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Solid Lipid Nanoparticles: Tuneable Anti-Cancer Gene/Drug Delivery Systems

Tranum Kaur and Roderick Slavcev

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1. Introduction

With the advent of multifunctional nano delivery systems, simultaneous imaging and therapy aspires to detect and treat tumors at a very early stage with promising outcomes. In this context, numerous anti-cancer drug/gene delivery systems have been explored with the primary aim to increase the treatment efficacy without compromising safety. Secondary goals include enhancing bioavailability, specific targeting, apart from the enhanced stability of the formulation [1]. The multifaceted applications of nanoparticles are the direct result of their ability to deliver high pay loads of drugs or biomarkers to the desired sites within the body. Design and development of tumor specific nanoparticles could significantly amplify the delivering capacity to a specific target of interest, without affecting healthy cells [2]. Technological advances in nanomaterials and nanotechnology have paved the way for several carriers such as liposomes [3], dendrimers [4], and micelles [5], solid lipid nanoparticles (SLN) [6] and recently nanostructured lipid carriers [1, 7]. Polymeric micelles, or nanosized (~10–100 nm) supramolecular constructs composed of amphiphilic block-copolymers, are emerging as powerful drug delivery vehicles for hydrophobic drugs. Liposomes are currently the most popular nanosized drug delivery systems, with one or several lipid bilayers enclosing an aqueous core. Liposome-encapsulated formulations of doxorubicin earlier approved for the treatment of Kaposi's sarcoma, are now used against breast cancer and refractory ovarian cancer. Breast cancer in particular has been the focus of many studies involving liposome-based chemotherapeutics, in part due to the clinical success of various drugs such as Doxil, which is a liposomal formulation currently used to treat recurrent breast cancer [7]. The anthracycline doxorubicin is the active cytotoxic agent and is contained within the internal aqueous core of the liposome. The encapsulation of doxorubicin within liposomes significantly re-

duces the cardiotoxicity that commonly results from the use of unencapsulated anthracyclines by decreasing the amount of the drug being delivered to the heart [7]. As such, patients can receive much higher doses of the chemotherapeutic in the liposomal formulation compared to unencapsulated, thereby allowing tumor tissue to potentially be exposed to a lethal dose of the drug while minimizing deleterious side effects. This inherent advantage associated with the use of liposomes as drug delivery vehicles also serves to minimize the many other toxic side effects associated with doxorubicin including gastrointestinal toxicity and complications arising from myelosuppression.

Each delivery system however, has its advantages and limitations. Advantages afforded for drug delivery include the presence of an inner core for lipophilic drug entrapment, as well as a hydrophilic outer shell that prevents particle aggregation and opsonisation [8]. This complexation prevents uptake by the reticuloendothelial system (RES), thereby improving circulation times which, combined with nanoscale sizing, confers preferential accumulation in tumor tissue. In general, nanovectors can be targeted to tumors by passive and active targeting approaches, where a passive strategy takes advantage of a nanvector's small size permitting it to penetrate and accumulate in the tumor. Most solid tumors are sustained by extensive angiogenesis leading to hypervascular tissue with an incomplete vascular architecture. They also have an impaired lymphatic drainage and an increased production of permeability factors resulting in the accumulation and inefficient clearance of nanoparticles leading to the enhanced permeability and retention effect [9]. The hyperpermeable nature of tumor vasculature is characterized by a pore cut off size ranging between 380 and 780 nm allowing particles less than 780 nm to extravasate into the tumor interstitium [10]. In addition, active targeting to various tissues may be achieved *via* utilization of ligands on the surface of nanoparticles, reducing the side effects to the normal tissue by limiting drug/gene distribution to the target organ [11]. An excellent example is Abraxane, an albumin bound nanoparticle formulation of Paclitaxel (PTX), approved by FDA in January 2005 for the treatment of metastatic breast cancer. Abraxane has been shown to outperform standard PTX in the treatment of breast cancer [12]. Utility of this drug was initially limited due to its poor aqueous solubility [13], requiring use of an excipient, Cremophor, which is satisfied by novel engineered nanovectors. A recent Gynecologic Oncology Group Phase II evaluation of albumin-bound paclitaxel nanoparticles to treat recurrent or persistent platinum-resistant ovarian, fallopian tube, or primary peritoneal cancer, concluded that these nanoparticles are as effective and tolerable in their cohort of refractory ovarian cancer patients previously treated with paclitaxel [14]. Nanoparticles fabricated with albumin [15], poly(lactic-co-glycolic acid) [16] and poly lactic acid have also been loaded with PTX and used to passively target tumors. Albumin has been shown to be nontoxic, non-immunogenic, biocompatible and biodegradable making it an ideal candidate to fabricate nanoparticles for drug delivery. Site-specific drug delivery allows for the clinical translation of chemotherapeutic agents with safer targeted cell killing, that are otherwise abandoned due to insolubility, toxicity and safety concerns. Moreover, these new delivery devices can preferentially confine treatments to tumors within the nodal space while sparing healthy tissues.

2. Solid lipid nanoparticles

Solid lipid nanoparticles [17] or lipospheres are rapidly emerging as new class of safer and efficient gene/drug delivery vectors. SLNs are sub-micron colloidal carriers, ranging from 50 nm to 1 μm, that are composed of physiological lipid dispersed in water or in aqueous surfactant solution (Figure 1). SLNs function as an alternative drug carrier system to other novel delivery approaches such as emulsions, liposomes, and polymeric nanoparticles. SLNs offer several advantages conferred by their colloidal dimensions including: i) feasibility of incorporation of lipophilic and hydrophilic drugs; ii) improved physical stability; iii) controlled release; iv) improved biocompatibility; v) potential for site specific drug delivery; vi) improved drug stability; vii) better formulation stability; viii) the ability to freeze dry and reconstitute; ix) high drug payload; x) controllable particle size; xi) the avoidance of carrier toxicity; xii) low production cost; and xiii) easy scale-up and manufacturing [18]. In addition, significant toxicity and acidity associated with a number of biodegradable polymeric materials are not observed with SLNs. And, in contrast to emulsions and liposomes, the particle matrix of SLNs is composed of solid lipids. SLNs can be prepared using wide variety of lipids including lipid acids, mono- (glycerol monostearate), di- (glycerol bahenate) or triglycerides (tristearin), glyceride mixtures or waxes (e.g. cetyl palmitate) and stabilized by the biocompatible surfactants(s) of choice (non-ionic or ionic). Lipids most commonly used are triglyceride esters of hydrogenated fatty acids, including hydrogenated cottonseed oil (Lubritab™ or Sterotex™), hydrogenated palm oil (Dynasan™ P60 or Softisan™ 154), hydrogenated castor oil (Cutina™ HR), and hydrogenated soybean oil (Sterotex™ HM, or Lipo™) as typical examples [19]. Various emulsifiers and their combination (Pluronic F 68, F 127) have also been added to stabilize the lipid dispersion by more efficiently preventing particle agglomeration.
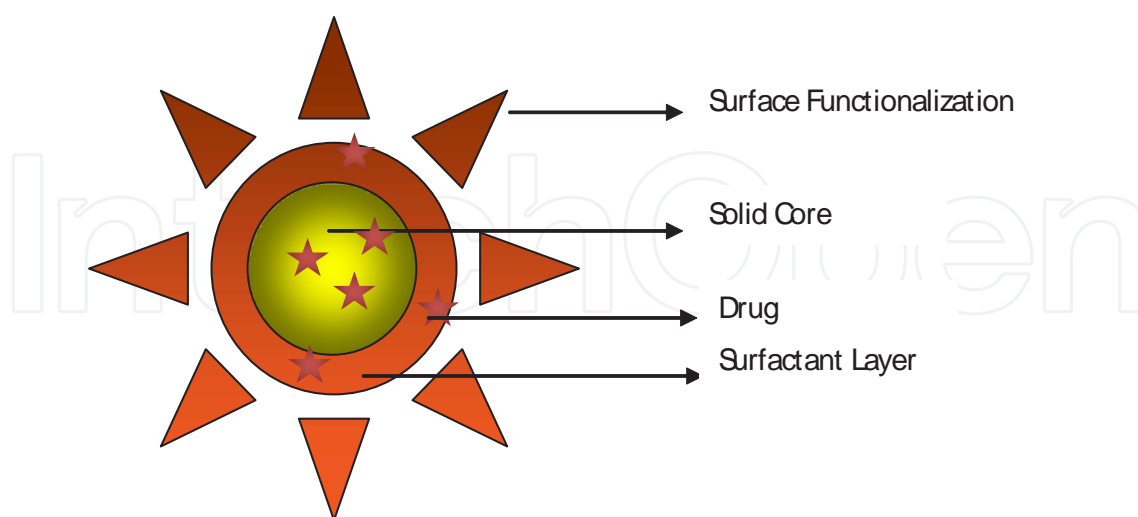

