to LNCaP cell induced xenograft mouse models. Aptamer surface density was varied using distinct ratios of PLGA-*b*-PEG-*b*-Apt TCP with PLGA-*b*-PEG and optimized the aptamer surface density for maximum cellular uptake with maximum exposure of PEG and its antibiofouling properties. A maximum tumor accumulation of nanoparticles was observed with aptamer density of 5 %, while increase in density to 10 % resulted into higher accumulation of nanoparticle to liver and spleen. They suggested that a proper balance between tumor targeting ligand surface density and the antibiofouling surface properties of PEG are

required to achieve tumor targeting with less accumulation of nanoparticle to RES system. These groups have also evaluated the effectiveness of RNA aptamer conjugated PEG-PLA nanoparticle to target prostate tumors (Omid et al., 2006). In vivo study carried out via intra-tumoral injection of the micelles into LNCaP xenografts in a nude mouse model showed significant increase in anti-tumor efficacy over their non-targeted control. In addition, aptamer conjugated nanoparticles were able to induce total tumor regression in five of the seven mice in the group compared to the non-targeted nanoparticles resulted in only two of the seven mice.

vi) Hyaluronic acid and biotin functionalization

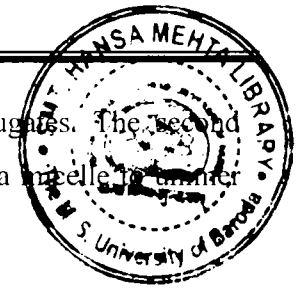
Hyaluronic acid and biotin were also utilized as ligand for tumor targeted drug delivery of block copolymeric nanoparticles. Kim et al. (2007) developed biotin conjugated PEG-PCL nanoparticles loaded with anticancer drug paclitaxel. A significant increase in cytotoxicity of biotin conjugated nanoparticles against HeLa cells was observed compared to nanoparticles without biotin. Hyaluronic acid conjugated PEG-PLGA nanoparticles (HA-PEG-PLGA) were developed by Yadav et al. (2007) for doxorubicin delivery to Ehrlich ascites tumor–bearing mice. The biodistribution study by radiolabeling technique showed higher accumulation of HA-PEG-PLGA nanoparticles compared to MPEG-PLGA nanoparticles. A tumor growth inhibition study showed significant reduction in tumor volume with HA-PEG-PLGA nanoparticles compared to MPEG-PLGA.

2.2.5.2 Stimuli-responsive Polymeric Micelles

The words 'intelligent', 'smart,' or 'stimulus-responsive' are synonyms with regard to polymer nanoparticle systems that should present large, sharp changes in response to physical stimuli (such as temperature, solvents, or light) or to chemical stimuli (such as reactants, pH, ions in solution, or chemical recognition). Stimuli-responsive polymeric systems (micelles, vesicles, etc.) are progressive in the way to enhance the efficacy of drugs with selectively delivery on the targeted organ as well as to reduce side effects.

i) pH Dependent Systems

Basically, two different strategies to induce a controlled drug release with pH have been developed. The first one concerns the grafting of the drug using a pH-sensitive



cleavable group, and thus forming block copolymer-drug conjugates. The second method consists of using a pH-sensitive polymer, able to induce a micelle to unimer transition at the targeted pH value.

Yoo et al. (2002) prepared PEO-*b*-PLLA with doxorubicin conjugated to the terminal group of the PLLA block by an acid-sensitive cis-aconityl or hydrazone linkage. Hydrazone linkage of micelles with doxorubicin showed higher cytotoxicity than free doxorubicin *in vitro*. Bae et al. (2003) reported copolymer doxorubicin conjugate via hydrazone linkage to the aspartic acid units of a PEO-*b*-PAsp and the resulted polymeric micelles showed pH-dependent drug release. In addition, confocal microscopy analysis revealed that the micelles were entrapped in lysosomes and released doxorubicin due to the acidic cleavage of hydrazone in the acidic environment of endocytic vesicles. Acid-labile bonds have also been used as a structural component of the micelle polymer backbone (Gillies & Frechet 2005; Gillies et al., 2004). In this way, the micelle itself can degrade as a function of pH resulting in a micelle that should be pH sensitive regardless of the drug use. Xu et al. (2006) developed cisplatin loaded nanoparticles consist of pH sensitive polymer poly[2-(*N,N*-diethylamino)ethyl methacrylate]-*block*-poly(ethylene glycol) (PDEA-PEG) and their cellular uptake as well localization in lysosomes were visualized by confocal microscopy with help of lysotracker. Confocal images showed the localization of nanoparticle in lysosomes which was assumed to dissolve immediately after protonation of its amine group at acidic pH.

