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Review Article

Impact of Nanoparticles on Cancer Therapy

Mayson H Alkhatib*, Hayat M Albishi and Sawsan H Mahassni
Department of Biochemistry, College of Science, King Abdulaziz University, PO Box 42801, Jeddah 21551, Saudi Arabia

Abstract

The tremendous contribution of nanotechnology to the treatment and diagnosis of medical diseases has recently attracted the attention of anticancer researchers. Most of the new nanoparticle carriers have improved drug bioavailability and reduced the cytotoxic effects of the drugs. This article presents an overview of the recent advances of nanotechnology in cancer therapy. It covers the mechanisms of cellular uptake for anticancer drugs delivered in nanoscale systems by either active or passive targeting. The various nanoscale systems employed in drug delivery and their immense potential in diagnosis and imaging of cancerous tumors are also addressed.

Keywords: *Nanocarriers, Drug delivery, Liposomes, Micelles, Dendrimers, Emulsification systems*

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Corresponding author:* **Email: *mhalkhatib@kau.edu.sa;* ***Tel:*** *+966599240526.*

INTRODUCTION

Cancer is considered one of the most common causes of death all over the world [1]. In spite of the research advances in anti-cancer therapy, higher mortality rates are still associated more with the adverse side effects of therapeutic intervention than the cancer itself [2]. In fact, most anti-cancer drugs are very hydrophobic and have poor bioavailability. Therefore, they reach the tumor tissue with poor specificity and dose-limiting toxicity. Usually, anticancer drugs are administered to patients via oral or intravenous route. The disadvantages of oral administration of tablets or capsules is the resulting disorderly pharmacokinetics due to the exposure of these agents to the metabolic pathways of the body, which can result in larger than necessary doses being administered and further cause increased toxicity. On the other hand, the specificity of some conventional intravenous drugs is low; resulting in harmful effects to healthy tissues and are often more problematic.

Recent studies have demonstrated that encapsulation of anti-cancer drugs in a nanodroplet system would help in improving their cellular uptake and reducing their cytotoxic side effects [3]. These nanoparticulate systems are created at an incredibly small scale between 1 and 400 nm. They have showed a great potential by serving as carriers for drugs, genes and imaging agents that would bind efficiently and selectively to specific targets on injured or neoplastic tissue. Nanocarriers may include liposomes, micelles, dendrimers and emulsification systems.

CELLULAR UPTAKE OF NANO-SCALE MATERIALS

The various mechanisms of intracellular uptake of nanoparticles can be by phagocytic cells, non-phagocytic cells or drug resistant cancer cells [4]. Nanomaterials with a lower size are preferable to those in the upper submicron and micron ranges to achieve longer-circulation half-lives with reduced uptake of mononuclear phagocyte system (MPS) and more efficient cellular uptake with increased internalization. In particular, the cellular uptake of drug nanocarriers by phagocytic cells depends on the size that should not exceed 200 nm, surface charge and molecular interactions that might influence both the uptake and subsequent clearance by the MPS cells. On the other hand, internalization of particles by non-phagocytic cells such as tumor cells would happen if the particles sizes are even > 500 nm. Internalization of nanomedicines into the target cells can occur via a diverse range of endocytic pathways including phagocytosis, macropinocytosis, clathrin-mediated endocytosis, and non–clathrin-mediated, such as, caveolae-mediated endocytosis. Another proposed mechanism of the cellular uptake that recently has gained interest is overcoming drug resistance in cancer chemotherapy by using nano-scale delivery systems [5]. Major mechanisms that have been proposed include enhanced intracellular concentration of the drug by endocytosis, inhibition of multidrug resistance proteins by carrier component materials such as Pluronic block copolymers, adhesion of nanoparticles to the cell surface, promotion of other uptake mechanisms such as receptor-mediated cellular internalization, and increased drug concentrations at the vicinity of target cancer cells. Furthermore, both drug and inhibitors of multidrug resistance proteins can be incorporated into the same carriers for simultaneous delivery to the cancer cells. For example, doxorubicin and cyclosporine A, encapsulated in polyalkylcyanoacrylate nanoparticles have been discovered to reverse resistance synergistically [6].