Figure 1. Schematics of Functionalized Solid Lipid Nanoparticles

The disavantages associated with SLNs relate mostly to their preparation, which generally involves high pressure and rapid temperature changes that can lead to high pressure-induced

drug degradation, lipid crystallization, gelation phenomena and the co-existence of several colloidal species [20]. The drug loading capacity of a conventional SLN is limited by the solubility of drug in the lipid melt, the structure of the lipid matrix and the polymeric state of the lipid matrix. If the lipid matrix consists of highly similar molecules (i.e. tristearin or tripalmitin), a perfect crystal with few imperfections is formed. Since incorporated drugs are located between fatty acid chains, between the lipid layers and also in crystal imperfections, a highly ordered crystal lattice cannot accommodate large amounts of drug. This may also lead to the fast release of a large dose of drug initially, generally known as "burst effect", followed by slow and incomplete release of drug. Since high lipid crystallinity is the major cause of burst release of drug from SLNs, this undesirable phenomenon may be minimized by choosing lipids that do not form good crystals, including mono- or di-glycerides, or triglycerides with chains of different lengths. For this reason, in formulation design use of more complex lipids is recommended for higher drug loading. Nanostructured lipid carriers or NLCs were designed to overcome these disadvantages with the main goal to increase drug loading and prevent drug expulsion [21]. For NLCs, the highest drug load could be achieved by mixing solid lipids with small amounts of liquid lipids (oils). These types of NLCs are called multiple types NLC, and are analogous to w/o/w emulsions since it is an oil-in-solid lipid-in-water dispersion.

3. SLN preparation methods

There are two main established SLN synthesis techniques, namely, the high-pressure homogenisation technique described by Müller and Lucks [21], and the microemulsion-based technique described by Gasco [22, 23]. SLNs are prepared from lipid, emulsifier and water/solvent using different methods, discussed below.

3.1. High Pressure Homogenization (HPH)

High Pressure Homogenization (HPH) is a very reliable technique in the production of SLNs. High pressure homogenizers are employed to push a liquid with high pressure (100–2000 bar) and the fluid accelerates on a very short distance to very high velocity (>1000 Km/h).. Very high shear stress and cavitation forces disrupt the particles down to the submicron range. Generally 5-10% lipid content is used but up to 40% lipid content has also been investigated. Typical SLNs production conditions are 500 bar and two or three homogenisation cycles. Two general approaches of HPH are hot and cold homogenization, both working on the same concept of mixing the drug in bulk of lipid melt.

3.1.1. Hot homogenization

Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion (Figure 2). A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device. The quality of the pre-emulsion affects the quality of the final product to a great extent and it is desirable to obtain droplets in the size range of a few

micrometers. HPH of the pre-emulsion is carried out at temperatures above the melting point of the lipid. Usually, lower particle sizes are obtained at higher processing temperatures because of lowered viscosity of the lipid phase [24].

Hot homogenisation is the most frequently applied technique in which even temperature sensitive compounds can be processed because of the short exposure time to the elevated temperatures [25]. However, high temperatures increase the degradation rate of the drug and the carrier. Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size due to high kinetic energy of the particles. The cold homogenisation technique is therefore recommended for extremely temperature sensitive compounds and hydrophilic compounds, which might partition from the liquid lipid phase to the water phase during the hot homogenisation.

3.1.2. Cold homogenization

During cold homogenization, the drug containing lipid melt is cooled and, after solidification, the lipidic mass is ground to yield lipid microparticles [26]. The lipid microparticles are dispersed in cold surfactant solution by stirring, yielding a macro-suspension. This suspension is then passed through a high-pressure homogeniser at or below room temperature, where the microparticles are broken down to solid lipid nanoparticles. However, compared to hot homogenization, larger particle sizes and a broader size distribution are typical of cold homogenized samples.

3.1.3. Ultrasonication or high speed homogenization

SLNs are also developed by high speed stirring or sonication [27]. The ultrasonic dispersion may offer an appropriate alternative for laboratory scale productions due to its rapid nature and the relatively low cost of required apparatus. So far, its suitability has only been evaluated for SLN [17, 28]. The primary disadvantage of this method is the broader particle size distribution that is yielded, ranging into the micrometer range. Potential metal contamination due to ultrasonication is another issue presented by this method. To generate more stable formulations, high speed stirring and ultrasonication may be used in combination at high temperature.

3.2. Solvent emulsification/evaporation

In this method, the lipidic material, such as glyceride is dissolved in an organic solvent (e.g. chloroform, cyclohexane) and the solution is emulsified in an aqueous phase [29]. After evaporation of the solvent the lipid precipitates to form nanoparticles with a mean diameter of around 30 nm using cholesterol acetate as a model drug and lecithin/sodium glycocholate blend as an emulsifier [30]. The solution is emulsified in an aqueous phase by high pressure homogenization and the organic solvent is removed from the emulsion by evaporation under reduced pressure (40–60 mbar).

3.3. Supercritical fluid

This platform technology, with several variations for powder and nanoparticle preparation, is a relatively new technique for SLN production and offers the advantage of solvent-less

processing [31]. SLNs can be prepared by the rapid expansion of supercritical carbon dioxide solutions (RESS) method, where carbon dioxide (99.99%) is a good choice as solvent.

4. Microemulsion method

This method is based on the dilution of microemulsions that are two-phase systems composed of an inner and outer phase (e.g. o/w microemulsions) [32]. They are made by stirring an optically transparent mixture at 65-70°C, which typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol) and water. The hot microemulsion is dispersed in cold water (2-3°C) with stirring. SLN dispersion can be used as granulation fluid for transferring into solid product (tablets, pellets) by granulation process, but in case of low particle content, excess water must first be removed. High-temperature gradients facilitate rapid lipid crystallization and prevent aggregation. Due to the dilution step, achievable lipid contents are considerably lower compared with the HPH based formulations.
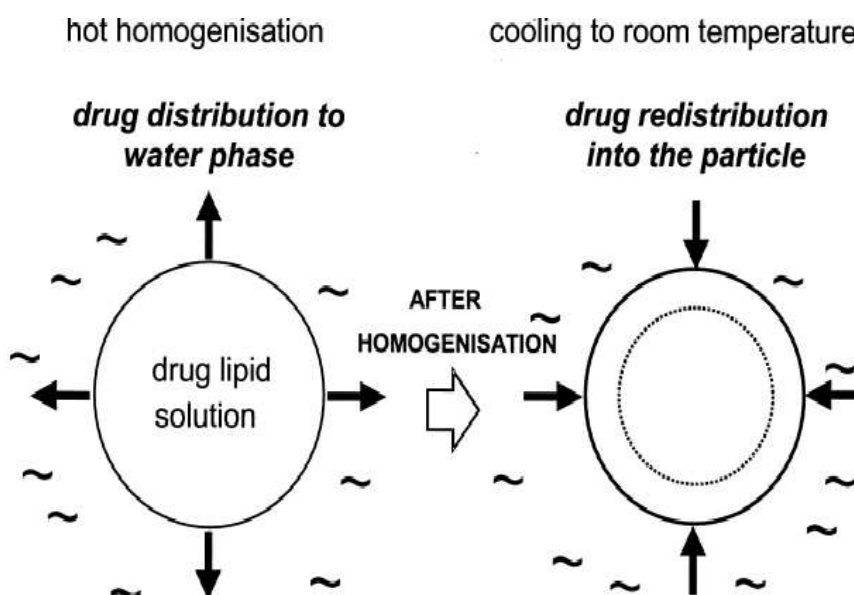

Figure 2. Partitioning effects on drug during the hot homogenization technique production of SLNs. *Left:* Partitioning of drug from the lipid phase to the water phase at increased temperature. *Right:* Re-partitioning of the drug to the lipid phase during cooling of the produced O/W nanoemulsion. Source: Muller RH et al. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. European Journal of Pharmaceutics and Biopharmaceutics 50: (2000) 161-177.