Regardless of the general impression that the body has a uniform pH of 7.4, intravenously administered nanodevices can encounter several instances of pH change that can facilitate drug delivery. Tumors tend to have lower pH values (as low as 5.7) than normal tissue environment (pH 7.4) (Bae et al., 2003). This acidification is due to the general characteristic of cancer cells to rely on glycolysis for metabolism. Changes in pH are also encountered once nanodevices enter cells via endocytosis. Endocytosis is the sequestration of the nanocarriers into an early endosome, which is accompanied by an increase of acidity inside the vesicle as it matures into late endosomes and heavily degradative lysosomes (pH 5.0-5.5). Both the acidic nature of tumor tissue and endocytosis provide ample applications for pH responsive micelles which release their contents upon exposure to acidic environments.

ii) Temperature dependent systems

The ability to raise local temperatures inside the body makes temperature triggered drug release a viable strategy in site-specific drug release (Zee, 2002). Additionally, tumors have been shown to be more vulnerable to hyperthermia than normal tissue as a result of their chaotic vasculature. These two factors give temperature sensitive nanosystems with the capability of providing a synergistic therapy, whereupon the elevated temperature not only causes local drug release but also serves to inflict additional damage to tumor cells. Elevated temperatures have also been proposed as a way to induce micellar aggregation at the tumor site, improving biodistribution of the administered agent. Chung et al. (1999) encapsulated adriamycin in core of micelles made of poly(N-isopropylacrylamide-*b*-butylmethacrylate) (PNIPAM-*b*-PBMA) by optimizing adriamycin and PMBA hydrophobic interactions. The release of adriamycin was obtained above the lower critical solution temperature (LCST) of PNIPAM (32 °C) because of its structural deformation At low temperature, micellar doxorubicin did not interact with the cell because of steric hindrance due to the highly hydrophilic nature of the PNIPAM and no cytotoxicity was observed below the LCST, even at the high adriamycin concentration. However, above the LCST higher and selective cytotoxicity is observed compared to free adriamycin due to release of the drug and enhanced interaction of PNIPAM with the cell resulting from its structural change.

Liu et al. (2005b) also reported pNIPAM/PLGA micelles, in which pNIPAM was copolymerized with dimethylacrylamide to obtain desirable LCST of 39 °C of polymer. The low Tg core of these micelles was also able to be sufficiently deform for temperature sensitive drug release, in particular when the PLGA segment length was short when compared to the hydrophilic region. This explained that a balance must be considered when designing these systems, for longer hydrophobic segments appear to result in larger drug loading contents but a lesser intensity of temperature sensitivity to the release kinetics.

iii) Ultrasound dependent system

Ultrasound used in medicine for diagnostic and therapeutic applications has proven a non-invasive method to access and treat many problems including strokes, osteoporosis, and cardiovascular disease (Mitragotri, 2005) The use of ultrasound as a

means to induce drug release of doxorubicin from pluronic micelles was reported by groups of scientist (Ghaleb et al., 2002; Marin et al., 2002). In absence of ultrasound, pluronic has a synergistic effect with the doxorubicin, increasing the toxicity of the chemotherapeutic agent two fold in spite of decreased uptake of the doxorubicin into the cells. Ultrasound treatment further increased the toxicity of the drug containing micelles to another six fold (Rapoport et al., 1999). In order to explain this, it was proposed that the presence of pluronic unimers aided the toxicity of the drug (Marin et al., 2002) but the micelles actually sequestered drug away from the cells until ultrasound induced drug release (Ghaleb et al., 2002; Marin et al., 2002).

iv) Magnetic responsive Systems

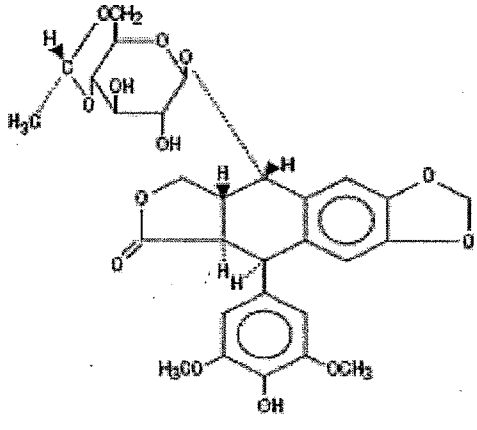
The use of magnetic field toward the development of iron oxide magnetic nanoparticles (γ-Fe2O3) and their use in biomedical applications for magnetic resonance imaging (MRI), magnetic drug targeting, or magnetic fluid hyperthermia is one of the promising approach developed so far (Mornet et al., 2004). Hong et al. (2008) developed folate conjugated PEG-PCL micelles incorporated with doxorubicin and super-paramagnetic ion oxide Fe_3O_4 (SPIO) in the core of micelles. The results of Folate-SPIO-DOX-Micelle acquired by MRI T2 signal intensity suggested that cells treated with the folate targeting micelles decreased significantly while, T2 signal did not show obvious decrease for cells treated with the folate-free micelles. Recently, Chen et al. (2007) developed temperature-responsive magnetic nanoparticles from magnetite nanoparticles and PEO-*b*-PPO-*b*-PEO (Pluronic) block copolymer. Because of the self-assembly of Pluronic copolymers on the magnetite solid surface, the copolymer shell could act as a temperature-controlled "gate" for the upload and release of guest species.

