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Review Article

Solid Lipid Nanoparticles as Efficient Drug and Gene Delivery Systems: Recent Breakthroughs

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Abstract

In recent years, nanomaterials have been widely applied as advanced drug and gene delivery nanosystems. Among them, solid lipid nanoparticles (SLNs) have attracted great attention as colloidal drug delivery systems for incorporating hydrophilic or lipophilic drugs and various macromolecules as well as proteins and nucleic acids. Therefore, SLNs offer great promise for controlled and site specific drug and gene delivery. This article includes general information about SLN structures and properties, production procedures, characterization. In addition, recent progress on development of drug and gene delivery systems using SLNs was reviewed.

Introduction

Recently, nanotechnological sciences brought considerable progresses in the diagnosis and treatment of disease. Drug and gene delivery, production of improved biocompatible materials and *in vitro* and *in vivo* diagnostics are examples of nanotechnology application.[1-3] Nanoparticles can improve drug efficacy in cancer and other diseases, while simultaneously diminishing side effects, due to features such as more targeted localization in tumors and active cellular uptake.[4-6] The reason why nanomaterials are excellent candidates for medical purposes is based on their unique properties, such as their surface to mass ratio that is much larger than that of other colloidal particles, high chemical reactivity, tunable properties, and their ability to carry other compounds. The relatively large surface of nanoparticles provides the possibility of binding and carrying of other compounds such as drugs, probes and proteins.[7-9] The main purposes for nanomaterials application in drug and gene delivery consist of: a) Biocompatibility and safety, b) Specific drug targeting and delivery, c) Toxicity decreasing while maintaining therapeutic effects, and d) Development of safe medicines.[7]

In order to omit limitations associated with traditional formulations, lipid based drug delivery systems are introduced.[10] In comparison to liposomes, solid particles have some advantages as well as prevention of incorporated active compounds from chemical degradation and more flexibility in modulating the release of the compound.[11]

Solid lipid nanoparticles (SLNs) as example of lipid based drug delivery system have distinctive property such as high drug loading, large surface area and can protect the drug from the environment, while increasing its bioavailability. Consequently, two noticeable advantages can be attained; first, less active drug consumption during the formulation process owing to the enhanced encapsulation efficiency. Second, the preferred effects of the drug are accelerated owing to the initial release increasing, which is because of the homogenization production process of SLNs.

Drug incorporation models of SLNs including solid solution model and core-shell model (drug-enriched shell and drug-enriched core) has been shown in Figure 1. Drug is molecularly dispersed in the lipid matrix in solid solution model when the particles are formed by application of neither any surfactant nor drug-solubilizing surfactant using cold homogenization technique. The drug has robustly distinct interactions with the lipid. In the drug-enriched shell model, a solid lipid core forms once the recrystallization temperature of the lipid is achieved and the drug concentrates in the still liquid outer shell of the SLN due to the dispersion temperature reduction.[12,13] Finally, in the drug-enriched core model, cooling the nanoemulsion results in a super-saturation of the drug which is dissolved in the melted lipid at or close to its saturation solubility and the drug

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precipitation occurs before lipid recrystallization. Extra cooling ultimately leads to the recrystallization of the lipid surrounding the drug as a membrane.[13]
This review presents the preparation and characterization methods of SLNs and the recent advances in application of them as drug/gene delivery system are discussed in more details.

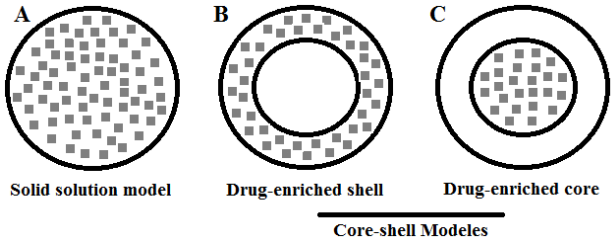

Figure 1. Drug incorporation models into SLN: A) homogeneous matrix of solid solution, B) drug-free core with drug-enriched shell and C) drug-enriched core with lipid shell.