4.1. Spray drying method

Spray drying is an alternative procedure to lyophilization in the transformation of an aqueous SLN dispersion into a solid drug product. This method results in particle aggregation due to high temperature, shear forces and partial melting of the particle. The use of lipid with melting point >70°C for spray drying is recommended [33]. Best results are obtained with an SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol-water mixtures (10/90 v/v).

4.2. Double emulsion method

For the preparation of hydrophilic loaded SLN, double emulsion method, a novel approach based on solvent emulsification-evaporation can be employed. Here, the drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase during solvent evaporation in the external water phase of w/o/w double emulsion [34]

5. SLNs cellular uptake, pharmacokinetics and bio-distribution

Research on cellular uptake mechanisms has repeatedly demonstrated that endocytosis is the preferred route of internalization of non-viral gene vectors via a number of distinct endocytic processes. The most studied mechanisms include macropinocytosis, circular dorsal ruffles, clathrin-mediated endocytosis and several clathrin-independent endocytic pathways [35]. Endocytic uptake mechanisms are highly dependent on cell type and on the nature of gene vectors [36]. Clathrin-mediated processes are limited to particles under 200 nm in size, whereas caveolae-dependent uptake prevails for particles between 200 and 500 nm [37]. The prevalent pathway for the cell internalization of PEI polyplexes is however, clathrin-dependent [38].

Apart from overcoming cellular barriers of uptake, an **anticancer** drug must be specifically targeted to the tumor in order to maximize its therapeutic effect, and therefore biodistribution studies are critical to assess the safety of a nanomedicine. However, since most groups work on healthy instead of tumor-bearing animals, it is difficult to confirm whether SLNs can lead to increased tumor drug concentrations by way of enhanced permeability and retention [9]. Recently, Zhang *et al.* (2010) evaluated antitumor efficacy of docetaxel-loaded **solid lipid nanoparticles** (DSN) in a murine ovarian **cancer** model [39]. In this study, SLN biodistribution from RES more toward the circulation system was observed. Moreover, SLNs in comparison to the free drug demonstrated more potent *in vivo* anti-ovarian **cancer** activity with improved pharmacokinetics. In contrast, paclitaxel loaded in pegylated **solid lipid** nanoparticles were mainly taken up by the RES after intravenous administration in rats, showing 8-fold and 3-fold higher levels in liver and spleen, respectively, 8 h after administration compared to paclitaxel in Taxol® [40]. Moreover, paclitaxel levels in kidney, heart and lung were indistinguishable between the two formulations. The difference in biodistri-

bution of SLNs reported in literature may be due to several factors including variations in size, surface functionalization and composition.

The biodistribution of an anticancer drug delivered by SLN may be further manoeuvred by route of injection to achieve the desired therapeutic goal. Harivardhan Reddy *et al.* (2005) compared the biodistribution of free [99] mTechnetium-labeled etoposide and radio-labeled etoposide loaded SLNs in Dalton's lymphoma tumor-bearing mice [41]. They showed that administration via the subcutaneous route resulted in high tumor uptake of etoposide and etoposide loaded tripalmitin nanoparticles and was the preferred route as compared to intravenous or intraperitoneal administration. However, elevated tumor drug concentrations were also found with intravenously administered etoposide loaded SLN in comparison to the free drug, (approximately 67% increase 1 h post-injection, 30% increase 24 h post-injection). In yet another study by Zara *et al.* (2002) duodenal administration of idarubicin-loaded SLN led to higher bioavailability than intravenously administered SLNs [42]. Also, idarubicin and its main metabolite, idarubicinol, were detected in the brain after IDA-SLN administration, indicating that the SLNs were able to pass the blood-brain barrier; an attractive attribute in the tratement of brain tumors. Thus, the route of administration of SLN formulation is a key consideration in the design of animal or clinical anti-cancer drug delivery studies.

6. SLNs as anti-cancer gene/drug delivery vectors: Challenges and successes

Solid lipid nanoparticles have rapidly established themselves during the past decade as stable, reliable and easy to produce vectors. SLN advantages over other existing transfection vectors include safety, good storage stability, possibility of lyophilization and a high degree of flexibility in design and optimization [25]. Cationic SLNs can efficiently bind DNA directly *via* ionic interaction and mediate gene transfection. However, as with all non-viral vectors, many cellular obstacles have to be overcome to achieve satisfactory levels of transfection activity: i) binding to the cell surface; ii) cellular internalization; iii) escape from the endolysosomal compartment; and iv) translocation through the nuclear envelope. In order to surmount these barriers, cationic SLNs are designed as multifunctional "smart" carriers for efficient gene expression [43]. Components such as chitosan [44] and surface functionalization moieties e.g. poly(styrene-4 sodium sulfonate) (PSS) and poly(L-lysine hydrochloride) (PLL) [45], folate–chitosan and cholesterol derivative (CHETA) [46], cetyltrimethyl ammonium bromide (CTAB) [47] and a phyto-ceramide [48] and TAT peptides [49], may each individually assist in overcoming the barriers of efficient transfection. In addition, protamine a cationic small protein rich in arginine exerts both DNA condensation activity and proton sponge effect facilitating endosomal escape as well as assisting nanovectors to enter the nucleus owing to its nuclear localization signal (NLS) [50]. Table 1 lists some of the successful SLN formulations evaluated as anti-cancer agents in various cancers.

SLN Composition	Characterization	Indication	Drug/Gene	Reference
Stearic acid, DOTAP, Pluronic F68 and dioleoylphosphatidylethanolamine (DOPE)	Size, Zeta Potential	Prostate Cancer	Plasmid DNA	[s51]
Tricaprin as a core, 3beta[N-carbamoyl] cholesterol (DC-Chol), DOPE and Tween 80	Size, Zeta Potential, Differential Scanning Calorimetry	Lung Cancer	Plasmid DNA	[52]
Stearic acid, Lecithin and PS	Transmission electron microscopy	Lung Cancer	Phospho-Sulindac	[53]
Poloxamer 188 and Tween 80	Size, Zeta potential	Breast Cancer	Emodin	[54]
Precirol, Compritol, soybean Phosphatidylcholine, Tween 80	Size, Zeta Potential	Breast Cancer	Tryptanthrin	[55]
Myristic acid, Stearic acid, Palmitic acid, lauric acid, poly(ethylene glycol)-100-stearate (PEG100SA), poly(ethylene glycol)-40-stearate (PEG40SA), Hydrolyzed polymer of epoxidized soybean oil, Pluronic F68 (PF68) (non-ionic block copolymer)	Size, Zeta Potential, Transmission Electron Microscopy	Breast Cancer	Doxorubicin and Mitomycin -C	[56]
Stearyl alcohol and cetyltrimethylammonium bromide (CTAB), Ceramide VI, polysorbate 60	Size, Zeta Potential	Ovarian Cancer	Doxorubicin and mixed-backbone GCS antisense oligonucleotides (MBO-asGCS)	[57]
1,2-Dioleoyl-sn-glycero-3-ethylphosphocholine 1,2-diphytanoyl-sn-glycero-3-phosphatidylethanolamine (DPhPE), 3â[N-(N′,N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol), 1,2-Dioleoyl-sn-glycero-3-ethylphosphocholine (EDOPC), and methoxypolyethylene glycol 2000-distearoyl phosphatidylethanolamine (mPEG-DSPE), glyceryl trioleate.	Zeta potential and Gel retardation	Epithelial Cancer	Paclitaxel and siRNA	[58]
Cholesteryl oleate, glyceryl trioleate, DOPE, Chol , and DC-Chol	Size, Zeta Potential	Brain Cancer (glioblastomas)	c-Met siRNA	[59]
Stearic acid, Glyceryl behenate	Size, Zeta Potential	Skin Cancer	Doxorubicin	[60]

Table 1. Solid Lipid Nanoparticles loaded with DNA/ Drug as anti-cancer delivery systems in various cancers.