2.3 DRUG PROFILE

Etoposide [VP 16] is a semi-synthetic derivative of epipodopyhllotoxin. It is mainly used for the treatment of lung cancer, testicular cancer, lymphoma and several type of leukemia. ETO was synthesized from podophyllotoxin in 1963. Several phodophyllin components possess considerable anti-neoplastic activity but are very toxic for human use. Out of various semi synthetic derivatives synthesized, ETO proved to be one of the most promising compounds (Keller et al., 1971).

2.3.1 Physicochemical properties

a. **Appearance:** White to off-white, crystalline powder
b. **Category:** Antineoplastic
c. **Generic name:** Etoposide
d. **IUPAC name:** 4′-O-demethyl-1-O-[4,6-O-ethylidene-β-D-glucopyranosyl] epipodophyllotoxin.
e. **Emperical formula:** $C_{29}H_{32}O_{13}$
f. **Molecular weight:** 588.6
g. **Melting point:** 221-222 °C
h. **Solubility:** Very slightly soluble in water; slightly soluble in alcohol, chloroform, dichloromethane and ethyl acetate; sparingly soluble in methyl alcohol.
i. **Structure:**



2.3.2 Mechanism of action

ETO is known to cause single-strand breaks in DNA. ETO also causes DNA damage through inhibition of topoisomerase II and activation of oxidation-reduction reactions to produce derivatives that bind directly to DNA. Topoisomerase II carries out breakage and reunion reactions of DNA which are necessary for normal cellular function. ETO is cell cycle phase specific with predominant activity occurring in late S-phase and G2. (Isseh & Crooke, 1979)

2.3.3 Pharmacokinetics

The pharmacokinetics of ETO is subject to a considerable inter-individual variation, which resulted that after oral administration, about 50% of the ETO is absorbed. It is

rapidly distributed and concentrations in plasma fall in a biphasic manner, with a terminal half-life of 4 to 11 h. ETO is metabolized by the cytochrome P450 iso-enzyme CYP3A4. ETO is excreted in urine and feces as unchanged drug and metabolites: about 45% of a dose is reported to be excreted in urine over 72 hours. It crosses the blood-brain barrier poorly; concentrations in CSF are 1 to 10% of those in plasma. Approximately 74% of the drug was shown to be bound to human serum protein at ETO concentrations of 10μg/ml (Isseh & Crooke, 1979).

2.3.4 Therapeutic uses

It is effective in the treatment of testis and lungs cancer alone and with combination with other drugs. It has been also used to treat other solid tumors including those of the brain, gastrointestinal tract, ovary, thymus and some childhood neoplasm's; in lymphomas, acute leukemia and in the treatment of Kaposi's sarcoma associated with AIDS.

2.3.5 Side effects and toxicity

The common side effects observed with ETO are as follows (Isseh & Crooke, 1979; Aisner & Lee, 1991)

- Decreased white blood cell count with increased risk of infection
- Decreased platelet count with increased risk of bleeding
- Mild nausea and vomiting with loss of appetite
- Changes in taste including metallic taste of foods
- Fetal damage, if pregnancy occurs while taking this drug
- Constipation, diarrhea and pain in stomach
- Allergic reactions
- Temporary loss of hair

2.3.6 Doses and administration

It is given by slow intravenous infusion over at least 30 min, as a solution in sodium chloride 0.9 % or glucose 5 % injection. In the UK, manufacturers recommend that the concentration of the drug in infusion should not exceed 250 μg/ml to avoid the risk of the drug crystallizing out of solution, while in USA an upper limit of 400 μg/ml is considered as acceptable. The usual intravenous dose is 50 to 120

mg/m^2/day of ETO for 5 days. Somewhat lower doses have been suggested in lung cancer. Alternatively 100 mg/ m^2 have been given on alternate days to a total of 300 mg/m2. The usual oral dose of ETO is 100 to 240 mg/ m^2/d for 5 consecutive days. Courses may be repeated after 3 to 4 weeks. Doses should be reduced if renal impairment persists.

2.3.7 Analytical method

There are numbers of analytical methods reported for analysis of ETO in various working solutions and biological fluids. Some of them are mentioned as under.