ROLE OF NANOPARTICLE SYSTEMS IN DIAGNOSIS AND IMAGING

The National Cancer Institute (NCI) has recognized the immense potential that nanotechnology holds for the cancer diagnosis and treatment [7]. A good example for the application of nanotechnology in

oncology is the immunoassays that employ the nanoparticles as markers in detecting the presence of tumors. These nanoparticles, containing quantum dots and molecular beacons (MBs), are synthetic, stable inorganic fluorophore semiconductors and have narrow emission spectra with wide excitation range [8, 9]. The quantum dot-conjugated probes can be used to measure the expression levels of several specific malignant tumor biomarkers and at the same time, provide valuable data for treatment. The MBs are hairpin-shaped oligonucleotides that act like switches. They undergo conformational changes and fluoresce when turned on. When used as probes, MBs have valuable applications in the nucleic acid and gene expression monitoring and as biosensors. They also have the potential to become very useful tools in genomics and proteomics as they enable real-time detection of protein–RNA–DNA interaction with high sensitivity and specificity. Other diagnostic probes may include nanoshells, gold nanoparticles, paramagnetic nanoparticles and carbon nanotubes.

ROLE OF NANOPARTICLE SYSTEMS IN DRUG DELIVERY

The major aim of pharmaceutical research is the proper distribution of drugs and other therapeutic agents within the patient's body and delivering of any drug at the right time in a safe and reproducible manner to a specific target at the required level [10]. Oral route is considered as one of the preferred methods for drug delivery due to its non-invasive feature. However, adequate peptide or protein drug delivery has not yet been attained via this route [11]. This is partly due to the acidic conditions of the stomach, the first-pass effect of the liver that diminishes the drug by the metabolic processes before it goes through the systemic circulation, and the resistance exerted by the intestine which alter, destroy, or reduce absorption of nearly all therapeutic agents and hence, reduce the drug bioavailability. Accordingly, engineered nanodevices and nanostructures systems can improve the stability, absorption, and therapeutic concentration of the drug within the target tissue, as well as permit reproducible and long-term release of the drug at the target site. In addition to reducing the frequency of drug administration and thus improving patient comfort, novel drug delivery systems would offer protection and improve the pharmacokinetics of easily degradable peptides and proteins, which often have short half-lives *in vivo*.

Nanoconjugates can also surmount the draw backs of the conventional chemotherapy which is non-specific in killing rapidly dividing cells whether within the tumor or in the normal tissues [12]. Selective targeting of tumor tissue is possible with modern macromolecular nanodelivery systems that deliver high drug concentrations and maximal effects to tumor tissue and minimal drug concentrations and negligible side effects to healthy tissue. Polymers as platforms for delivering agents into tumor cells have increasingly gained importance because they are unaffected by the multidrug resistance, MDR, which is an effect produced by cancer cells to the multiple different distinct drugs. The MDR have minimal immunogenicity and are able to maintain effectiveness with each cycle of tumor treatment. General macromolecular targeting of tumor tissue is referred to as "passive", whereas site-specific targeting of cell surface molecules and receptors is referred to as "active". In the following sections, both targeting will be discussed in more details.

Passive targeting

Passive targeting of injured tissue occurs due to extravasation of the nanocarriers at the diseased site where the microvasculature of tumor and inflamed tissue is leaky as shown in Figure 1. Tumor vascular leakiness is the result of increased angiogenesis and the presence of cytokines, bradykinin and other vasoactive factors that enhance permeability [13]. Tumor angiogenesis is characterized by vessels with irregular diameters and

branching, and tumors lacking defining structures of vasculature such as arterioles, capillaries, or venules. The majority of solid tumors exhibit a vascular pore cutoff size between 380 and 780 nm. Therefore elevated levels of bradykinin result in vasodilatation and enhance the extravasation of nanocarriers of a size much smaller than the cutoff pore diameter to the target tumor sites and their retention in tumors which is called the passive enhanced permeability retention effect [14]. Tumors lack an effective lymphatic drainage system to clear these extravasated substances. As a consequence, macromolecules and nanoparticles, that enter the tumor, will accumulate. By contrast, normal vasculature is impermeable to drug associated carriers larger than two to four nanometer compared to free unassociated drug molecules (Figure 1). This nanosize window offers the opportunity to increase drug accumulation and local concentration in target sites by extravasation, and significantly to reduce drug distribution and toxicity to normal tissues.

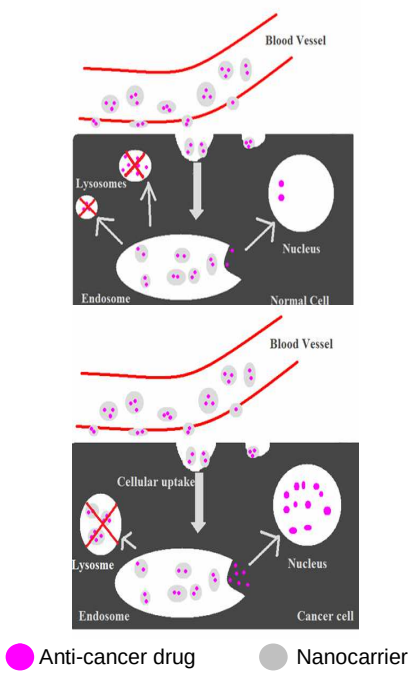

Figure 1: The uptake of the anticancer drug by cancerous and normal cells via passive targeting.