Nanovectors offer the potential to both detect and treat cancer at a very early stage, thereby maximizing survival rates. The NCI (National Cancer Institute) Alliance for Nanotechnology in Cancer provides up-to-date information in nano-cancer research and its promise for cancer diagnosis and treatment (http://nano.cancer.gov/). Using siRNA molecules loaded in nano-vectors, early proof-of-principle experiments in various tumor cells suggest that RNA silencing may have great potential as a strategy for treating cancer. However, siRNA therapeutics are hindered by poor intracellular uptake, limited blood stability and undesirable non-specific immune stimulation [61]. An interesting strategy used to target the vector employs three-amino-acid peptide, arginine-glycine-aspartic acid (known by its amino acid code RGD) that binds to integrins, which in turn are involved in angiogenesis, tumor cell growth, metastasis, and inflammation. Intravenous administration into tumor-bearing mice of nanoparticles combined with a dual strategy of siRNA inhibiting vascular endothelial growth factor receptor-2 and RGD peptide ligand attached at the distal end of the polyethylene glycol [40], conferred selective tumor uptake, and inhibition of both tumor angiogenesis and growth rate, achieving both tissue and gene selectivity [62]. In February 2012, Calando Pharmaceuticals, in Pasadena, Canada, and the National Cancer Institute (NCI) entered into a collaborative development program for a nanoparticle-based siRNA therapeutic aimed at treating neuro-blastoma, the most common extracranial solid tumor in children less than five years of age. Previous attempts to develop targeted nanoparticles were unsuccessful due to the inherent difficulties of designing and scaling up a particle capable of targeting, long-circulating via immune-response evasion and controlled drug release. Very recently, Hrkach *et al.* (2012) reported the preclinical development and clinical translation of a docetaxel nanoparticle with prostate-specific membrane antigen, a clinically validated tumor antigen expressed on prostate cancer cells and on the neovasculature of most non-prostate solid tumors including breast, head, lung, neck, prostate and stomach [63]. This targeted nanoparticle-based compound called "BIND-014" is currently the first one to enter clinical trial, although with small number of only 17 patients. Patients with advanced or metastatic cancer receive an injection of the nano-drug once every three weeks and are showing signals of efficacy even at relatively low doses. This initial but positive result shows promise and the potential impact of nanomedicines as a paradigm shift in the treatment of cancer.

Very recently, Vighi E *et al.* (2012) developed a multicomponent cationic SLN as a pDNA delivery vehicle. The formulations were prepared using stearic acid as the main component in the lipid phase, stearylamine, the main component in the aqueous phase, as cationic agent and protamine as transfection promoter along with the phosphatidylcholine (SLN–PC), cholesterol (SLN–Chol) or both (SLN–PC–Chol). Transfection results on various cell lines in this study revealed the best transfection for SLN–PC–Chol on COS-1 cells (African green monkey kidney cell line) [64]. However, lower transfection levels than poly [62] were observed on HepG2 cells (human hepatocellular liver carcinoma cell line), regardless of the SLN composition. Using COS-1 monkey kidney fibroblast-like cells, SLNs and liposomes formu-lated from the same cationic lipids, demonstrated equipotent *in vitro* transfection efficiencies [65]. This study suggests that only the lipid composition in the tested lipid-based formulations affected transfection efficiencies. The intrinsic toxicity that is common in cationic gene delivery vehicles may also be minimized, while maintaining high transfection efficiency, by selecting

good combinations of two-tailed cationic lipids and matrix lipids. Hence, structural or compositional design changes of nanovectors may influence the outcome in relation with cell physiology, cell internalization pathways and transfection efficiency. The above results support the use of SLNs to serve as nano/microcarriers for anti-cancer gene therapies.
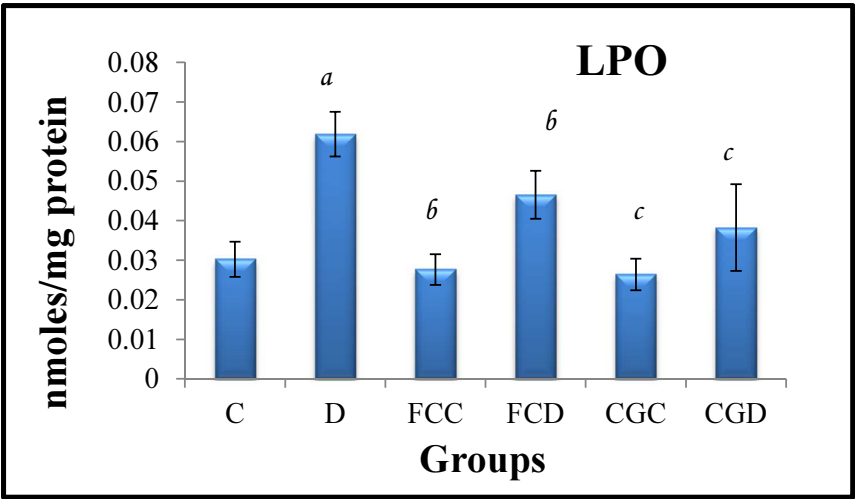
Under optimised conditions SLNs can be designed to incorporate lipophilic or hydrophilic drugs and seem to fulfil the requirements for an optimum particulate carrier system. Stability studies were performed on SLNs loaded with all-*trans* retinoic acid (ATRA), another compound that is sensitive to light, heat and oxidants, and quickly degrades into less active products such as isotretinoin and all-*trans*-4-oxo [66]. After 3 months of storage at 4 °C, more than 90% of the ATRA drug molecules in SLN remained chemically intact. This can be compared to approximately 50% drug degradation when stored at the same temperature in the form of methanol solution or 1% polysorbate-80 solution for only 1 month. Hence, SLNs are useful for the protection of anticancer compounds that are sensitive to light, and probably heat and oxidants as well. In a study conducted by our group, modulatory effects of encapsulated and free forms of sesamol (anti-oxidant and anti-cancer compound) were evaluated by the topical delivery systems in a skin cancer mice model. Both free sesamol and SLN dispersion were applied as gels (using 1% w/v of Carbopol 934P®) on the skin of mice. Encapsulated or nanosesamol was found to safely exert chemopreventive effects by decreasing the lipid peroxidation levels and increasing the anti-oxidant levels, thereby decreasing the development and promotion of skin tumors. Immunofluorescence studies of pro- and anti-apoptotic markers, bcl-2 and bax protein expression revealed higher expression of anti-apoptotic protein, bcl-2, in the tissue sections of tumor bearing mice in comparison to their control counterparts and groups which received sesamol treatment, reinforcing the role of bcl-2 in skin carcinogenesis. Higher expression of bax was also observed in sesamol treated animals as compared to the tumor bearing mice. Up-regulation of bax in the control and sesamol treated groups suggests that it follows the intrinsic pathway of apoptosis (unpublished results).