- Reddy & Murthy (2005) assayed ETO using methanol:chloroform (1:1) at 286 nm by UV visible spectroscopy .
- Kato et al. (2004) developed HPLC method for quantification using C 18-AR, 55μm column using acetonitrile: acetic acid: distilled water (27:1:72) at 254 nm in biological samples.
- Manouilov et al. (1998) assayed the ETO from the human serum using solid phase extraction using HPLC with fluorescence detector. Drug was extracted in the presence of ammonium acetate and methanol and detected at α_{ex}230 nm and α_{em} 330 nm using fluorometric detector. The mobile phase was comprised of methanol, 40 mM KH_2PO_4 (pH 6.9) and 0.14 mM 1-heptansulphonic acid in the ratio 400:600:6.
- Tranchand et al. (1999) proposed the HPLC method in the cancer patients. The drug extracted from plasma by dichloromethane and the detection was done by UV detector at wavelength of 230 nm.
- Shirazi et al. (2001) developed a rapid, simple and sensitive isocratic HPLC method to measure the concentration of ETO in plasma samples with UV detection at 220 nm. The method uses a Bondapac C18 column at 60°C. The mobile phase consists of methanol: water (45:55 v/v) using phenacetin as an internal standard.
- Chen & Uckun (2000) developed method to estimate the ETO in human plasma and serum by LC-MS method. ETO was separated using Lichospher 100 RP-18 (5mm) column (250mmX34mm) with the mobile phase of acetonitrile–water containing 0.1% acetic acid (45/55 v/v) at flow-rate of 0.5 ml/min. Selected-ion monitoring (SIM) mode was performed on *m/z* 589 (positive ion mode) using a fragmentor of 75 V.

2.4 PEPTIDE PROFILE

2.4.1 YIGSR-NH_2

The Tyr-Ile-Gly-Ser-Arg (YIGSR) peptide derived from the residues 929–933 on the β1 chain laminin has been shown to decrease tumor growth and metastasis (Nomizu et al., 1993; Graf et al, 1987). It binds to a high affinity, non-integrin, laminin receptor with an apparent molecular weight of 67 kDa and shown to promote cell attachment and migration as well as to reduce the formation of lung colonies in mice injected with melanoma cells (Iwamoto et al., 1987; Graf et al., 1987). The expression of 67 kDa Laminin Receptor (LR) appears to be more frequent in human tumor cells, compared to their normal counterparts, and directly correlates with an enhanced invasive and metastatic potential (Satoh et al., 1990)

2.4.2 EILDV-NH_2

The pentapeptide sequence, Glu-Ile-Leu-Asp-Val (EILDV), found in an alternatively spliced segment of fibronectin, is recognized by the α4β1 and α4β7 integrin receptor (Hynes, 1992). EILDV peptides have been used as inhibitors of cell adhesion and migration by competition with the ECM protein for the specific cellular integrin (Singleton & Menino, 2005). EILDV also exhibited its antimetastatic activity in experimental metastasis of B16BL6 melanoma in mice (Kawasaki et al., 1996).

REFERENCES

Adams ML, Kwon GS. Relative aggregation state and hemolytic activity of amphotericin B encapsulated by poly(ethylene oxide)-block-poly(N-hexyl-l-aspartamide)-acyl conjugate micelles: effects of acyl chain length. J Control Release 2003; 87: 23-32.

Aisner J, Lee EJ. Etoposide current and future status. Cancer 1991; 67: 215-219.

Alexis F, Rhee, JW, Richie JP, Radovic-Moreno AF, Langer R, Farokhzad OC. New frontiers in nanotechnology for cancer treatment. Urol Oncol: Orig Inv 2008; 26: 74–85.

Allen C, Maysinger D, Eisenberg A. Nano-engineering block copolymer aggregates for drug delivery. Colloids Surf B 1999; 16: 3 –27.

Bae YH. Drug targeting and tumor heterogeneity. J Control Release 2009; 133: 2-3.

Bae Y, Jang WD, Nishiyama N, Fukushima S, Kataoka K. Multifunctional polymeric micelles with folate-mediated cancer cell targeting and pH-triggered drug releasing properties for active intracellular drug delivery. Mol BioSyst 2005; 36: 242-250.

Bae Y, Fukushima S, Harada A, Kataoka K. Design of environment sensitive supramolecular assemblies for intracellular drug delivery: polymeric micelles that are Responsive to Intracellular pH Change. Angew Chem Int Ed 2003; 42: 4640–4643.

Beeta E. Application of Nanotechnology in Cancer Research: Review of Progress in the National Cancer Institute's Alliance for Nanotechnology. Int J Biol Sci 2007; 3: 108-110.

Bin S, Chao F, Mei XY, Yan Z Shoukuan F, YuanYing P. Stealth MePEG-PCL micelles: effects of polymer composition on micelle physicochemical characteristics, in vitro drug release, in vivo pharmacokinetics in rats and biodistribution in S180 tumor bearing mice. Colloid Polym Sci 2005; 283: 954-967.