SLN preparation

Solid lipid, emulsifier and water/solvent have been used in preparation of SLNs. The lipids used may be triglycerides (compritol), partial glycerides, fatty acids (stearic acid, palmitic acid), steroids (cholesterol) and waxes (cetyl palmitate). In order to stabilize the lipid dispersion, a variety of emulsifiers and their combination have been utilized. The particle agglomeration can be avoided by application of mixture of emulsifiers.[14] There are numerous methods of SLN preparation like high shear homogenization, hot homogenization, cold homogenization, ultrasonication or high speed homogenization, micro emulsion based SLN preparations, SLN preparation by using supercritical fluid, SLN prepared by solvent emulsification/evaporation, double emulsion method, spray drying method.

High shear homogenization

High shear homogenization techniques were initially applied for the preparation of SLNs. Dispersion quality is often compromised by the presence of micro particles. Olbrich et al investigated different process parameters, which affect the particle size and zeta potential as well as stirring rate, emulsification time and cooling condition. The particle size has not been influenced by higher stirring rates, but slightly improved the polydispersity index.[15,16]

Hot homogenization

Hot homogenization method is performed at higher temperature than the melting point of the lipid and it is similar to homogenization of emulsion. It can be performed by both high pressure homogenizers and high intensity ultrasound. A pre-emulsion of the drug incorporated lipid melt and the aqueous emulsifier phase is attained by high-shear mixing device at the same temperature. High pressure homogenization of the pre-emulsion is done above the lipid melting point. The quality of the final product to a great extent has been affected by the quality of the pre-emulsion and it is desirable to achieve droplets in the size range of a few micrometers. Lower particle sizes are acquired because of lower viscosity of the lipid phase at higher processing temperatures, which results in drug and carrier degradation acceleration. Good product is acquired due to several passes through the high-pressure homogenizer, typically 3-5 passes. Increasing the homogenization period, results in particle size enlarging due to particle coalescence, which occurs because of the high kinetic energy of the particles. Degradation of active compound and metal contamination due to high intensity ultrasound is disadvantage of this method.[3,17]

Cold homogenization

Cold homogenization method has been carried out to omit the following problems of the hot homogenization technique like temperature mediated drug and carrier degradation acceleration and consequently release of drug into the aqueous phase during homogenization. First stage in cold homogenization is the same with hot homogenization method but the next steps are different. The drug loaded lipid melt is cooled quickly by ice or liquid nitrogen for distribution of drug in the lipid matrix. The acquired particle sizes are in the range 50-100 microns for this method. Disadvantages of cold homogenized samples are larger particle sizes and a broader size distribution. However, this method reduces the thermal exposure of the sample.[17]

Ultrasonication or high speed homogenization

Sonication or high speed stirring method can be used for production of SLNs as well. It is very simple and the applied equipment in this technique is very common in every lab. Large particle size and metal contamination due to ultrasonication are examples of this method disadvantage.[18]

Microemulsion based SLN preparations

This technique is based on the dilution of microemulsions in which SLNs are produced by stirring an optically transparent mixture at 65-70° which is normally composed of a low melting fatty acid (stearic acid), an emulsifier (polysorbate 20, polysorbate 60, soy phosphatidylcholine, and sodium taurodeoxycholate), co-emulsifiers (sodium monooctylphosphate) and water. After that, the hot micro-emulsion is dispersed in cold water under stirring.[19] The volume ratios of the hot micro emulsion to cold water usually are in the range of 1:25 to 1:50. The dilution process is verified by the composition of the micro emulsion. As reported previously, the droplet structure is already contained in the microemulsion and as a result, no energy is needed to get submicron particle sizes.[19]

Supercritical fluid technique

This novel technique has the advantage of solvent-less processing for SLN preparation. SLN preparation is

based on the fast expansion of supercritical carbon dioxide solutions (RESS) method. Carbon dioxide (99.99%) was the fine candidate as a solvent for this method.[20,21]