Ongoing work by our group compared neutraceutical curcumin-loaded SLNs to the free form as a chemopreventive topical delivery system in 7,12-dimethylbenz [*a*]anthracene (DMBA) induced skin cancer model mice. In order to understand the molecular events underlying nanocurcumin-mediated chemoprevention, protein expression of various biomolecules e.g. anti and pro inflammatory cytokines (Il-4 and Il-1β) were analyzed by Western immunoblotting and immunoflourscence. For cancer induction, male Balb/c mice were subcutaneously injected with 30 mg/Kg body weight of DMBA (in olive oil) once a week for three weeks. DMBA skin cancer induced mice were topically applied free and encapsulated curcumin (50mg/Kg b.w) as a chemopreventive agent from one week before DMBA injection to the experiment's end (18 weeks). We found that free and nanocurcumin treatment of DMBA treated mice reduced the levels of malondialdehyde, a by-product of lipid degradation (Figure 3). Antioxidant analysis revealed increased levels of enzymes (SOD, Catalase, Reduced Glutathione, Total Glutathione) in encapsulated nanocurcumin treated group as compared to free curcumin group (Figure 4-7). Immunofluorscence studies and western blot analysis of Il-4 and Il-1β suggest enhanced anti-inflammatory potential of encapsulated curcumin in comparison to mice treated with free curcumin. Mice bearing skin tumors showed increased expression of

pro-inflammatory interleukins when compared to the control, which was decreased on treatment with curcumin (Figure 8). Furthermore, the immunoflourscence assay of anti-inflammatory interleukin (IL-4) showed a far greater increase in IL-4 expression by topical treatment with encapsulated curcumin as compared to the free curcumin in mice bearing skin tumors (Figure 9).
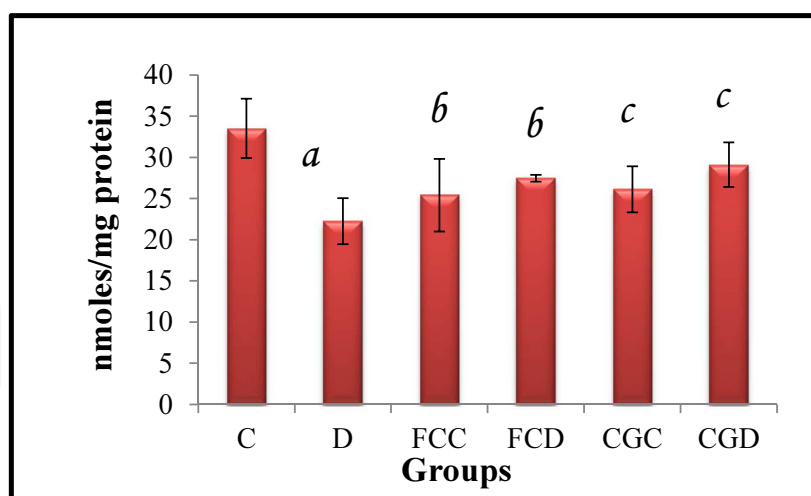
7. Conclusion

Solid Lipid Nanoparticles serve as efficient and safe DNA/ drug loaded nanosystems in both the imaging and treatment of cancer. Traditional drug delivery systems are often hindered by their low bioavailabilty, low solubility, toxicity and rapid clearance. In future, clinicians and researchers will be able to "**tune and time**" the amount of DNA/Drug delivery by controlling the release at specific location thereby minimizing their toxicity and side- effects.

The value are presented as Mean ± SEM., N=8-10

Statistical significance : Control vs DMBA $p \leq 0.001^a$

:DMBA vs FCD and FCC $p \leq 0.001^b$

:DMBA vs CGD and CGC $p \leq 0.001^c$

Figure 3. Effect of encapsulated and free curcumin on lipid peroxidation (LPO) in control and experimental groups. Control (C), DMBA (D), Free Curcumin (FCC), Free Curcumin + DMBA (FCD), Encapsulated Curcumin (CGC), Encapsulated Curcumin + DMBA (CGD).
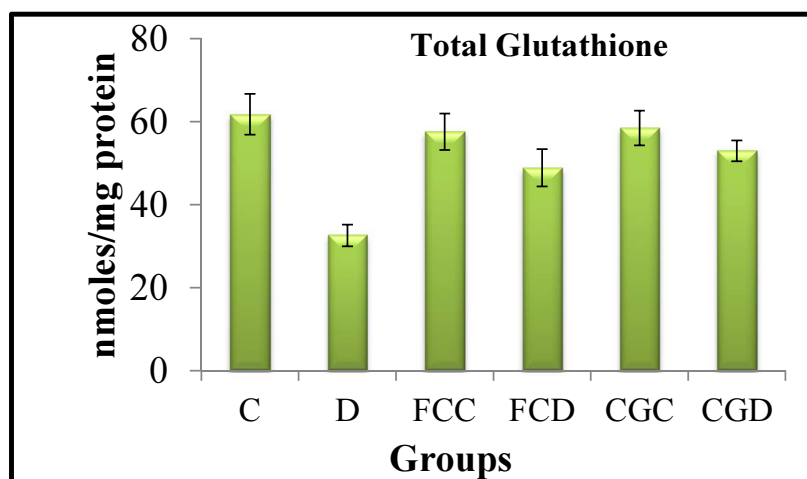
The value are presented as Mean ± SEM., N=8-10

Statistical significanc: Control vs DMBA   p≤0.001[a]

:DMBA vs FCD and FCC   p≤0.001[b]

:DMBA vs CGD and CGC   p≤0.001[c]

Figure 4. Effect of encapsulated and free curcumin on reduced glutathione in control and experimental groups. Control (C), DMBA (D), Free Curcumin (FCC), Free Curcumin + DMBA (FCD), Encapsulated Curcumin (CGC), Encapsulated Curcumin + DMBA (CGD).
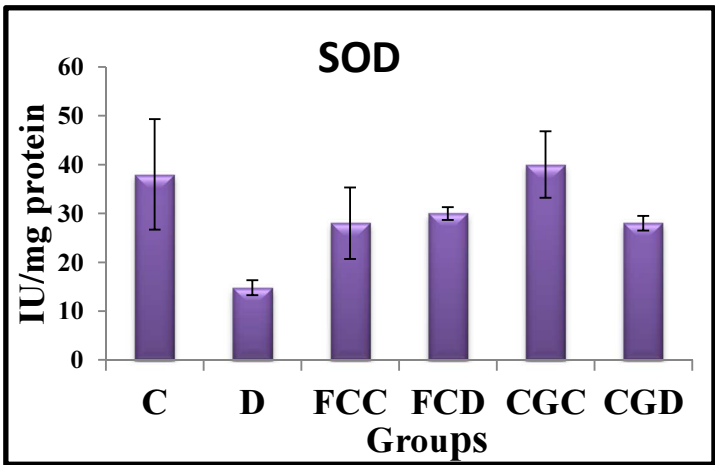

The value are presented as Mean ± SEM., N=8-10

Statistical significance: Control vs DMBA   p≤0.001[a]

:DMBA vs FCD and FCC   p≤0.001[b]

:DMBA vs CGD and CGC   p≤0.001[c]

Figure 5. Effect of encapsulated and free curcumin on total glutathione in control and experimental groups. Control (C), DMBA (D), Free Curcumin (FCC), Free Curcumin + DMBA (FCD), Encapsulated Curcumin (CGC), Encapsulated Curcumin + DMBA (CGD).

The value are presented as Mean± SEM., N=8-10

Statistical significance: Control vs DMBA $p \leq 0.05^a$
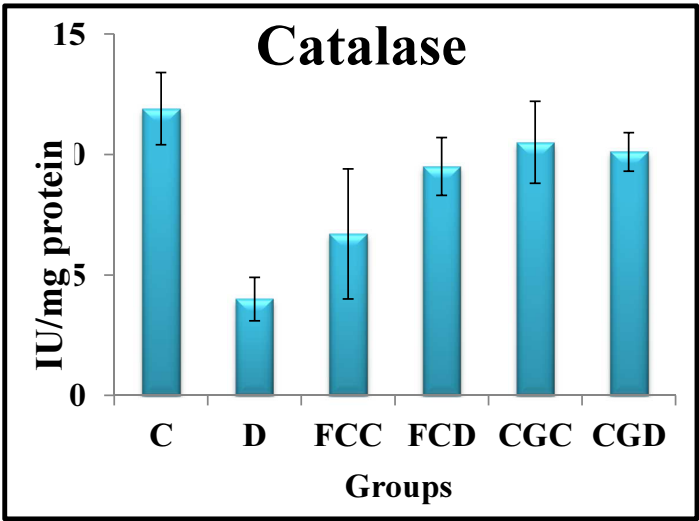
:DMBA vs FCD and FCC $p \leq 0.01^b$

:DMBA vs CGD and CGC $p \leq 0.01^c$

Figure 6. Effect of encapsulated and free curcumin on superoxide dismutase in control and experimental groups. Control (C), DMBA (D), Free Curcumin (FCC), Free Curcumin + DMBA (FCD), Encapsulated Curcumin (CGC), Encapsulated Curcumin + DMBA (CGD).