Burt HM, Xichen Z, Philip T, Leanne E, Willium LH. Development of copolymers of poly(DL-lactide) and methoxypolyethylene glycol as micellar carriers of paclitaxel. Colloids Surf B 1999; 16: 161-171.

Chen S, Li Y, Guo C, Wang J, Ma J, Liang X, Yang LR, Liu HZ. Temperature- responsive magnetite/PEO-PPO-PEO block copolymer nanoparticles for controlled drug targeting delivery. Langmuir 2007; 23:12669–12676.

Chen CL, Uckun FM. Highly sensitive liquid chromatography-electrospray mass spectrometry (LC-MS) method for the determination of etoposide levels in human serum and plasma. J Chromatogr B Biomed Sci Appl 2000; 744: 91-98.

Chung JE, Yokoyama M, Yamato M, Aoyagi T, Sakurai Y, Okano T. Thermo-responsive drug delivery from polymeric micelles constructed using block copolymers of poly(N-isopropylacrylamide) and poly(butylmethacrylate). J Control Release 1999; 62:115–127.

Damon S, Norased N, Elvin B, Jinming G. Functionalized micellar systems for cancer targeted drug delivery. Pharm Res 2007; 24: 1029-1046.

Danhier F, Lecouturier N, Vroman B, Jerome C, Marchand-Brynaert J, Feron O, Preat V. Paclitaxel-loaded PEGylated PLGA-based nanoparticles: In vitro and in vivo evaluation. J Control Release 2009; 133: 11–17.

Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature 1990; 346: 818-822.

Ferrari M. Cancer nanotechnology: Opportunities and challenges. Nat Rev Cancer 2005; 5: 161–171.

Forrest LM, Won CY, Malick AW, Kwon GS. In vitro release of the mTOR inhibitor rapamycin from poly(ethylene glycol)-b-poly((-caprolactone) micelles. J Control Release 2006a; 110: 370-377.

Forrest LM, Zhao A, Won CY, Malick AW, Kwon GS. Lipophilic prodrugs of Hsp90 inhibitor geldanamycin for nanoencapsulation in poly(ethylene glycol)-b-poly(ε-caprolactone) micelles. J Control Release 2006b; 116: 139-149.

Frank G, Liangfang Z, Benjamin AT, Nina M, Andrew W, Aleksandar FR, Robert L, Omid CF. Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. Proc Natl Acad Sci USA 2008; 105: 2586–2591.

Galindo-Rodriguez S, Allemann E, Fessi H, Doelker E. Physicochemical parameters associated with nanoparticle formation in the salting-out, emulsification-diffusion, and nanoprecipitation methods. Pharm Res 2004; 21:1428–1439.

Genevieve G, Marie-Helene D, Vinayak PS, Ning K, Dusica M, Jean-Christophe L. Block copolymer micelles: preparation, characterization and application in drug delivery. J Control Release 2005; 109:169-188.

Ghaleb AH, Christopher MR, Willium GP. Investigating the mechanism of acoustically activated uptake of drugs from pluronic micelles. BMC Cancer 2002; 2.

Gilbert A, Joe H. Carbohydrate-specific receptors of the liver. Annu Rev Biochem 1982; 51: 531-554.

Gillies ER, Frechet MJ. pH-responsive copolymer assemblies for controlled release of doxorubicin. Bioconjug Chem 2005; 16: 361-368.

Gillies ER, Goodwin AP, Frechet MJ. Acetals as pH-sensitive linkages for drug delivery. Bioconjug Chem 2004; 15:1254-1263.

Goto M, Yura H, Chang CW, Kobayashi A, Shinoda T, Maeda A, Kojima S, Kobayashi K, Akaike T. Lactose carrying polystyrene as a drug carrier investigation of body distributions to parenchymal liver-cells using I-125 labeled lactose-carrying polystyrene. J Control Release 1994; 28: 223-233.

Graf J, Iwamoto Y, Sasaki M, Martin GR, Kleinman HK, Robey FA, Yamada Y. Identification of an amino acid sequence in laminin mediating cell attachment, chemotaxis and receptor binding. Cell 1987; 48: 989-996.

Grodzinski P, Silver M, Molnar LK. Nanotechnology for cancer diagnostics: promises and challenges. Expert Rev Mol Diagn 2006; 6: 307-318.

Hong G, Yuan R, Liang B, Shen J, Yang X, Shuai X. Folate-functionalized polymeric micelle as hepatic carcinoma-targeted, MRI- ultrasensitive delivery system of antitumor drugs. Biomed Microdevices 2008; 10: 693-700.

Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992; 69: 11-25.

Iwamoto Y, Robey FA, Graf J, Sasaki M, Kleinman HK, Yamada Y, Martin GR. YIGSR, a synthetic laminin pentapeptide, inhibits experimental metastasis formation. Science 1987; 238: 1132-1134.

