Chapter 1

Introduction

Cancer is a global disease and its burden is growing fastest in low and middle income countries like India. In a report from the international agency for research on cancer, about 12.7 million cases and 7.6 million deaths occurred in 2008 worldwide. It is reported that by 2030 there will be 26 million new cancer cases and 17 million cancer deaths per year globally (Jemel et al., 2011). Cancer is a class of disease, characterized by uncontrolled division of cells and the ability of these cells to invade other tissues, through invasion or by implantation into distinct sites by metastasis. In normal cell cycle progression, growth regulating mechanism endeavors to maintain homeostasis. Homeostasis within a cell is regulated by the balance between proliferation, growth arrest and apoptosis. Imbalance between cell growth and death may result in cancer. Most of the cancers are caused by abnormalities in the genetic material of the transformed cells. These abnormalities may be due to the effects of carcinogens, such as tobacco, smoke, radiation, chemicals or infectious agents. Other cancer promoting genetic abnormalities may be randomly acquired through errors in DNA replication or are inherited, and thus present in all cells from birth. Genetic abnormalities found in cancer typically affect two general classes of genes. Cancer promoting oncogenes are often activated in cancer cells, giving those cells new properties, such as hyperactive growth and division, protection against apoptosis, loss of respect for normal tissue boundaries, and the ability to establish in diverse tissue environments. Tumor suppressor genes are often inactivated in cancer cells, resulting in the loss of normal functions in those cells, such as accurate DNA replication, control over the cell cycle, orientation and adhesion within tissues, and interaction with protective cells of the immune system. There are many types of cancer like lung, ovarian, skin, breast, prostate, colorectal and bladder.

Treatment options for cancer include surgery, radiotherapy, hormone therapy, immunotherapy and chemotherapy. Among them chemotherapy is the most commonly
used option for the treatment of cancer. Chemotherapy is performed by administering drugs that can destroy cancer cells. Presently available chemotherapy broadly faces two challenges, one related to dosage forms and another related with toxicity and efficacy of anti-cancer drugs used. Currently, anti-cancer drugs for the treatment of different types of cancer are administered using conventional dosage forms like intravenous (i.v.) injection, infusion, tablets and capsules. Conventional dosage forms not only distribute the drug in target tumor tissue, also throughout the body, including vital organs such as heart, liver and kidney. This leads to very severe side effects like cardiotoxicity, myelosuppression and mucositis. In addition to this, very little amount of drug reaches target tumor tissue resulting in very poor therapeutic efficacy. Poor intracellular uptake of anti-cancer drugs is another major problem in the cancer chemotherapy, which leads to the failure of cancer chemotherapy (Tannock et al., 2001). The resistance of solid tumors to anti-cancer drugs is most often attributed to gene mutations, gene amplification or epigenetic changes that influence the uptake or export of drug from cells. Another important cause of anti-cancer drug resistance is the limited ability of drugs to penetrate tumor tissue. To reach all viable cells in the tumor, anti-cancer drugs must be delivered efficiently through the tumor vasculature, cross the vessel wall and transverse the tumor tissue. Major drug related limitations are poor aqueous solubility of some of the key anti-cancer drugs, short biological half life, poor bioavailability, low therapeutic index and development of resistance.