The value are presented as Mean± SEM., N=8-10

Statistical significance: Control vs DMBA $p \leq 0.05^a$

:DMBA (D) vs FCD and FCC $p \leq 0.05^b$

:DMBA (D) vs CGD and CGC $p \leq 0.01^c$

Figure 7. Effect of encapsulated and free curcumin on Catalase in control and experimental groups. Control (C), DMBA (D), Free Curcumin (FCC), Free Curcumin DMBA (FCD), Encapsulated Curcumin (CGC), Encapsulated Curcumin + DMBA (CGD).
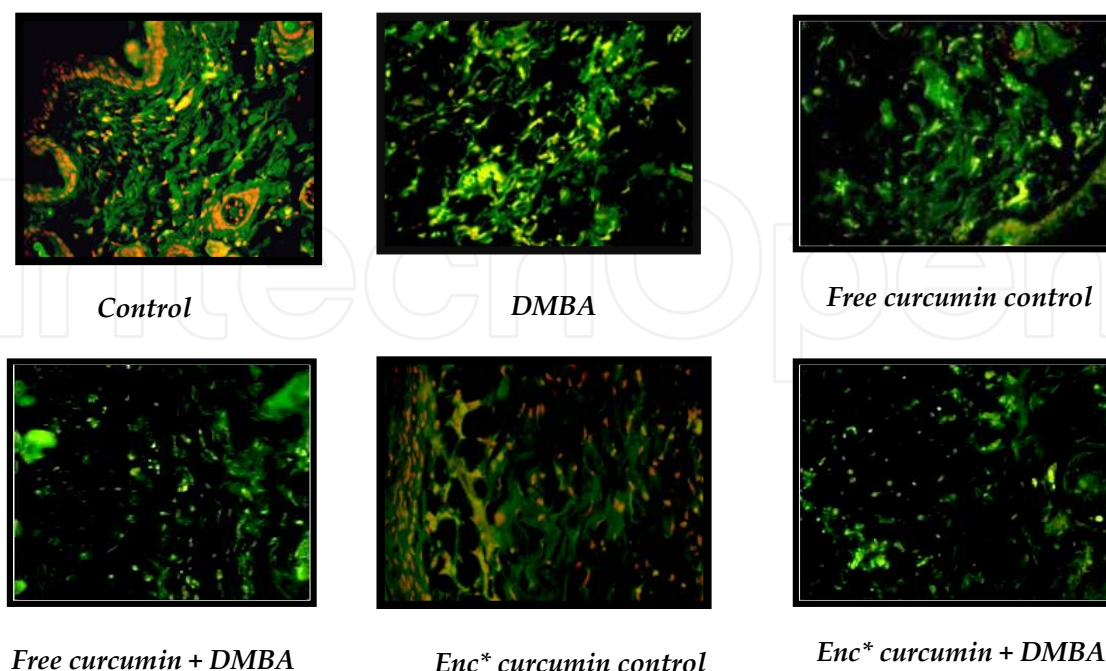
Immunofluorscence Expression of IL-1β

Control   *DMBA*   *Free curcumin control*

Free curcumin + DMBA   *Enc* curcumin control*   *Enc* curcumin + DMBA*

Figure 8. Photomicrographs (20X) showing expression of IL-1Beta in paraffin sections by immunofluoroscence after 18 weeks of treatment.
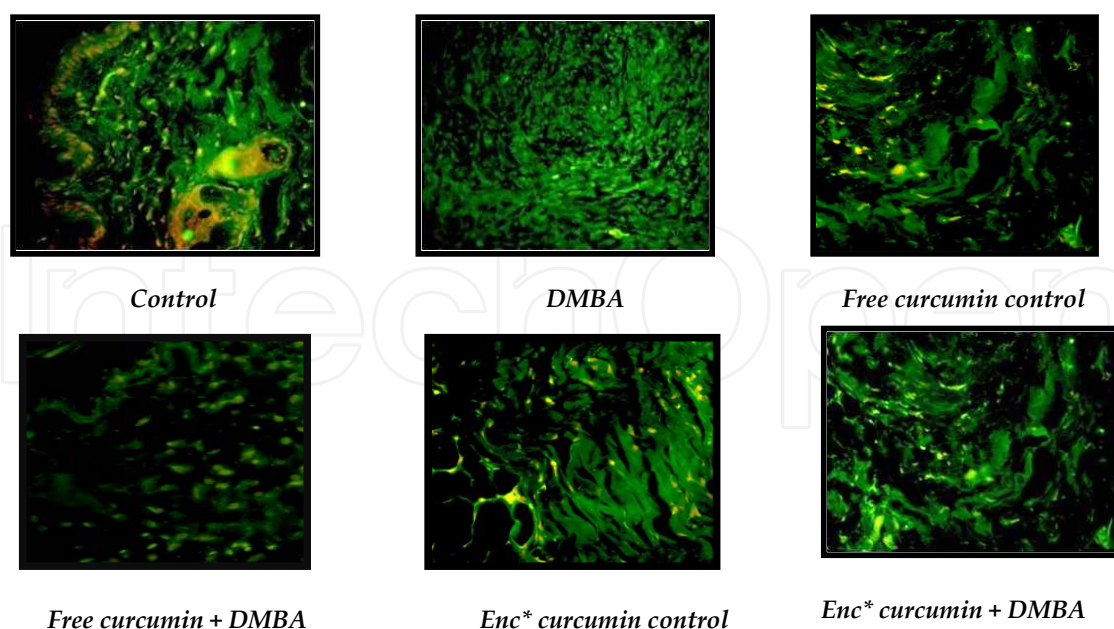
Immunofluorscence expression of IL-4

Control   *DMBA*   *Free curcumin control*

Free curcumin + DMBA   *Enc* curcumin control*   *Enc* curcumin + DMBA*

Figure 9. Photomicrographs (20X) showing expression of IL-4 in paraffin sections by immunofluoroscence after 18 weeks of treatment.

Author details

Tranum Kaur[1] and Roderick Slavcev[2*]

*Address all correspondence to: slavcev@uwaterloo.ca

1 Department of Biophysics, Panjab University, Chandigarh, India

2 School of Pharmacy, University of Waterloo, Kitchener, Ontario, Canada

References

[1] Petkar, K. C, Chavhan, S. S, Gatonovik-kustrin, S, & Sawant, K. K. Nanostructured materials in drug and gene delivery: a review of the state of the art. Crit Rev Ther Drug Carrier Syst (2011). , 28(2), 101-64.

[2] Arias, J. L, Clares, B, Morales, M. E, Gallardo, V, & Ruiz, M. A. Lipid-based drug delivery systems for cancer treatment. Curr Drug Targets (2011). Jul;, 12(8), 1151-65.

[3] Kozako, T, Arima, N, Yoshimitsu, M, Honda, S. I, & Soeda, S. Liposomes and nano-technology in drug development: focus on oncotargets. Int J Nanomedicine (2012). doi:IJN.S30726. Epub;%2012 Sep 14.:4943-51., 7, 4943-51.

[4] Pearson, R. M, Sunoqrot, S, Hsu, H. J, Bae, J. W, & Hong, S. Dendritic nanoparticles: the next generation of nanocarriers? Ther Deliv (2012). Aug;, 3(8), 941-59.

[5] Blanco, E, Kessinger, C. W, Sumer, B. D, & Gao, J. Multifunctional micellar nanomedi-cine for cancer therapy. Exp Biol Med (Maywood) (2009). Feb;, 234(2), 123-31.

[6] Mehnert, W, & Mader, K. Solid lipid nanoparticles Production, characterization and applications. Adv Drug Deliv Rev (2012). Sep;(12):10.

[7] Prados, J, Melguizo, C, Ortiz, R, Velez, C, Alvarez, P. J, Arias, J. L, Ruiz, M. A, Gallardo, V, & Aranega, A. Doxorubicin-Loaded Nanoparticles: New Advances in Breast Cancer Therapy. Anticancer Agents Med Chem (2012). Feb.

[8] Torchilin, V. P. Structure and design of polymeric surfactant-based drug delivery systems. J Control Release (2001). Jun;73(2-3):137-72.

[9] Greish, K. Enhanced permeability and retention (EPR) effect for anticancer nanomedi-cine drug targeting. Methods Mol Biol (2010). , 624, 25-37.