Solvent emulsification/evaporation method

This technique is based on SLN dispersions by precipitation in oil/water emulsions. The lipophilic compound is dissolved in water immiscible organic solvent such as cyclohexane, which is emulsified in an aqueous phase. SLN dispersion is formed by precipitation of the lipid in the aqueous medium after evaporation of the solvent. The mean diameter of 25 nm with cholesterol acetate as model drug and lecithin/sodium glycocholate mixture as emulsifier has been reported for the prepared SLN. The reproducibility of the result was verified by Siekmann and Westesen, who produced the cholesterol acetate SLN with mean size of 29 nm.[22,23]

Double emulsion method

This procedure is based on solvent emulsification evaporation for incorporating hydrophilic drug into SLNs. In order to avoid drug partitioning to outer water phase during solvent evaporation in the external water phase of w/o/w double emulsion, the drug is encapsulated with a stabilizer. After evaporation of organic solvent by rotary, SLNs were recovered by centrifugation at 12000 ×g for 30 min at 4°C.[24]

Spray drying technique

This method leads in particle aggregation because of high temperature, shear forces and partial melting of the particle. The most favorable result was acquired in SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol water mixtures (10/90 v/v).[25]

Characterization of SLNs

The prepared SLNs should be evaluated to distinguish if they are appropriate for the intended type of administration or not. Particle size and the (solid) state of the particle matrix are the main factors to be considered. Other important characteristics of SLNs are its shape, surface characteristics, and in particular, its interaction with incorporated drugs. Application of various characterization techniques is the most promising approach to attain a practical image of the sample properties due to the complexity of the systems.[26]

Particle size distribution is one of the most widely employed physical characteristic methods of SLNs, which is affected by numerous factors like lipid matrix, surfactant blend, lipid viscosity, drug to lipid ratio and aqueous phase and production parameters. Photon correlation spectroscopy and laser diffraction are main techniques which have been used for measurement of particle size.[3,17]

The zeta potential is a measurement of surface charge and is important factor in preventing aggregation and imparts the physical stability to formulation. Due to electrical repulsion at higher zeta potential, particle aggregation is less likely to occur.[3]

To study the morphology like sphericity and aggregation of SLNs, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) have been performed.[3,27]

Drug encapsulation and release rate strongly has been affected by the cristallinity and the polymorphic behavior of lipids. The application of emulsifiers, the preparation procedure, and the high dispersity such as the small particle size of the colloidal system affected crystallinity of lipids in the SLNs. Differential scanning calorimetry and X-Ray diffractometry is basic techniques, which is used to investigate behavior of the lipids.[28]

There are three basic models for the drug and ingredients loading into SLNs: homogeneous matrix model, drug-enriched shell model and drug-enriched core model. The obtained construction of SLNs is depend on the formulation combination (lipid, active compound, surfactant) and of the fabrication conditions and methods.[29]

SLNs have capability for encapsulation of enormous amount of lipophilic compounds like steroids, retinol, and sunscreens but for hydrophilic ones, the payload amount is low. The low solubility of hydrophilic compounds into lipidic phase and crystalline structure of the lipid matrix can be reason of this effect. Two methods were reported to increase the payload of hydrophilic drugs. The first approach was the development of oil loaded SLN and the second strategy is modification of the lipid matrix by incorporation of amphiphilic compounds as well as phosphatydilcholine, polyglyceryl-3-diisostearate and sorbitan.[29]

The SLN suspension's ability to retain homogeneity (suspension stability), particle size and the lipid crystalline state are perspectives that should be considered in SLNs stability. The instability of the above processes results in disturbing the uniform suspension and increase of size. Besides, the recrystallization process causes cross-linking of crystallized particles, thereby changing the suspension into the gel.[3,30]