Isseh BF, Crooke ST. Etoposide (VP-16-213). Cancer Treat Rev 1979; 6: 107-124.

Jansen RW, Molema G, Ching TL, Oosting R, Harms GMF, Hardonk MJ, Meijer DK. Hepatic endocytosis of various types of mannose-terminated albumins. what is important, sugar recognition, net charge, or the combination of these features. J Biol Chem 1991; 266: 3343-3348.

Jette KK, Law D, Schmitt EA, Kwon GS. Preparation and drug loading of poly(ethylene glycol)-block-poly(qcaprolactone) micelles through the evaporation of a cosolvent azeotrope. Pharm Res 2004; 21:1184-1191.

Kang N, Perron ME, Prud'homme RE, Zang Y, Gaucher G, Leroux JC. Stereocomplex block copolymer micelles: core-shell nanostructures with enhanced stability. Nano Lett 2005; 5 : 315-319.

Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. Adv Drug Deliv Rev 2001; 47: 113-131.

Kato T, Yamaoka K, Takakura Y. An evaluation method for nonlinear local disposition in rat liver and kidney. Drug Metab Dispos 2004; 32: 230-234.

Kawasaki ES, Player A. Nanotechnology, nanomedicine, and the development of new, effective therapies for cancer. Nanomedicine: Nanotech Biol Med 2005; 1: 101-109.

Kawasaki K, Maeda M, Inoue S, Yamashiro Y, Kaneda Y, Mu Y, Tsutsumi Y, Nakagawa S, Mayumi T. Amino acids and peptides. XXIX. Synthesis and antimetastatic effects of peptide and peptide-poly(ethylene glycol) hybrids related to the core sequence of the type III connecting segment domain of fibronectin. Biol Pharm Bull 1996; 19: 1574-1579.

Keller JC, Kuhn M, von WA. Synthesis and antimitotic activity of glycosidic lignan erivatives related to podophyllotoxin. J Med Chem 1971; 14: 936-940.

Kim SY, Cho SH, Lee YM. Biotin-conjugated block copolymeric nanoparticles as tumor-targeted drug delivery systems. Macromol Res 2007; 15: 646-655.

Kim S, Lee YM. Taxol-loaded block copolymer nanospheres composed of methoxy poly(ethylene glycol) and poly(ε-caprolactone) as novel anticancer drug carriers. Biomaterials 2001; 22: 1697-1704.

Kumar N, Ravikumar MNV, Dom AJ. Biodegradable block copolymers. Adv Drug Deliv Rev 2001; 53: 23-44.

Lee H, Hu M, Reilly RM, Allen C. Apoptotic epidermal growth factor (EGF)-conjugated block copolymer micelles as a nanotechnology platform for targeted combination therapy. Mol Pharmaceutics 2007; 4: 769-781.

Lee J, Cho EC, Cho K. Incorporation and release behavior of hydrophobic drug in functionalized poly(d,l-lactide)- block-poly(ethylene oxide) micelles. J Control Release 2004; 94: 323-335.

Letchford K, Burt H. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. Eur J Pharm Biopharm 2007; 65: 259-269.

Liu J, Zeng F, Allen C. Influence of serum protein on polycarbonate-based copolymer micelles as a delivery system for a hydrophobic anti-cancer agent. J Control Release 2005a; 103: 481-497.

Liu J, Zeng F, Allen C. In vivo fate of unimers and micelles of a poly(ethylene glycol)-block-poly(caprolactone) copolymer in mice following intravenous administration. Euro J Pharm Biopharm 2007; 65: 309–319.

Liu SQ, TongYW, Yang YY. Incorporation and In vitro release of doxorubicin in thermally sensitive micelles made from poly(N-isopropylacrylamide-co-N,N- imethylacrylamide)- b-poly(D,L-lactide-co-glycolide) with varying compositions. Biomaterials 2005b; 26:5064-5074.

Lukyanov AN, Gao Z, Torchilin VP. Micelles from polyethylene glycol/ phosphatidylethanolamine conjugates for tumor drug delivery. J Control Release. 2003; 91:97–102.

Maeda H, Wu J, Sawa T, Y. Matsumura, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review. J Control Release 2000; 65: 271– 284.

Manouilov KK, McGuire TR, Gordon BG, Gwilt PR Assay for etoposide in human serum using solid-phase extraction and high-performance liquid chromatography with fluorescence detection. J Chrom B 1998; 707: 342-346.

Mariano L, Gaetano G, Jianzhong D, Steven PA, Yiqing T, Andrew LL. New folate-functionalized biocompatible block copolymer micelles as potential anti-cancer drug delivery systems. Polymer 2006; 47: 2946-2955.