Active targeting

Cancerous and inflamed tissues are not only having leaky vasculature but also over-express some epitopes or receptors that can be used as targets. Accordingly, it can be actively targeted by nanoconjugated ligands via targeting these receptors. Ligands that specifically bind to surface epitopes or receptors usually couple to the surface of long circulating nanocarriers [15]. Various methods have been employed to couple ligands to the surface of the nanocarriers with reactive groups. These can be divided into covalent and noncovalent couplings. Common covalent coupling methods involve formation of a disulfide bond, cross-linking between two primary amines, reaction between a carboxylic acid and primary amine, reaction between maleimide and thiol, reaction between hydrazide and aldehyde, and reaction between a primary amine and free aldehyde. Non-covalent binding by physical association of targeting ligands to the nanocarrier surface has the advantage of eliminating the use of rigorous, destructive reaction agents. However, there are potential problems, such as low and weak binding, and the ligands may not be in the desired orientation after binding.

Ligand-mediated active binding to sites and cellular uptake are particularly valuable to therapeutics that are not taken up easily by cells and require facilitation by fusion, endocytosis, or other processes to access their cellular active sites. An important consideration when selecting the type of targeting ligand is its immunogenicity. For example, whole antibodies that expose their constant regions on the liposomal surface are more susceptible to Fc-receptor-mediated phagocytosis by the mononuclear phagocyte system [16].

Active targeting nanocarriers have a number of advantages over targeting ligand-drug conjugates [17]. First, high concentrations of drug within the carrier can be delivered to the target cell when a ligand interacts with its receptor. Second, the ligand is associated

with the carrier, and the drug is not modified with the coupling of ligands. Third, ligand molecules can be attached to the nanocarrier to increase probability of binding to target cells, particularly for therapeutics of lower binding affinities. Fourth, active targeting enables more efficient distribution of the carriers in the tumor interstitium and reduces return of drug back to the circulation due to high intratumoral pressure. Last, but also important, is that when ligand is only attached to the carrier due to the small size of the conjugate, it can only extravasate at the disease site but not at normal vasculature and therefore, the ligand cannot interact with the target epitopes of normal tissues and show side effects. Thus, nanocarriers can play an important role in reducing the toxicities of the drug and targeting ligand. Accordingly, nanoconjugates can be designed for sustained release of drug, passive enhanced permeability retention effect-based targeting of macromolecules to tumor tissue, ligand-based targeting of cell surface antigens and modules active in endosomal uptake and membrane disruption, drug release into the cytoplasm, and protection of drugs from enzymatic degradation.

NANOPARTICLE PLATFORMS FOR ANTICANCER DRUG DELIVERY

Nanoparticulate systems for drug delivery can be produced by two different methods. The first method of nanosuspension production involves the breaking down of bigger particles to nanosize using high-pressure homogenization of drug suspensions in the presence of surfactants [18]. The second method involves crystallization that build the nanoparticles up from the supersaturated solution state, or solid nanoparticles which can further be sub-classified according to their composition: mainly polymer-based, lipid-based, and ceramic-based materials, albumin nanoparticles and nanogels [19]. The types of nanocarrier systems covered in this review article (see Figure 2) include liposomes, micelles, emulsification systems and dendrimers.

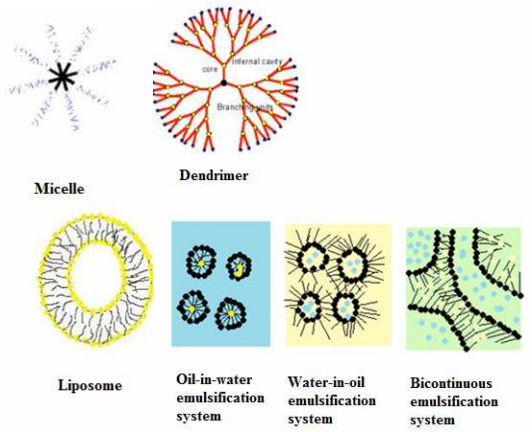

Figure 2: Schematic presentation of some nanoparticle platforms used for drug delivery.