[10] Hobbs, S. K, Monsky, W. L, Yuan, F, Roberts, W. G, Griffith, L, Torchilin, V. P, & Jain, R. K. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. Proc Natl Acad Sci U S A (1998). Apr;, 95(8), 4607-12.

[11] Brigger, I, Dubernet, C, & Couvreur, P. Nanoparticles in cancer therapy and diagnosis. Adv Drug Deliv Rev (2012). Sep;(12):10.

[12] Miele, E, Spinelli, G. P, Miele, E, Tomao, F, & Tomao, S. Albumin-bound formulation of paclitaxel (Abraxane ABI-007) in the treatment of breast cancer. Int J Nanomedicine (2009). Epub;%2009 Apr;%20.:99-105., 4, 99-105.

[13] Liggins, R. T, Hunter, W. L, & Burt, H. M. Solid-state characterization of paclitaxel. J Pharm Sci (1997). Dec;, 86(12), 1458-63.

[14] Coleman, R. L, Brady, W. E, Mcmeekin, D. S, Rose, P. G, Soper, J. T, Lentz, S. S, Hoffman, J. S, & Shahin, M. S. A phase II evaluation of nanoparticle, albumin-bound (nab) paclitaxel in the treatment of recurrent or persistent platinum-resistant ovarian, fallopian tube, or primary peritoneal cancer: a Gynecologic Oncology Group study. Gynecol Oncol (2011). Jul;, 122(1), 111-5.

[15] Ibrahim, N. K, Desai, N, Legha, S, Soon-shiong, P, Theriault, R. L, Rivera, E, Esmaeli, B, Ring, S. E, Bedikian, A, Hortobagyi, G. N, Ellerhorst, J. A, & Phase, I. and pharmacokinetic study of ABI-007, a Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel. Clin Cancer Res (2002). May;, 8(5), 1038-44.

[16] Su, W. P, Cheng, F. Y, Shieh, D. B, Yeh, C. S, & Su, W. C. PLGA nanoparticles codeliver paclitaxel and Stat3 siRNA to overcome cellular resistance in lung cancer cells. Int J Nanomedicine (2012). Epub;%2012 Aug 3.:4269-83., 7, 4269-83.

[17] Dasgupta, S, Mazumder, B, Ghosh, S. K, & Kaurav, S. S. Solid lipid nanoparticles (SLNs) for topical delivery of aceclofenac by using xanthan gum: Ex vivo and in vivo evaluation. Curr Drug Deliv (2012). Sep.

[18] Mukherjee, S, Ray, S, & Thakur, R. S. Solid lipid nanoparticles: a modern formulation approach in drug delivery system. Indian J Pharm Sci (2009). Jul;, 71(4), 349-58.

[19] Weyhers, H, Ehlers, S, Hahn, H, Souto, E. B, & Muller, R. H. Solid lipid nanoparticles (SLN)--effects of lipid composition on in vitro degradation and in vivo toxicity. Pharmazie (2006). Jun;, 61(6), 539-44.

[20] Mehnert, W, & Mader, K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev (2001). Apr;47(2-3):165-96.

[21] Iqbal, M. A, Md, S, Sahni, J. K, Baboota, S, Dang, S, & Ali, J. Nanostructured lipid carriers system: Recent advances in drug delivery. J Drug Target (2012). Aug.

[22] Mu, R. H. Èller, J.S.Lucks. Arzneistofftraèger aus festen Lipidteilchen, Feste Lipidnanosphaèren (SLN). European Patent (1996). Ref Type: Journal (Full)(0605497)

[23] Ugazio, E, Cavalli, R, & Gasco, M. R. Incorporation of cyclosporin A in solid lipid nanoparticles (SLN). Int J Pharm (2002). Jul;, 241(2), 341-4.

[24] Lander, R, Manger, W, Scouloudis, M, Ku, A, Davis, C, & Lee, A. Gaulin homogenization: a mechanistic study. Biotechnol Prog (2000). Jan;, 16(1), 80-5.

[25] Muller, R. H, Mader, K, & Gohla, S. Solid lipid nanoparticles (SLN) for controlled drug delivery- a review of the state of the art. Eur J Pharm Biopharm (2000). Jul;, 50(1), 161-77.

[26] Muller, R. H, Schwarz, C, Mehnert, W, & Lucks, J. S. Production of solid lipid nano-particles (SLN) for controlled drug delivery. Proc.Int.Symp.Control.Release Bioact.Ma-ter. (1993). Ref Type: Journal (Full), 20, 480-481.

[27] Eldem, T, Speiser, P, & Hincal, A. Optimization of spray-dried and-congealed lipid micropellets and characterization of their surface morphology by scanning electron microscopy. Pharm Res (1991). Jan;, 8(1), 47-54.

[28] Julia, C. Schwarz AWEPMLGPRCV. Nanocarriers for dermal drug delivery: Influence of preparation method, carrier type and rheological properties. International Journal of Pharmaceutics 437 [(2012). Ref Type: Journal (Full), 1-2.

[29] Sjostrom, B, & Bergenstahl, B. Preparation of submicron drug particles in lecithin-stabilized o/w emulsions: I: Model studies of the precipitation of cholesteryl acetate. Int J Pharm. (1992). Ref Type: Journal (Full), 88, 53-62.

[30] Siekmann, B, & Westesen, K. Investigations on solid lipid nanoparticles prepared by precipitation in o/w emulsions. Eur J Pharm Biopharm (1996). Ref Type: Journal (Full), 43, 104-109.

[31] Chen, Y. J, Jin, R. X, Zhou, Y. Q, Zeng, J, Zhang, H, & Feng, Q. R. Preparation of solid lipid nanoparticles loaded with Xionggui powder-supercritical carbon dioxide fluid extraction and their evaluation in vitro release]. Zhongguo Zhong Yao Za Zhi (2006). Mar;, 31(5), 376-9.

[32] Gasco, M. R. Method for producing solid lipid microspheres having narrow size distribution. United State Patent.US 188837. (1993). Ref Type: Journal (Full)

[33] Freitas, C, & Mullera, R. H. Spray-drying of solid lipid nanoparticles (SLN TM). Eur J Pharm Biopharm (1998). Sep;, 46(2), 145-51.

[34] Yang, R, Gao, R, Li, F, He, H, & Tang, X. The influence of lipid characteristics on the formation, in vitro release, and in vivo absorption of protein-loaded SLN prepared by the double emulsion process. Drug Dev Ind Pharm (2011). Feb;, 37(2), 139-48.

[35] Garaiova, Z, Strand, S. P, Reitan, N. K, Lelu, S, Storset, S. O, Berg, K, Malmo, J, Folasire, O, & Bjorkoy, A. de LD. Cellular uptake of DNA-chitosan nanoparticles: The role of clathrin- and caveolae-mediated pathways. Int J Biol Macromol (2012). Aug;, 51(5), 1043-51.

[36] Pichon, C, Billiet, L, & Midoux, P. Chemical vectors for gene delivery: uptake and intracellular trafficking. Curr Opin Biotechnol (2010). Oct;, 21(5), 640-5.

[37] Rejman, J, Oberle, V, Zuhorn, I. S, & Hoekstra, D. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Biochem J (2004). Jan;377(Pt 1):159-69.

[38] Midoux, P, Breuzard, G, Gomez, J. P, & Pichon, C. Polymer-based gene delivery: a current review on the uptake and intracellular trafficking of polyplexes. Curr Gene Ther (2008). Oct;, 8(5), 335-52.

[39] Zhang, P, Chen, L, Zhang, Z, Lin, L, & Li, Y. Pharmacokinetics in rats and efficacy in murine ovarian cancer model for solid lipid nanoparticles loading docetaxel. J Nanosci Nanotechnol (2010). Nov;, 10(11), 7541-4.

[40] Li, R, Eun, J. S, & Lee, M. K. Pharmacokinetics and biodistribution of paclitaxel loaded in pegylated solid lipid nanoparticles after intravenous administration. Arch Pharm Res (2011). Feb;, 34(2), 331-7.

