

Current Trends in Liposomal Drug Delivery Systems

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Introduction

Since the early 1970's it has been recognised that liposomes have significant potential as carriers for therapeutic agents. Specific attractions and benefits of liposomal drug delivery systems include:

- Effective encapsulation of both small and large molecules, with a wide range of hydrophobicity levels and pKa values;
- Dose control. Minimising both the amount of drug administration required to achieve therapeutic levels and toxicity effects; and
- Enhanced and targeted delivery. Control of circulation time and delivery site of therapeutic agent by modification of liposome surface/functionality (see Figure 1).

Extensive investment has been made into the research and development of liposome delivery systems for a wide range of applications, including chemotherapeutics, genetic material, vaccines, therapeutic proteins and anti-inflammatory agents. However, the number of successful commercial products has been limited and liposomes are widely considered to have underperformed their potential.

Difficulties in optimisation of formulation, stability and delivery performance have conspired to increase time to market.

Over the past decade, considerable progress has been made in liposome technology with both sustained and targeted delivery in mind, including:

- The development of lipids with improved performance and cost effectiveness;
- New insight into the mechanisms of liposome interactions with biointerfaces and the *in vivo* fate of liposomes, hence the design of novel targeting strategies; and
- Improved physicochemical characterisation of liposomes and hence more effective formulation strategies.

A resurgence of interest in liposomal delivery has resulted and we are now observing the next generation of liposome-based products under development. Increasingly, consideration is being given to alternate delivery routes to the more common parenteral (i.v.) mode, including; inhalation, implantable, depot technology and oral delivery based technology. In the following sections we highlight recent improvements in the understanding of liposomal delivery systems, new delivery concepts and the development pipeline.

Improved Drug Release, Circulation and Targeting of Liposomes

It is well established that by balancing circulation time, release rate and dose, long circulating liposomes may be used to prolong drug activity (Woodle *et al.*, 1992). However, drug release from liposomes is highly complex and subtly controlled by the liposome composition, drug loading method, drug properties and dose, and particularly the biological environment at the delivery site (Takagi *et al.*, 1996). These complexities and experimental difficulties (Vemuri *et al.*, 1995) often conspire, leading to poor *in vitro-in vivo* correlations. Our knowledge of the kinetics

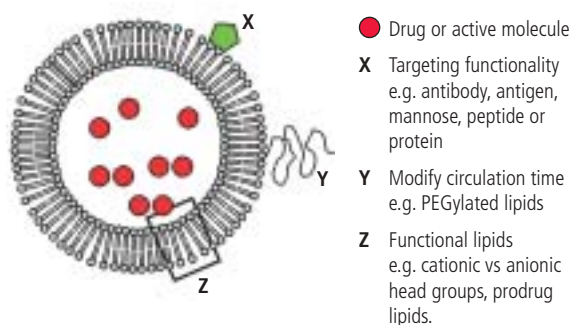


Figure 1 – Liposomal drug delivery.

and thermodynamics of encapsulation and release from liposomes for both small molecular weight drugs (Margalit *et al.*, 1991) and proteins (Dhoot and Wheatley, 2003) has significantly improved in recent years. Surface modification and microencapsulation of liposomes are leading to new opportunities for extended drug delivery.

Blood-born liposomes are rapidly cleared by the reticular endothelial system, which can result in drugs accumulating in the liver, spleen or marrowbone. Increased circulation times are facilitated by the incorporation of steric stabilisers that extend from the liposome membrane. Receiving most attention have been polyethylene glycol (PEG)-conjugated lipids which form the basis of Stealth™ liposome technology (**Schering, Canada, Inc.**); initially marketed as Caelyx® and Doxil® (Europe and US, respectively), a doxorubicin treatment for patients with Kaposi's sarcoma, a cancer found most commonly in people living with AIDS. Other ligands, such as polyvinyl alcohol (PVA), O-palmitoylpullulan, poloxamer 338, poly(vinylpyrrolidone), carboxymethylchitin or block copolymers have also shown potential for controlling liposome stability and drug release (Takeuchi *et al.*, 1998). Incorporating a steric stabilising surface functionality has become an essential component of the majority of liposomal formulations under development. Theoretical and experimental studies (e.g. Čeh *et al.*, 1997; Er *et al.*, 2004) have quantified the relationship between PEG size and density, and liposome interactions with protein, cell and biosurfaces. Insight into the mechanisms of steric stabilisation is leading to improved formulation strategies for specific liposomal applications.