Paclitaxel is one of the commonly used anti-cancer drug for the treatment of various solid tumors including breast, ovarian, lung and AIDS related Kaposi’s sarcoma. The major limitation of paclitaxel is its insolubility in water (≤ 2µg/mL). Due to its poor aqueous solubility, paclitaxel is dissolved in a mixture of Cremophor EL (Polyoxyethylated castor oil): ethanol (1:1) in the marketed parenteral dosage form and is administered as intravenous (i.v) infusion after dilution. Cremophor EL is a non ionic surfactant and toxic in nature. It produces vasodilatation, labored breathing, lethargy, hypersensitivity, cardiotoxicity, nephrotoxicity and neurotoxicity in many patients. Although a premedication regimen with corticosteroids and antihistamine reduces the incidence of serious hypersensitivity, milder reactions have still been reported in 5-30%
of patients (Weiss et al., 1990). Although Cremophor EL has been used as solubilizer for other drugs, the amount necessary to deliver the required doses of paclitaxel is significantly higher than that administered with any other marketed drug (Singla et al., 2002). The reduction in side effects of Cremophor EL based paclitaxel formulation are possible, only if alternative formulation of paclitaxel is developed without using Cremophor EL. In the past decade, an alternative formulation for paclitaxel was studied intensively by different scientists and some products were developed and marketed by different pharmaceutical companies. Abraxane® is first US Food and Drug Administration (USFDA) approved albumin-bound paclitaxel formulation for the treatment of breast cancer (Desai et al., 2006). Abraxane® improved toxicity profile as compared to marketed Cremophor EL based formulation, but only a limited improvement in breast cancer progression-free was achieved (Gradishar et al., 2005). The second product Nanoxel™ is albumin nanoparticles based paclitaxel formulation from Dabur India Ltd. Still, the search for an ideal paclitaxel drug delivery system, which is Cremophor EL free, cost effective and easy to use is underway. Various novel paclitaxel delivery systems that have been studied include polymeric micelles (Zhang et al., 2009; Tsallas et al., 2011), emulsions (Narnoo et al., 2008), nanoparticles (Dong et al., 2004; Desai et al., 2006; Liang et al., 2012), polymer based nanoshells (Zahr and Pishko, 2007), carbon nanotubes (Liu et al., 2008), dendrimers (Ooya et al., 2003) and liposomes (Koudelka et al., 2009; Heney et al., 2010). These approaches have some unique inherent difficulties like toxicity associated with the use of dendrimer and tedious multistep preparation method and poor yield with nanoparticles. Stable micro and nanoemulsions are hard to achieve and due to poor drug loading, high volumes are needed to achieve therapeutic concentrations. Satisfactory entrapment efficiency can sometimes be problematic with microsphere and liposomes. As for micelles based formulation, their disassembly upon injection due to dilution can result in burst release and toxic effects.

Liposomal drug delivery systems are the most established with regulatory approvals for several formulations. Liposomes are small lipid vesicles of spherical shape with a membrane composed of phospholipid bilayers capable of encapsulating hydrophilic and lipophilic drugs. Several FDA approved liposomal formulations like
cyclophosphamide liposomal injection (Depocyt), Doxorubicin PEGylated liposomal injection and Doxorubicin liposomal formulation (Doxil) are available for cancer treatment. Liposomal formulations have greatest impact on oncology field because of their biocompatibility, biodegradability, low toxicity and immunogenicity.

In addition to its poor water solubility, paclitaxel also has poor lipid affinity (Yang et al., 2007a). Due to this reason, it is difficult to encapsulate clinically effective concentration of paclitaxel in conventional lipid based carrier systems like liposomes and lipid nanocapsules. Several authors reported the paclitaxel liposomal formulation as alternate to Cremophor EL based formulation (Gerald and Robert, 2006, Yang et al., 2007a). Low solubility of paclitaxel in the lipid bilayer of liposomes and poor physical stability hindered the development of commercial liposomal based formulation. Elastic liposomes are vesicular carrier system, morphologically similar to liposomes but differ in composition. Elastic liposomes consist of phospholipid and sub-lytic concentration of surfactant. Surfactant is used for increasing the fluidity of vesicle membrane and due to this reason elastic liposomes found to increase the drug loading of highly lipophilic drugs like melatonin (Dubey et al., 2006) and isotretinoin (Kaur et al., 2010).