Liposomes with their long history starting from the mid-1980s were the first products to be used as nanocarriers and are available in the market (Table 1 and Figure 2). Daunorubicin and vincristine are anticancer drugs that have been loaded in liposomes and clinically used with the commercial names such as Daunoxome and Onco TCS, respectively. Doxorubicin loaded in liposomes is commercially marketed as Mayocet, and while that loaded in polyethyleneglyol-liposomes is branded Doxil [17]. The liposomes's vesicle size plays a critical role in complement activation and mononuclear phagocyte systems clearance of liposomes [20]. Vesicles larger than 100 nm require additional strategies to prevent surface opsonization. Liposomal anthracyclines have achieved highly efficient drug encapsulation, resulting in significant anticancer activity with reduced cardiotoxicity, and include versions with greatly prolonged circulation such as liposomal daunorubicin and pegylated liposomal doxorubicin. Pegylated liposomal doxorubicin has shown substantial efficacy in breast cancer treatment as monotherapy and in combination with other chemotherapeutics.

Table 1: Characteristics of some nanocarriers and their current stage of development for use in cancer therapy

Nanocarrier type & size (nm)	Properties	Advantages	Disadvantages	Clinical trials
Liposomes (85-100)	Composed of one or multiple natural non-toxic phospholipids bilayer surrounding an aqueous hydrophilic core.	1- Both water and poorly soluble drugs are incorporated into the core or within the lipophilic phase of the bilayer. 2- Toxicity of the phospholipids used is low.	1-Complex manufacturing process 2-Rapid clearance from the blood stream 3- Instability 4- Non-specific cellular uptake.	Several liposomal carriers for mainly daunorubicin, doxorubicin and vincristine, have already entered the market [17].
Micelles (5-100)	Unique core-corona assemblies of amphiphilic block copolymers.	1- High solubilization capacity without the need of additional solvents for both hydrophilic and hydrophobic drugs. 2- Great thermodynamic stability.	1-Inappropriate procedures may lead to supersaturated states with subsequent instability and drug precipitation during storage. 2- The micelle morphology affects the biodistribution.	Several polymeric micelles in clinical trials (phase I) are used for delivering mainly doxorubicin and paclitaxel [17, 27, 28].
Dendrimer (1-10)	Polymeric complexes of well-defined branches around an inner core.	Both hydrophobic and hydrophilic drugs are associated with dendrimers by either encapsulation or conjugation.	Fabrication of dendrimers are difficult to synthesize and lengthy.	Doxorubicin and cisplatin were delivered in dendrimers and are under the *in vitro* evaluation [31, 32, 33].

Liposomes with Sialyl Lewis X (SLX) proved to work as imaging reagent and drug delivery agent for fluorescent markers, proteins and genes [21,22]. Some liposomes were produced by encapsulating perfluorocarbon nanoparticle to be utilized as an imaging contrast agent. When doxorubicin was encapsulated in the formulation of human epidermal growth factor receptor 2-specific antibody (ZHER2:342-Cys) conjugated thermosensitive liposome, its targeting and triggering potential for breast cancer treatment was improved [23].

Oxaliplatin, an anticancer drug, was delivered sequentially in a cationic liposome coated with polyethylene glycol and revealed dual targeting to both endothelial cells and tumor cells in solid tumor [24]. Another example on the cationic liposomes is the trilysinoyl oleylamide -based liposomes which displayed the greatest delivery efficiency of the anticancer drug suberoylanilide hydroxamic acid with minimal cytotoxicity compared to the other oligolysine-based cationic lipid derivatives [25].

Micelles are nanosized assemblies of amphiphilic block copolymers exhibiting a unique core-corona structure (Figure 2 and Table 1). The hydrophilic corona is important to stabilize the micelles in an aqueous environment whereas the hydrophobic core functions as a drug reservoir [26]. Nanosized

micelles have polarity gradients from the highly hydrated corona to the hydrophobic core, and are used for solubilization of both hydrophilic and hydrophobic drugs. The hydrophobic interactions are the predominant driving force in the assembly of the amphiphiles in the aqueous medium when their concentrations exceed the critical micelle concentration, which is defined as the concentration of surfactants above which micelles are spontaneously formed. Micellar drug delivery systems can be divided into four classes that share a similar molecular architecture, phospholipid micelles, Pluronic micelles, Poly (L-amino acid) micelles and Polyester micelles. The clinical assessments for using the micelles are still in the early stages (phase 1). Recent studies have showed that assembling human tumor necrosis factor (TNF)-related apoptosis-inducing ligand (Apo2L/TRAIL) and doxorubicin (Dox, an anti-cancer drug) or paclitaxel with micellar nanoparticles from a biodegradable cationic copolymer P(MDS-co-CES) showed great potential in cancer therapy as it induces cell death selectively in cancer cells with limited toxicity to normal tissues [27,28].