Biocompatibility and safety of prepared SLNs should be assessed using various *in vitro* cyto/genotoxicity assays such as with MTT, DAPI staining and DNA fragmentation assays and flow cytometry analysis.[3,31] Dolatabadi et al (2014) confirmed the safety and lack of toxicity of alendronate sodium loaded SLNs to A549 cells by various cytotoxicity methods[3]. In order to identify non toxic concentration of SLN suspension, Perugini et al (2010) determined the EC50 values of aqueous suspension that can to be use in topical formulation.[32]

SLNs application in drug and gene delivery systems

SLNs have been considered as an efficient and non-toxic alternative lipophilic colloidal carrier for delivery of various drugs and other biological macromolecules like DNA and peptides. SLNs are biodegradable and are stable for a long period of time and easy to scale up

compared to other colloidal systems. Drug delivery by SLNs to the liver cells has been reported, which are actively phagocytic. Their colloidal dimensions and the controlled release actions facilitate drug protection and administration by both parenteral and non-parenteral routes. The application of lipid nanoparticles by parenteral routes as well as biodistribution and pharmacokinetic studies upon intravenous administration has been described in literature.[33-36]

Drug delivery using SLNs

SLNs have been reported to be useful as drug carriers to treat various diseases as well as cancer. They offer a distinctive drug delivery system to prevent rapid clearance by the immune system. Stealth SLNs can be utilized to target specific tissues in accessible cells. Fluorescent markers together with drugs (Fluorescent SLNs) have been successfully evaluated in animal models. Methotrexate, paclitaxel and camptothecin- loaded SLNs have been reported for tumor targeting. Longer circulation times have been achieved by paclitaxel.[37-40]

SLNs can penetrate the blood–brain barrier (BBB) owing to adsorption of blood proteins such as apolipoproteins on their surface which can facilitate the adherence to endothelial cells. This effect was studied for the drugs like tobramycin, doxorubicin and idarubicin.[41-44]

Various administration routes such as oral, topical, parenteral and pulmonary have been reported for drug delivery using SLNs.[5]

Oral administration of SLNs

Oral administration of SLNs is feasible as aqueous dispersion or after transforming into dosage form i.e. tablets, pellets, capsules or powder in sachets. Antitubercular drugs like rifampicin, isonizide, pyrazinamide-loaded SLN systems have been reported for oral administration, which were able to reduce the dose amount and improve patient compliance. Formulation of poor orally bioavailable drug lopinavir into SLNs were reported using hot self nano-emulsification technique by Negi et al. They demonstrated that the oral bioavailability of lopinavir was improved due to higher intestinal lymphatic uptake of lopinavir-loaded SLNs.[45] In another study, Silva et al formulated a risperidone (an atypical antipsychotic drug) into SLNs and investigated its long-term stability, biocompatibility and drug transport. They believed that the prepared drug delivery system has potential for the oral delivery of poorly water-soluble drugs like risperidone.[46] In order to prevent rifampicin from hydrolyzes at the acidic pH of the stomach; Singh et al. encapsulated it into SLNs and reduced the risk of failure of therapy.[47] In addition, various anticancer drugs loaded SLNs such as camptothecin and tamoxifen were reported for this administration route as well.[48]

Parenteral administration of SLNs

Because of SLNs small size, they can be administered intravenously, intramuscularly, subcutaneously or to the target organ. Miao et al reported that SLN drug delivery system can enhance the transport of standard anticancer drugs like paclitaxel and doxorubicin into cancer cells and improve the cytotoxicity effect against sensitive cancer cells and their multi-drug resistant variant cells, compared to free drug solutions.[49] Also pharmacokinetic studies of doxorubicin loaded SLNs showed higher blood levels compared to a commercial drug solution after intravenous injection in rats. SLN loaded with the cationic amphiphilic doxorubicin and the anionic lipophilic docosahexaenoic acid (DHA), a polyunsaturated fatty acid that enhances the activity of anticancer drugs, has been developed with Mussi et al. They reported higher cytotoxicity and improved cellular uptake of doxorubicin-DHA-loaded SLN on human lung tumor cell line compared to free doxorubicin and DHA and suggested that DHA-doxorubicin-loaded SLN is a promising alternative for the treatment of cancer.[50] Regarding to the body distribution, SLNs showed high drug concentrations in lung, spleen and brain, whilst the solution causes a distribution more into liver and kidneys.[51] The distribution of camptothecin incorporated SLNs showed increased uptake in some organ especially in brain following intravenous administration.[12,52]