The research in localized delivery of anti cancer drugs directly to tumor sites has evoked a considerable interest recently due to obvious advantages over conventional routes. Localized drug delivery is a way to deliver the drug from a dosage form to a particular site in the biological system, where its entire pharmacological effect is desired. Localized delivery of anti cancer drug will give sustained drug exposure to tumor cells and increase its tumor penetration and decrease the rate of replication of tumor cells. Local administration of anti cancer drug at the tumor site is also thought to enhance the chemo responsiveness by exposing tumors and adjacent metastasis to high drug concentration while reducing its systemic exposure. Two approaches have been widely studied for localized delivery of anti-cancer drugs. First is the dermal delivery and second is subcutaneous (SC) and intratumoral administration of hydrogel formulation (Khandavilli et al., 2007; Ruel-Gariepy et al., 2002).
Administration through dermal route is one strategy studied by drug delivery specialists with the aim to improve the therapeutic efficiency of chemotherapy by targeting the drug directly to tumor site, especially in breast and skin cancers (Panchagnula et al., 2005; Xiao et al., 2012). As skin is the largest organ of human body and easily accessible, relatively it is easy to administer anti-cancer drugs for skin cancer and breast cancer through this route. The major challenge in the localized delivery of anti-cancer drugs through skin is to increase the penetration of the anti-cancer drugs through stratum corneum in therapeutic effective concentration to kill tumor cells. Skin is mainly composed of three primary layers: the epidermis, dermis and hypodermis. The epidermis plays an important role in the penetration of substances into the skin. It is the outer vascular layer of the skin, primarily composed of keratinocytes. Because of cellular differentiation, the epidermis is divided into different layers, which are formed by the division of basal cells from the inner part of the body toward the surface. Hence, basal cells undergo progressive maturation, giving rise to the spinous layer or squamous cells. These cells also differentiate, forming the granular layer and finally the stratum corneum, which is the outermost layer of the skin. The stratum corneum is the major barrier for the penetration of substances into the skin because of its heterogeneous composition, packed organization of corneocytes and the intracellular lipid matrix. The corneocytes are flat anucleated squamous cells packed primarily with keratin filaments and surrounded by a lipid matrix composed primarily of ceramides, cholesterol, and free fatty acids (Bouwstra et al., 2003). It is important to understand the mechanisms of drug penetration through the stratum corneum in order to determine methods or strategies that can increase drug penetration such that the drug reaches sufficient concentrations to kill tumor cells. Current available topical formulations for localized delivery to skin cancer include semi-solid formulations of 5-fluorouracil and Imiquimod. Another topical formulation approved by the USFDA is photodynamic therapy (PDT). These therapies are used to treat non-melanoma skin cancers.

Paclitaxel is highly effective against many skin cancers (Sqadari et al., 2000), but no topical formulation is commercially available due to its poor skin permeation and irritation potential. Different approaches have been developed to increase skin
penetration of paclitaxel, such as the use of chemical permeation enhancers (Panchagnula et al., 2005), the application of electric field (e.g., iontophoresis and electroporation) (Xiao et al., 2012) and the use of nanocarriers (e.g., lamellar liquid crystalline phase, and polymeric and solid lipid nanoparticles (Kim, et al., 2011, Serpe, et al., 2004). The common goal of these methods is to overcome the barrier of stratum corneum and increase the skin penetration of paclitaxel. The major limitation in these approaches was the very poor skin penetration of paclitaxel.

One strategy to achieve the high local drug concentration at tumor vicinity and also sustain the drug release is to encapsulate paclitaxel in elastic liposomes. Elastic liposomes are basically modified liposomes and developed to increase the skin permeation of encapsulated drug. Elastic liposomes are more elastic than the conventional liposomes up to several orders of magnitude and thus well suited for the skin penetration (Cevc et al., 1997; Jain et al., 2003). The proposed vesicular carrier system has advantages like high transdermal flux, ability to accommodate drug molecules with a wide range of solubility, suitability for high as well as low molecular weight drugs, high entrapment efficiency, biocompatibility and biodegradability. Elastic liposomes can deliver drugs in zero-order fashion for prolonged period of time and are easy to scale up as the method of preparation is simple.