Another subclass of the nanoparticle systems is the dendrimers (Figure 2 and Table 1), that have been extensively investigated and under preclinical assessments for delivering various anticancer drugs [29,30]. Drug molecules can be associated with dendrimers by either encapsulated in the void spaces of the dendrimer interior or conjugated on the surface of the dendrimer.

The proper choice of dendrimer as a drug delivery system could improve the circulation half-life and hence, develop the enhanced permeation retention effect phenomena that result in increasing the efficacy of the drug. For example, doxorubicin is one of the anticancer drugs that was encapsulated in a 2, 2-bis (hydroxymethyl) propanoic acid based dendritic scaffold by a hydrazone linkage. Its cytoxicity was reduced and its half-life has increased compared to the free drug [31]. Doxorubicin was also conjugated into the polyamidoamine (PAMAM) dendrimer and coated with superparamagnetic iron oxide (Fe_3O_4) nanoparticles through acid-cleavable hydrazone bonds which would make this dendritic platform a good candidate for cancer diagnosis *in vivo* [32].

Cisplatin is another anticancer drug that was also incorporated into generation 3.5 PAMAM dendrimer conjugated through the sodium carboxylate surface giving a dendrimer–platinate (dendrimer–Pt; 20–25 wt% platinum), which resulted in a fairly water soluble nanoformulation with the ability to release cisplatin slowly *in vitro*. It showed greater antitumor activity over free cisplatin when injected into mice bearing B16F10 tumor cells [33]. The saccharide-terminated generation 3 PAMAM dendrimer conjugated to the drug methotrexate proved to have a great potential in specifically targeting and killing the folate receptor-expressing tumor cells [34].

In an attempt to improve the bioavailability, efficacy and half-life of anticancer drugs, dendrimers were attached to polyethylene glycols (PEG) which shield the body's immune system. A research study suggested that the proper design of the size and surface characteristics of PEGylated Polylysine dendrimers would determine the rout of the delivery system. It demonstrated that if dendrimer size was increased by increasing the chain length of attached polyethylene glycol chains, a dramatic increase in absorption efficiency after subcutaneous injection could be achieved and transported into the lymphatic system. Conversely, a shorter PEG chain was shown to lead to rapid absorption into the blood [35]. Doxorubicin was conjugated into generation 5 PEGylated polylysine dendrimers comprising an outer generation of L-lysine or succinimyl dipropyldiamine. When this conjugate was evaluated in rats bearing Walker 256 tumors, it revealed higher uptake into tumor tissue compared to control tissue such as muscle and heart [36].

Nanoscale systems also include emulsification systems that are still under preclinical investigations. They can incorporate drug compounds and modify their bioavailability, stability, and reduce their side effects (Figure 2 and Table 2). They are colloidal systems that consist of two or more immiscible liquids stabilized by surfactants which are chemical compounds with polar head and non-polar tail [37]. These dispersion systems might produce different structures with distinct morphologies and sizes range from 1 – 400 nm. They are mainly classified according to their differences in the composition, appearance, kinetic and thermodynamic stability into emulsions, microemulsions and nanoemulsions.

The structure of the single-phase for emulsification systems produced is affected by the fraction of oil and water. The structure can be oil-in-water (o/w), water-in-oil (w/o) or bicontinuous. In each type, there is an interfacial surfactant monolayer separating the water and oil domains. The presence of o/w droplets is likely to be produced where the volume fraction of oil is low while the w/o droplets are formed at a lower fraction of water. In systems where the amounts of water and oil are similar, a bicontinuous structure may result. The existence of microdomains of different polarity within the same single-phase solution enables both water-soluble and oil-soluble materials to be solubilized.

Table 2: Characteristics of emulsification systems and their current stage of development for use in cancer therapy

Characteristic	Emulsion	Microemulsion	Nanoemulsion
Appearance	Opaque	Transparent	Transparent to slightly opaque
Size (nm)	>200	1 -100	1 -100
Advantages	Require fewer amounts of surfactants (~ 5%)	Spontaneously formed Thermodynamically stable under certain conditions (pH, temperature…)	Very thermodynamically and kinetically stable Require fewer amounts of surfactants (~ 5%)
Disadvantages	Thermodynamically unstable and thus do not form spontaneously Require input of energy through shaking, homogenizing or exposure to power ultrasound	Require more surfactants (>20%) Stability of microemulsions is easily compromised by dilution, heating or changing pH levels.	Require input of energy and specialized equipment to be produced
Clinical trials	Preclinical stages for evaluating vinorelbine-loaded lipid emulsion [38].	In vivo and in vitro evaluation for the mitomycin C, diallyl trisulfide and adriamycin, when loaded in microemulsion [40, 41, 42].	Preclinical assessment for nanoemulsion formulation of paclitaxel and decarbazine [43, 44, 45].