Topical administration of SLNS

Topical applications of SLNs have been reported with promising results for therapeutic purposes. It has a potential advantage of direct drug delivery to the site of action, which will generate higher tissue concentrations. Development and evaluation of SLNs of terbinafine hydrochloride for sustained release and skin targeting has been carried out by Vaghasiya et al. *In vivo* pharmacodynamic investigation indicated that the SLNs based gel decreased fungal burden of *Candida albicans* in rats as compared to commercial product in shorter duration of time.[53] Carbopol gels of aceclofenac SLNs have been prepared by Chawla et al. They demonstrated that the prepared gel could preserve the concentration of bioactives such as aceclofenac in desirable levels at sites of inflammation and injury.[54] A variety of drug such as anticancers, vitamin-A, isotretinoin, flurbiprofen has been incorporated in SLNs for topical applications as well.[55-57]

Pulmonary administration of SLNS

Pulmonary administration of drugs has some exclusive characteristics as well as large surface area, avoidance of the first-pass effect, high capacity for solute exchange, excellent vascularisation, and ultra-thinness of the alveolar epithelium (0.1-0.5 mm) that can facilitate systemic delivery. Moreover, degradation of drugs in the lung is slow because of low extracellular and intracellular enzyme activity. Thus, even compounds with low absorption rates can be absorbed to a relative high amount after pulmonary application. For the local treatment of airway diseases, the pulmonary application stands out by direct reaching the lung epithelium and thus the site of action which indicates that there is a fast onset of action and the necessary dose is reduced

compared with traditional administration routes like the oral route. Furthermore, after local delivery of poorly absorbed drugs, high-dose exposures to the systemic circulation and therefore systemic adverse effects are minimized or avoided.[58-61]

One of the proposed strategies that can decrease both doses and the side effects of drugs is administration of them through dry powder aerosol form. Insulin-loaded SLN by micelle-double-emulsion method has been prepared by Liu et al. they demonstrated the stability of SLN during nebulization, the deposition properties and the hypoglycaemic effect after intrapulmonary application and showed higher bioavailability of insulin in lungs due to deposition of insulin-SLN.[62] Paclitaxel-loaded SLNs has been delivered to the lung lymphatics through the pulmonary route in experimental mouse mammary carcinoma. Paclitaxel-loaded SLNs was 20-fold more effective than a paclitaxel-solution on MXT-B2 cells, which was related to a higher internalization rate of paclitaxel-loaded SLNs than of free paclitaxel.[63] Budesonide-loaded SLNs were prepared by Zhang et al. for asthma therapy.[64] Also, SLNs of glucocorticoids, beclomethasone, dipropionate and alendronate sodium (bone resorption inhibitor) are examples of drugs, which have been developed for administration through this method.[5,65-67]

The published data show that SLNs could offer an extensive advantage for the local treatment for severe airway diseases as well as for the systemic drug delivery. Table 1 shows the application of SLNs in delivery of various drugs.

Table 1. Examples of SLNs based drug delivery systems.