[41] Harivardhan, R. L, Sharma, R. K, Chuttani, K, Mishra, A. K, & Murthy, R. S. Influence of administration route on tumor uptake and biodistribution of etoposide loaded solid lipid nanoparticles in Dalton's lymphoma tumor bearing mice. J Control Release (2005). Jul;%20;, 105(3), 185-98.

[42] Zara, G. P, Bargoni, A, Cavalli, R, Fundaro, A, Vighetto, D, & Gasco, M. R. Pharmaco-kinetics and tissue distribution of idarubicin-loaded solid lipid nanoparticles after duodenal administration to rats. J Pharm Sci (2002). May;, 91(5), 1324-33.

[43] Vighi, E, Leot, E, Montanari, M, Mucci, A, Hanuskova, M, & Iannuccelli, V. Structural investigation and intracellular trafficking of a novel multicomposite cationic solid lipid nanoparticle platform as a pDNA carrier. Ther Deliv (2011). Nov;, 2(11), 1419-35.

[44] Sandri, G, Bonferoni, M. C, Gokce, E. H, Ferrari, F, Rossi, S, Patrini, M, & Caramella, C. Chitosan-associated SLN: in vitro and ex vivo characterization of cyclosporine A loaded ophthalmic systems. J Microencapsul (2010). , 27(8), 735-46.

[45] Jayagopal, A, Sussman, E. M, & Shastri, V. P. Functionalized solid lipid nanoparticles for transendothelial delivery. IEEE Trans Nanobioscience (2008). Mar;, 7(1), 28-34.

[46] Liu, Z, Zhong, Z, Peng, G, Wang, S, Du, X, Yan, D, Zhang, Z, He, Q, & Liu, J. Folate receptor mediated intracellular gene delivery using the charge changing solid lipid nanoparticles. Drug Deliv (2009). Aug;, 16(6), 341-7.

[47] Doktorovova, S, Shegokar, R, Rakovsky, E, Gonzalez-mira, E, Lopes, C. M, Silva, A. M, Martins-lopes, P, Muller, R. H, & Souto, E. B. Cationic solid lipid nanoparticles (cSLN): structure, stability and DNA binding capacity correlation studies. Int J Pharm (2011). Nov;, 420(2), 341-9.

[48] Siddiqui, A, Patwardhan, G. A, Liu, Y. Y, & Nazzal, S. Mixed backbone antisense glucosylceramide synthase oligonucleotide (MBO-asGCS) loaded solid lipid nanopar-ticles: in vitro characterization and reversal of multidrug resistance in NCI/ADR-RES cells. Int J Pharm (2010). Nov;400(1-2):251-9.

[49] Musacchio, T, & Torchilin, V. P. Recent developments in lipid-based pharmaceutical nanocarriers. Front Biosci (2011). Jan;, 16, 1388-412.

[50] Vighi, E, Ruozi, B, Montanari, M, Battini, R, & Leo, E. pDNA condensation capacity and in vitro gene delivery properties of cationic solid lipid nanoparticles. Int J Pharm (2010). Apr;389(1-2):254-61.

[51] De Jesus, M. B, & Ferreira, C. V. de PE, Hoekstra D, Zuhorn IS. Design of solid lipid nanoparticles for gene delivery into prostate cancer. J Control Release (2010). Nov; %20;148(1):ee90., 89.

[52] Choi, S. H, Jin, S. E, Lee, M. K, Lim, S. J, Park, J. S, Kim, B. G, Ahn, W. S, & Kim, C. K. Novel cationic solid lipid nanoparticles enhanced gene transfer to lung cancer cells. Eur J Pharm Biopharm (2008). Mar;68(3):545-54., 53.

[53] Zhu, R, Cheng, K. W, Mackenzie, G, Huang, L, Sun, Y, Xie, G, Vrankova, K, Constantinides, P. P, & Rigas, B. Phospho-Sulindac (OXT-328) Inhibits the Growth of Human Lung Cancer Xenografts in Mice: Enhanced Efficacy and Mitochondria Targeting by its Formulation in Solid Lipid Nanoparticles. Pharm Res (2012). Jun.

[54] Wang, S, Chen, T, Chen, R, Hu, Y, Chen, M, & Wang, Y. Emodin loaded solid lipid nanoparticles: preparation, characterization and antitumor activity studies. Int J Pharm (2012). Jul;430(1-2):238-46.

[55] Fang, Y. P, Lin, Y. K, Su, Y. H, & Fang, J. Y. Tryptanthrin-loaded nanoparticles for delivery into cultured human breast cancer cells, MCF7: the effects of solid lipid/liquid lipid ratios in the inner core. Chem Pharm Bull (Tokyo) (2011). Feb;, 59(2), 266-71.

[56] Shuhendler, A. J, Cheung, R. Y, Manias, J, Connor, A, Rauth, A. M, & Wu, X. Y. A novel doxorubicin-mitomycin C co-encapsulated nanoparticle formulation exhibits anticancer synergy in multidrug resistant human breast cancer cells. Breast Cancer Res Treat (2010). Jan;, 119(2), 255-69.

[57] Siddiqui, A, Gupta, V, Liu, Y. Y, & Nazzal, S. Doxorubicin and MBO-asGCS oligonucleotide loaded lipid nanoparticles overcome multidrug resistance in adriamycin resistant ovarian cancer cells (NCI/ADR-RES). Int J Pharm (2012). Jul;431(1-2):222-9.

[58] Yu, Y. H, Kim, E, Park, D. E, Shim, G, Lee, S, Kim, Y. B, Kim, C. W, & Oh, Y. K. Cationic solid lipid nanoparticles for co-delivery of paclitaxel and siRNA. Eur J Pharm Biopharm (2012). Feb;, 80(2), 268-73.

[59] Jin, J, Bae, K. H, Yang, H, Lee, S. J, Kim, H, Kim, Y, Joo, K. M, Seo, S. W, Park, T. G, & Nam, D. H. In vivo specific delivery of c-Met siRNA to glioblastoma using cationic solid lipid nanoparticles. Bioconjug Chem (2011). Dec;, 22(12), 2568-72.

[60] Taveira, S. F, & Araujo, L. M. de S, Nomizo A, de Freitas LA, Lopez RF. Development of cationic solid lipid nanoparticles with factorial design-based studies for topical administration of doxorubicin. J Biomed Nanotechnol (2012). Apr;, 8(2), 219-28.

[61] Schiffelers, R. M. siRNA based approaches in medicinal chemistry and drug discovery. Curr Top Med Chem (2012). , 12(2), 67-8.

[62] Schiffelers, R. M, Ansari, A, Xu, J, Zhou, Q, Tang, Q, Storm, G, Molema, G, Lu, P. Y, Scaria, P. V, & Woodle, M. C. Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle. Nucleic Acids Res (2004). Nov; 32(19):e149.

[63] Hrkach, J. Von HD, Mukkaram AM, Andrianova E, Auer J, Campbell T, De WD, Figa M, Figueiredo M, Horhota A, Low S, McDonnell K, Peeke E, Retnarajan B, Sabnis A, Schnipper E, Song JJ, Song YH, Summa J, Tompsett D, Troiano G, Van Geen HT, Wright J, LoRusso P, Kantoff PW, Bander NH, Sweeney C, Farokhzad OC, Langer R, Zale S. Preclinical development and clinical translation of a PSMA-targeted docetaxel nano-particle with a differentiated pharmacological profile. Sci Transl Med (2012). Apr; 4(128):128ra39.

[64] Vighi, E, Montanari, M, Hanuskova, M, Iannuccelli, V, Coppi, G, & Leo, E. Design flexibility influencing the in vitro behavior of cationic SLN as a nonviral gene vector. Int J Pharm (2012). Sep;(12):10.

[65] Ho Lun Wong RBAMRYLXYWChemotherapy with anticancer drugs encapsulated in solid lipid nanoparticles. Advanced Drug Delivery Reviews 59 [6], (2007). Ref Type: Journal (Full), 491-504.

[66] Lim, S. J, Lee, M. K, & Kim, C. K. Altered chemical and biological activities of all-trans retinoic acid incorporated in solid lipid nanoparticle powders. J Control Release (2004). Nov;, 100(1), 53-61.