Some anticancer drugs were recently formulated in emulsion systems and were preclinically assessed (Figure 2 and Table 2). For example, the anticancer drug, vinorelbine, proved to have equivalent efficacy and lower toxicity compared to the commercial product Navelbine(®) when loaded in lipid emulsion as observed in tumor-bearing nude mouse models inoculated with A549 human lung cancer, hepatoma solidity (Heps) G2 cancer and BCAP-37 human breast cancer cells [38]. Furthermore, epirubicin, an anticancer drug, was encapsulated in water-in-oil-in-water emulsion containing glucose solution as the inner aqueous phase, lipiodol which consisted of polyglycerol esters of polycondensed fatty acids of castor oil, as the oily phase, and physiological saline with polyoxyethylene 60 stearate. The resulted formula revealed an effective treatment for patients with recurrent hepatocellular carcinoma after surgical resection [39].

The proper microemulsion delivery systems of some anticancer drugs have showed decreased cytotoxic effect and have improved the efficacy of the drug (Figure 2 and Table 2). The *in vitro* evaluation of mitomycin C loaded in microemulsion revealed safe effect when subjected unto Calu1 and A549 lung carcinoma cell lines [40]. Another promising study proved that an oil-in-water microemulsion containing cremophor EL, ethanol, propylene glycol and saline solution would be a good candidate as an intravenous delivery of diallyl trisulfide which is an oil-soluble sulfur compound that induces apoptosis in tumor cells [41]. Lipidol microemulsions with adriamycin anticancer drug in combination with the antioxidant diethyldithiocarbamate demonstrated a significant antitumor activity *in vivo* and higher liver adriamycin concentration as compared to free adriamycin, adriamycin liposome, and adriamycin microsphere groups [42].

Anticancer drugs loaded in nanoemulsion systems have greater activity against cancer cells in comparison to other emulsification systems (Figure 2 and Table 2). This is due to the decreased particle size and zeta potential, production of a stable water dispersion, reduced polydispersity index, and greater stability of drug with the nanoemulsion. When the anticancer drug dacarbazine was loaded in a nanoemulsion formula containing ethanol, soybean oil, polysorbate 80 and deionized water, its efficacy compared with the suspension formula was increased, revealed by the reduction of the tumor size in epidermoid carcinoma xenograft mice. A nanoemulsion formulation of dacarbazine reduces tumor size in a xenograft mouse epidermoid carcinoma model compared to dacarbazine suspension [43]. Paclitaxel, formulated in a self assembling nanoemulsion containing cremophor EL and ethanol solution, have showed improved inhibition effect of cell proliferation in breast, colon and pancreatic cell lines compared to blank nanoemulsion [44]. Another recent study has encapsulated the paclitaxel in perfluorocarbon nanoemulsions and showed excellent therapeutic properties characterized by tumor regression and suppression of metastasis [45].

CONCLUSION

Nanoparticle systems have shown great potential and promising result in cancer treatment and imaging. However, the physicochemical properties of nanoscale systems still need further in-depth research. In addition, the *in vivo* and *in vitro* evaluation of the nanocarriers capsulation for the anti-cancer drugs are still under investigations. Discoveries of new nanoscale systems that are biocompatible are still limited to specific applications in cancer therapy.

REFERENCES

1 *Anand P, Kunnumakara A, Sundaram C, Harikumar K, Tharakan S, Lai O, Sung B, Aggarwal B. Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharmaceut Res 2008; 25: 2097-2116.*

2 Nussbaumer S, Bonnabry P, Veuthey J, leury-Souverain, S. Analysis of anticancer drugs: A review. Talanta 2011; 85: 2265-2289.

3 Bawarski WE, Chidlowsky E, Bharali DJ, Mousa SA. Emerging nanopharmaceuticals. Nanomedicine 2008; 4: 273-282.

4 Kateb B, Chiu K, Yamamoto V, Khalsa B, Black KL, Ljubimova JY, Ding H, Patil R, Portilla-Arias JA, Modo M, Moore D, Farahani K, Okun M, Prakash N, Neman J, Ahdoot D, Grundfest W, Nikzad S, Heiss j. Nanoplatforms for constructing new approaches to cancer treatment, imaging, and drug delivery: What should be the policy?. NeuroImage 2011; 54:S106-S124.

5 Mamot C, Drummond DC, Hong K, Kirpotin DB, Park JW. Liposome-based approaches to overcome anticancer drug resistance. Drug Resist Update 2003; 6: 271 – 279.