Applicability of elastic liposomes for enhanced skin permeation of drug is well reported in literature. Cevc and coworkers reported first time the concept of elastic liposomes as a carrier for transdermal/topical drug delivery (Cevc and Blume, 1992). Since then, many investigations have been carried out on elastic liposomes and their possible application as drug carriers. Remarkable results have been claimed for better skin permeation ability of elastic liposomes e.g., as much as 50% of a topically applied dose of insulin-penetrated into the skin in vivo in 30 min (Cevc et al., 1998). Honeywell-Nguyen et al. (2002, 2003) observed enhanced skin delivery by using elastic liposomes formulation containing oestradiol and 5-Fluorouracil as model drugs. A combination of iontophoresis and ultra deformable liposomes was found to enhance the delivery of oestradiol (Essa et al., 2002). Similarly, elastic liposomes have been investigated for transcutaneous delivery of dipotassium glycyrrhizinate (Trotta et al., 2002) and
cyclosporin A (Guo et al., 2000). Hofer et al. (1999, 2000, and 2004) and Lehmann et al. (2002) reported enhanced delivery of immunomodulatory proteins Interleukin-2 and Interferon-α after topical application. Kim et al. (2004) prepared ultradeformable cationic liposomes for enhanced transfection efficiency in different cell lines. Vanden Bergh et al. (1998, 1999, 2001) have proved better skin permeation ability of elastic liposomes. Paul et al., 1998 reported the use of elastic liposomes for transdermal immunization. Gupta et al. (2005) also reported the use of elastic liposomal formulation for transdermal immunization. Better systemic delivery of dexamethasone (Jain et al., 2003a), norgestrel (Jain et al., 2005a), diclofenac (Jain et al., 2005b), zidovudine (Jain et al., 2005a), propranolol (Mishra et al., 2007) and sumatriptan succinate (Fernandez et al., 2011) has been observed using elastic liposomal formulations. Enhanced skin permeation and therapeutic effect of elastic liposomal formulation of colchicine were observed by Singh et al. (2009). Significant enhancement of intensity and duration of anaesthetic effect of benzocaine (BZC) and butamben (BTM) was found by elastic liposomal formulation (Maestrellia et al., 2010). Enhanced transdermal flux of valsartan was obtained with elastic liposomal formulation in comparison to rigid liposomes (Ahad et al., 2012). Significantly (5-10 fold) better skin permeation and deposition was observed from elastic liposomes as compared to that from conventional liposomal formulation. In the present study, elastic liposomes are selected as drug carrier for localized delivery of paclitaxel.

Another approach explored in last decade for localization of anti cancer drugs into tumor site is in situ thermosensitive hydrogel drug delivery systems. These in situ thermosensitive hydrogel are gels having special property of remaining liquid at room temperature and converting into semi solid gel, upon attaining body temperature. In literature, applications of this approach for enhanced localization of anti cancer drug have been reported. Obara et al. (2005) administered paclitaxel hydrogel subcutaneously beneath the tumor and observed strong inhibition of tumor in comparison to paclitaxel solution. Sustained release of doxorubicin from thermosensitive hydrogel formulation was observed by Wu et al. (2006). In another study, Mulik et al. (2009) reported thermosensitive hydrogel containing cytrabine loaded liposomes and found sustained release of drug with improved in vivo efficiency in comparison to marketed formulation.
Very recently, Lin et al. (2012) studied the *in situ* thermosensitive hydrogel of paclitaxel and suggested that injectable hydrogel could be employed for site-specific administration to achieve the sustained release of paclitaxel.

Many authors also studied the combination approach in which vesicular carrier was incorporated into hydrogel (Ruel-Gariepy et al., 2002; Mulik et al., 2009). The main rationale behind this type of approach is to further sustain the release of drug. The lipid bilayer present in liposomes provides physical barrier and slows down the diffusion of drug in the hydrogel. The well-known thermo sensitive materials are based either on synthetic (Peter et al., 1998; Elisseef et al., 1999) or natural polymers (Park et al., 2003 and Thein-Han and Stevens, 2004), but these polymers have some drawbacks like their resistance to metabolism, the need of high polymer concentrations, slow rate of conversion to gel. In last decade chitosan received attention for medical and pharmaceutical application. The addition of polyol salts into chitosan solution creates a viscous liquid at room temperatures or below but converts it into semi solid like gel when it approaches body temperature (Chenite et al., 2000 and Crompton et al., 2005).

In the present study attempt has also been made for preparation and characterization of *in situ* thermosensitive elastic liposomal hydrogel formulation by using chitosan for localized delivery of paclitaxel.