Marin A, Sun H, Husseini GA, Pitt WG, Christensen DA, Rapoport NY. Drug delivery in pluronic micelles: effect of high-frequency ultrasound on drug release from micelles and intracellular uptake. J Control Release 2002; 84: 39-47.

Mitragotri S. Innovation-Healing sound: the use of ultrasound in drug delivery and other therapeutic applications. Nature Rev Drug Dis 2005; 4:255-260.

Mornet S, Vasseur S, Grasset F, Duguet E. Magnetic nanoparticle design for medical diagnosis and therapy. J Mater Chem 2004;14: 2161–2175.

Nomizu M,Yamamura K, Kleinman HK, Yamada Y. Multimene forms of Tyr-Ile-Gly-Ser-Arg (YIGSR) peptide enhance the inhibition of tumor growth and metastasis. Cancer Res 1993; 53: 3459-3461.

Nasongkla N, Shuai X, Ai H, Weinberg BD, Pink J, Boothman DA, Gao J. cRGD-functionalized polymer micelles for targeted doxorubicin delivery. Angew Chem Int Ed 2004; 116: 6483-6487.

Oishi M,Nagasaki Y, Itaka K, Nishiyama N, Kataoka K. Lactosylated poly(ethylene glycol)-siRNA conjugate through acid-labile β-thiopropionate linkage to construct pH-sensitive

polyion complex micelles achieving enhanced gene silencing in hepatoma cells. J Am Chem Soc 2005; 127:1624– 1625.

Omid CF, Jianjun C, Benjamin AT, Ines S, Sangyong J, Philip WK, Jerome PR, Robert L. Targeted nanoparticle- aptamer bioconjugates for cancer chemotherapy in vivo. Proc Natl Acad Sci USA 2006; 103: 6315-6320.

Pang Z, Lu W, Gao H, Hu K, Chen J, Zhang C, Gao X, Jiang X, Zhu C. Preparation and brain delivery property of biodegradable polymersomes conjugated with OX26. J Control Release 2008; 128:120-127.

Park EK, Kim SY, Lee SB, Lee YM. Folate-conjugated methoxy poly(ethylene glycol)/poly(e-caprolactone) amphiphilic block copolymeric micelles for tumor-targeted drug delivery. J Control Release 2005; 109: 158– 168.

Presta LG. Engineering of therapeutic antibodies to minimize immunogenicity and optimize function. Adv Drug Deliv Rev 2006; 58: 640–656.

Rapoport NY, Herron JN, Pitt WG, Pitina L. Micellar delivery of doxorubicin and its paramagnetic analog, ruboxyl, to HL-60 cells: effect of micelle structure and ultrasound on the intracellular drug uptake. J Control Release 1999; 58:153-162.

Reddy LH, Murthy RSR. Etoposide-loaded nanoparticles made from glyceride lipids: Formulation, characterization, in vitro drug release, and stability evaluation. AAPS PharmSci Tech 2005; 6:.E1158-1166.

Ross JF, Chaudhuri PK, Ratnam M. Differential regulation of folate receptor isoforms in normal and malignanttissues in-vivo and in established cell-lines- physiological and clinical implications. Cancer 1994; 73: 2432-2443.

Satoh K, Narumi K, Abe T, Sakai T, Kikuchi T, Tanaka M, Shimo-Oka T, Uchida M, Tezuka F, Isemura M, Nukiwa T. Diminution of 37-kDa laminin binding protein expression reduces tumor formation of murine lung cancer cells. Br J Cancer 1990; 80: 1115-1122.

Sethuraman VA, Bae YH. TAT peptide-based micelle system for potential active targeting of anti-cancer agents to acidic solid tumors. J Control Release 2007; 118: 216-224.

Shao-Qiong L, Nikken W, Shu-Jun G, Yen WT, Yi-Yan Y. Bio-functional micelles self-assembled from a folate-conjugated block copolymer for targeted intracellular delivery of anticancer drugs. Biomaterials 2007; 28:1423-1433.

Shirazi, FH, Bahrami G, Stewart DJ, Tomiak E, Delorme F, Noel D, Goel R. A rapid reversed phase high performance liquid chromatographic method for determination of etoposide (VP-16) in human plasma. J Pharm Biomed Anal 2001; 25: 353-356.

Shuai X, Merdan T, Schaper AK, Xi F, Kissel T. Core-cross- linked polymeric micelles as paclitaxel carriers. Bioconjug Chem 2004; 15: 441-448.

Singleton C, Menino AR. Effects of inhibitor of integrin binding on cellular outgrowth from bovine inner cell masses in vitro. In Vitro Cell Dev Biol Anim 2005; 40: 29-37.

Slager J, Domb AJ. Biopolymer stereocomplexes. Adv Drug Deliv Rev 2003; 55: 549– 583.

Sudimack J, Lee RJ. Targeted drug delivery via the folate receptor. Adv Drug Deliv Rev 2000; 41: 147-162.