6 Soma CE, Dubernet C, Bentolila D, Benita S, Couvreur P. Reversion of multidrug resistance by co-encapsulation of doxorubicin and cyclosporin A in polyalkylcyanoacrylate nanoparticles. Biomaterials 2000; 21: 1-7.

7 Sahoo SK, Parveen S, Panda JJ. The present and future of nanotechnology in human health care. Nanomedicine 2007; 3: 20–31.

8 True LD, Gao X: Quantum dots for molecular pathology: their time has arrived. J Mo Diagn 2007; 1: 7–11.

9 Tan L, Li Y, Drake TJ, Moroz L, Wang K, Li J, Munteanu A, James Yang C, Martinez K, Tan W: Molecular beacons for bioanalytical applications. Analyst. 2005; 7: 1002–1005.

10 Hamman JH, Enslin GM, Kotze AF. Oral delivery of peptide drugs: barriers and developments. BioDrugs 2005; 19: 165-177.

11 Pawar R, Ben-Ari A, Domb AJ. Protein and peptide parenteral controlled delivery. Expert Opin Biol Ther 2004; 4: 1203-1212.

12 Luo Y, Prestwich GD. Cancer-targeted polymeric drugs. Curr. Cancer Drug Targets 2002; 2: 209–226.

13 Oeffinger BE, Wheatley MA. Development and characterization of a nano-scale contrast agent. Ultrasonics 2004; 42: 343-347.

14 Gao X, Cui Y, Levenson RM, Chung LW, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol 2004; 22: 969–976.

15 Nobs L, Buchegger F, Gurny R, Allemann E. Current methods for attaching targeting ligands to liposomes and nanoparticles. J Pharm Sci 2004; 93: 1980-1992.

16 Metselaar JM, Mastrobattista E, Storm G. Liposomes for intravenous drug targeting: design and applications. Mini Rev Med Chem 2002; 2: 319–329.

17 Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2007; 2: 751-760.

18 Koo OM, Rubinstein I, Onyuksel H. Role of nanotechnology in targeted drug delivery and imaging: a concise review. Nanomed Nanotech Biol Med 2005; 1:193-212.

19 Malinger KA, Maguer A, Thorel A, Gaunand A, Hochepied J. Crystallization of anatase nanoparticles from amorphous precipitate by a continuous hydrothermal process .Chem Eng J 2011; 174: 445-451.

20 Mohanraj VJ, Chen Y. Nanoparticles – A Review. Trop J Pharm Res 2006; 1: 561-573.

21 Hirai M, Minematsu H, Kondo N, Oie K, Igarashi K, Yamazaki N. Accumulation of liposome with Sialyl Lewis X to inflammation and tumor region: application to in vivo bio-imaging. Biochem Biophys Res Commun 2006; 353: 553–558.

22 Hashida N, Ohguro N, Yamazaki N, Arakawa Y, Oiki E, Mashimo H, Kurokawa N, Tano Y. High-efficacy site-directed drug delivery system using sialyl-Lewis X conjugated liposome. Exp Eye Res. 2008; 86: 138–149.

23 Smith B, Lyakhov I, Loomis K, Needle D, Baxa U, Yavlovich A, Capala J, Blumenthal R, Puri A. Hyperthermia-triggered intracellular delivery of anticancer agent to $HER2^+$ cells by HER2-specific affibody (ZHER2-GS-Cys)-conjugated thermosensitive liposomes ($HER2^+$ affisomes). J Control Release 2011; 153: 187-194.

24 Abu Lila A, Doi Y, Nakamura K, Ishida T, Kiwada H. Sequential administration with oxaliplatin-containing PEG-coated cationic liposomes promotes a significant delivery of subsequent dose into murine solid tumor. J Control Release 2010; 142: 167-173.

25 Shim G, Han S, Yu Y, Lee S, Lee H, Kim K, Kwon IC, Park TG, Kim YB, Choi YS, Kim C, Oh Y. Trilysinoyl oleylamide-based cationic liposomes for systemic co-delivery of siRNA and an anticancer drug. J Control Release 2011; 155: 60-66.

26 Nishiyama N, Kataoka K. Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. Pharmacol Therapeut 2006; 112: 630-648.

27 Lee AL, Dhillon SH, Wang Y, Pervaiz S, Fan W, Yang YY. Synergistic anti-cancer effects via co-delivery of TNF-related apoptosis inducing ligand (TRAIL/Apo2L) and doxorubicin using micellar nanoparticles. Mol Biosyst 2011; 7: 1512-1522.

28 Lee AL, Wang Y, Pervaiz S, Fan W, Yang YY. Synergistic anticancer effects achieved by co-delivery of TRAIL and paclitaxel using cationic polymeric micelles. Macromol Biosci 2011; 11: 296-307.