Drug	Disease type	Lipid Type	Preparation technique	Ref
Stavudine, delavirdine & saquinavir	Human immunodeficiency virus (HIV)	Compritol 888 ATO, tripalmitin & cacao butter	Microemulsion	68
Lopinavir	HIV infections	Stearic acid	Hot self nano-emulsification	45
Aceclofenac	Inflammation in rheumatoid arthritis	Compritol & precirol	Ultrasonication	54
Norfloxacin	Bacterial infection	Stearic acid	Hot homogenization and ultrasonication	69
Rifampicin	Bacterial infection	Compritol 888 ATO	Microemulsion	47
Doxorubicin	Cancer	Docosahexaenoic acid	Hot homogenization	50
Gemcitabine	Solid tumors	4-(N)-stearoyl	Solvent emulsification	70
Paclitaxel & doxorubicin	Solid tumors	Glyceryl monostearate	Solvent diffusion	49
Terbinafine hydrochloride	Skin infections	Compritol & precirol	Solvent injection	53
Alendronate sodium	Bone diseases	Compritol & precirol	Hot homogenization	3
Risperidone	Schizophrenia	Compritol 888 ATO	Hot high pressure homogenization	46
Insulin	Diabetes mellitus	Phosphatidylcholine	Micelle-double-emulsion method	62

Gene delivery using SLNs

Successful gene therapy is defined as expression of the therapeutic gene in the target organ or tissue. Viral gene delivery (or viral vector) and non-viral gene delivery (or non-viral vector) are two types of gene therapy technique. In first type, the genes are transferred by a virus into the cells because of virus ability in penetrating cells.[71-76] Even though high levels of gene expression reported with viral gene delivery but it can have oncogenic and immunogenic effects, and induce inflammation that render transgene expression transient.[71-73] Non-viral vectors can overcome some of these concerns and compared to viral vectors they have considerable manufacturing and safety advantages. A variety of non-viral delivery systems have been demonstrated for efficient delivery of small-interfering RNAs (siRNAs) and DNA including cationic polymers, liposomes, cationic lipids, cell-penetrating peptide, carbon nanotubes and so on.[72,73,77]

SLNs as gene delivery systems have attracted increasing attention in recent years. Cationic SLNs usually have been used for gene delivery due to the possible electrostatic interaction between the negative charges of the DNA and the positive charges of the lipid, which allow the formation of a complex called lipoplex. These lipoplexes can form a structure that protects the DNA and direct it towards the target cells (Figure 2).[36,78] Carrillo et al. demonstrated cationic SLNs capable of forming a complex with DNA plasmids.[36] Jin et al. developed the siRNA-PEG/SLN, which can cross the BBB to the tumor site with no apparent systemic toxicity.[79] In another study, Montana et al. utilized cationically modified SLN as *Paracentrotus lividus bep3* RNA carriers and evaluated their potential as a nonviral vector for gene delivery.[80]

Future gene expression study definitely will open a new horizon for the achievement of rational design approaches in the development of cationic SLNs as

effective gene delivery systems due to remarkable capability of them such as penetrating into cells, capability to achieve spatially- and temporally-controlled release for targeted gene silencing.

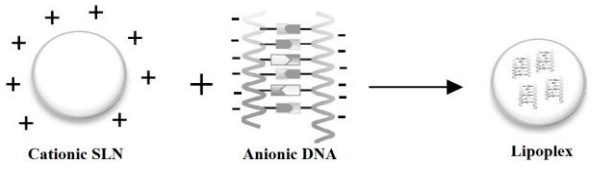

Figure 2. Lipoplexes complex formation due to the interaction between the negative charges of the DNA and the positive charges of the SLN.

Conclusion

SLNs are a novel and biocompatible colloidal drug and gene carrier system that merges the advantages of both liposomes and polymeric nanoparticles and simultaneously avoid some of their disadvantages. SLNs as an advanced drug and gene delivery nanosystems present significant opportunities for improving medical therapeutics. This article has reviewed various aspects of SLNs and their application in the encapsulation of various drugs and genes. Because of the SLN potential for facilitating controlled drug delivery to a target tissue and its biocompatibility, there will be much investigation in improvement of quality, efficacy, and safety profile of drugs using them in the future.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

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