Taiho N, Yeon HK, Chiyoung P, Hyewon Y, Hana K, Eun TO, Eun KC, Heon JP, Chulhee K. Block copolymer micelles conjugated with anti-EGFR antibody for targeted delivery of anticancer drug. J Polym Sci Part A: Polym Chem 2008; 46: 7321-7331.

Tranchand B, Amsellem C, Chatelut E, Freyer G, Iliadis A, Ligneau B, Trillet-Lenoir V, Canal P, Lochon I, Ardiet CJ. A limited-sampling strategy for estimation of etoposide pharmacokinetics in cancer patients. Cancer Chemother Pharmacol 1999; 43:316-322.

Thurmond KB, Haiyong H, Christopher GC, Tomasz K, Karen LW. Shell cross-linked polymer micelles: stabilized assemblies with great versatility and potential. Colloids Surf B 1999; 16: 45-54.

Toncheva V, Schacht E, Ng SY, Barr J, Heller J. Use of block copolymers of poly(ortho esters) and poly(ethylene glycol) micellar carriers as potential tumor targeting systems. J Drug Target 2003; 11: 345– 353.

Torchilin VP, Lukyanov AN, Gao Z, Papahadjopoulos-Sternberg B. Immunomicelles: targeted pharmaceutical carriers for poorly soluble drugs. Proc Natl Acad Sci USA 2003; 100: 6039-6044.

Vangeyte P, Gautier S, Jerome R. About the methods of preparation of poly(ethylene oxide)-b-poly(-caprolactone) nanoparticles in water. Analysis by dynamic light scattering. Colloids Surf A 2004; 242: 203-211.

Wang YC, Liu XQ, Sun TM, Xiong MH, Wang J. Functionalized micelles from block copolymer of polyphosphoester and poly(ε-caprolactone) for receptor-mediated drug delivery. J Control Release 2008; 128: 32–40.

Weiner LM, Adams GP. New approaches to antibody therapy. Oncogene 2000; 19: 6144-6451.

Weiss RB, Donehower RC, Wiernik PH. Hypersensitivity reactions from taxol. J Clin Oncol 1990; 8: 1263-1268.

Xiong XB, Mahmud A, Uludag H, Lavasanifar A. Conjugation of arginine-glycine-aspartic acid peptides to Poly(ethylene oxide)-*b*-poly(ε-caprolactone) micelles for enhanced intracellular drug delivery to metastatic tumor cells. Biomacromolecules 2007; 8: 874–884.

Xu P, Van Kirk EA, Murdoch WJ, Zhan Y, Isaak DD, Radosz M, Shen Y. Anticancer efficacies of cisplatin-releasing pH- responsive nanoparticles. Biomacromolecules 2006; 7: 829-835.

Yadav AK, Mishra P, Mishra AK, Mishra P, Jain S, Agrawal GP. Development and characterization of hyaluronic acid–anchored PLGA nanoparticulate carriers of doxorubicin. Nanomedicine: Nanotech Biology and Med 2007; 3: 246-257.

Yamamoto Y, Nagasaki Y, Kato Y, Sugiyama Y, Kataoka K. Long circulating poly(ethylene glycol)–poly(D,L-lactide) block copolymer micelles with modulate surface charge. J Control Release 2001; 77: 27–38.

Yoo HS, Lee EA, Park TG. Doxorubicin-conjugated biodegradable polymeric micelles having acid-cleavable linkages. J Control Release 2002; 82:17–27.

Yoo HS, Park TG. Folate receptor targeted biodegradable polymeric doxorubicin micelles. J Control Release 2004; 96: 273-283.

Young-Il J, Seog-Jin S, In-Kyu P, Hyun-Chul L, In-Chul K, Toshihiro A, Chong-Su C. Cellular recognition of paclitaxel loaded polymeric nanoparticles composed of poly(gamma-benzyl L-glutamate) and poly(ethylene glycol) diblock copolymer endcapped with galactose moiety. Int J Pharm 2005; 296:151-161.

Yu L, Glen SK. Methotrexate esters of poly(ethylene oxide)-block-poly(2-hydroxyethyl-l-aspartamide): Part 1. Effects of the level of methotrexate conjugation on the stability of micelles and on drug delivery. Pharm Res 2000; 17: 607-611.

Zee J. Heating the patient: a promising approach? Ann Oncol 2002; 13:1173-1184.

Zeng F, Lee H, Allen C. Epidermal growth factor-conjugated poly(ethylene glycol)-block-poly(delta-valerolactone) copolymer micelles for targeted delivery of chemotherapeutics. Bioconjug Chem 2006; 17:399-409.

Zhao H, Yung LY. Selectivity of folate conjugated polymer micelles against different tumor cells. Int J Pharm 2008; 349: 256–268.