29 Hu J, Su Y, Zhang H, Xu T, Cheng Y. Design of interior-functionalized fully acetylated dendrimers for anticancer drug delivery. Biomaterials 2011; 32: 9950-9959.

30 Wang Y, Guo R, Cao X, Shen M, Shi X. Encapsulation of 2-methoxyestradiol within multifunctional poly(amidoamine) dendrimers for targeted cancer therapy. Biomaterials 2011; 32: 3322-3329.
31 Padilla De Jesús OL, Ihre HR, Gagne L, Fréchet JM, Szoka FC. Polyester dendritic systems for drug delivery applications: in vitro and in vivo evaluation. Bioconjug Chem 2002; 13:453–461.
32 Chang Y, Meng X, Zhao Y, Li K, Zhao B, Zhu M, Li Y, Chen X, Wang J. Novel water-soluble and pH-responsive anticancer drug nanocarriers: Doxorubicin–PAMAM dendrimer conjugates attached to superparamagnetic iron oxide nanoparticles (IONPs). J Colloid Interf Sci 2011; 363: 403-409.
33 Malik N, Evagorou EG, Duncan R. Dendrimer-platinate: a novel approach to cancer chemotherapy. Anticancer Drug 1999; 10: 767-776.
34 Zhang Y, Thomas TP, Lee K, Li M, Zong H, Desai AM, Kotlyar A, Huang B, Banaszak Holl MM, Baker JR. Polyvalent saccharide-functionalized generation 3 poly(amidoamine) dendrimer–methotrexate conjugate as a potential anticancer agent. Bioorgan Med Chem 2011; 19: 2557-2564.
35 Kaminskas LM, Kota J, McLeod VM, Kelly BD, Karellas P, Porter CJ. PEGylation of polylysine dendrimers improves absorption and lymphatic targeting following SC administration in rats. J Control Release 2009; 140: 108-116.
36 Kaminskas LM, Kelly BD, McLeod VM, Sberna G, Owen DJ, Boyd BJ, Porter CJ. Characterisation and tumour targeting of PEGylated polylysine dendrimers bearing doxorubicin via a pH labile linker. J Control Release 2011; 152: 241-248.
37 Mason T, Wilking J, Meleson K, Chang C, Graves S. Nanoemulsions: formation, structure, and physical properties. J Phys: Condens Matter 2006; 18: R635–R666.
38 Su M, Zhao M, Luo Y, Lin X, Xu L, He H, Xu H, Tang X. Evaluation of the efficacy, toxicity and safety of vinorelbine incorporated in lipid emulsion. Int J Pharm 2011; 411: 188-196.
39 Ikushima I, Higashi S, Seguchi K, Ishii A, Ota Y, Shima M, Kanemaru M, Hidaka Y. Transarterial infusion chemotherapy with epirubicin in water-in-oil-in-water emulsion for recurrent hepatocellular carcinoma in the residual liver after hepatectomy. Eur J Radiol 2009; 69: 114-119.
40 Kotmakchiev M, Cetintas VB, Kantarci G. Determining in vitro anticancer activity of mitomycin C loaded microemulsion. Curr Opin Biotech 2011; 22: S127-S128.
41 Li X, Yue Y, Zhou Y, Fan Y, Fan C, Huang Y, Wu F, Liu Y. An oil-free microemulsion for intravenous delivery of diallyl trisulfide: Formulation and evaluation. Int J Pharm 2011; 407: 158-166.
42 Daocheng W, Mingxi W. Preparation of the core-shell structure adriamycin lipiodol microemulsions and their synergistic anti-tumor effects with diethyldithiocarbamate in vivo. Biomed & Pharmacother 2010; 64: 615-623.
43 Kakumanu S, Tagne JB, Wilson TA, Nicolosi RJ. A nanoemulsion formulation of dacarbazine reduces tumor size in a xenograft mouse epidermoid carcinoma model compared to dacarbazine suspension. Nanomed Nanotech Biol Med 2011; 7: 277-283.
44 Bagul M, kakumanu S, Wilson T, Nicolosi R. In vitro evaluation of anti proliferative effects of self-assembling nanoemulsion of paclitaxel on various cancer cell lines. Nano Biomed and Eng 2010; 2: 11-19.
45 Rapoport N, Nam K, Gupta R, Gao Z, Mohan P, Payne A, Todd N, Liu X, Kim T, Shea J, Scaife C, Parker DL, Jeong E, Kennedy AM. Ultrasound-mediated tumor imaging and nanotherapy using drug loaded, block copolymer stabilized perfluorocarbon nanoemulsions. J Control Release 2011; 153: 4-15.