Specific targeting of liposomes is a key focus of ongoing research receiving considerable attention. Passive targeting of liposomes exploits their tendency to accumulate in certain tissues. The enhanced accumulation of liposomes at tumour and inflammation sites is due to the leaky vasculature present (Jain, 1997), which is unable to prevent species <100 nm crossing the capillary membrane. Similarly, liposomes of diameter <120 nm facilitate enhanced penetration of probe molecules into human skin (Verma *et al.*, 2003). Clearly, size control of liposomes may be used to optimise passive targeting.

Active targeting of liposomes to specific cell receptor sites involves modifying the basic liposome surface with the incorporation of specific ligands such as sugars, lectins, peptide hormones, haptens, antibodies, vitamins and other proteins (Torchilin, 1985). For example, the conjugate of poly-L-lysine with transferrin has been used to target cancer cells (Zenke *et al.*, 1990). Tri-mannose modification is also a popular route for targeting, for example to dendritic cells (Copland *et al.*, 2003). Liposomes enter a cell via phagocytosis or endocytosis; however, the liposomes and their payload are normally broken down in lysosomes prior to reaching their target, the cytoplasm. One current approach is to prepare virosomes (Kaneda, 2000), where viral surface proteins are incorporated into the surface of liposomes, enabling the virosome to interact directly with and pass through the cell wall. DNA-loaded liposomes have

been fused with a UV-inactivated hemagglutinating virus of Japan (HVJ). The HVJ-liposomes are able to directly fuse with a cell membrane enabling DNA delivery directly into the cytoplasm.

Many additional novel approaches are now being exploited to extend liposomal delivery technology. One approach is to couple liposomes with other delivery systems for enhancement of sustained release of an active component, for example liposome encapsulation within microparticles (Stenekes *et al.*, 2001). Triggered-release technologies for liposomes are also receiving considerable interest and offer great potential for the delivery of chemotherapeutics.

Commercial Development of Liposomal Products

During the 1980's, companies such as **The Liposome Company, Alza, Sequus, Liposome Technology, Inc.** and **NeXstar** pioneered the commercialisation of liposomal drug delivery systems. Liposome-based formulations were developed for drugs such as amphotericin B, doxorubicin and daunorubicin. In the late 1990's, a new wave of companies emerged with a range of novel liposome-based delivery systems. Notable examples include:

- **Biozone Labs** marketed a liposome-based lignocaine formulation, now sold under the trade name L.M.X.4. Biozone Labs currently market a range of liposome delivery systems including Qusomes™, a cosmetic delivery system that may also be delivered transdermally using iontophoresis (Lipophoresis™), and Lipospray™ a liposomal-based oral spray for delivery to the oral mucosal membranes.
- **DELEX Therapeutics** are currently undertaking Phase IIa clinical trials of their AeroLEF™ (aerosolised liposome encapsulated fentanyl) formulation based on their ROSE-DS™ (Rapid Onset and Sustained Effect Delivery System) technology platform. The therapeutic dose of fentanyl has been shown to be delivered over 12 hours, significantly longer than obtained from a typical intravenous dose.
- **Lipoxen Technologies Ltd** has developed the ImuXen™ liposome-based vaccine delivery system. Active targeting is obtained with the incorporation of mannose residues on the liposome surface, to specifically interact with mannose receptors present on the surface of immune cells.
- **NeoPharm, Inc.** developed NeoLipid™ LE-SN38 for the treatment of colorectal cancer, which is currently undergoing Phase I clinical trials. (SN-38 is the active metabolite of irinotecan (CPT-11); however, CPT-11 must be converted by the body into SN-38. Alone, SN-38 is not readily bioavailable to the body, while the efficiency of the CPT-11 to SN-38 conversion rate varies from person to person.) Another NeoLipid based delivery system (LErafAON) is for the delivery of c-raf antisense oligonucleotide, to inhibit the production of the Raf-1 protein in tumours and inhibit cancer growth, and is also in Phase I/II clinical trials.

- **SkyePharma** has developed DepoFoam™ technology as an extended-release drug delivery system based on vesicles or liposomes. DepoCyt® is a sustained-release injectable product for the treatment of lymphomatous meningitis and DepoDur™ is a sustained-release morphine formulation that has recently received both US and European regulatory approval.
- **Novosom AG** was founded to commercialise nano-capsulated liposomes. Currently, three liposome delivery system platforms have been developed; Cagicels®, Smarticles® and Micromethason®. Cagicels provide a sustained-release liposome-based delivery system for therapeutic proteins such as leuprolide acetate with no initial burst release observed and high encapsulation efficiencies. Leuprolide acetate is a synthetic antagonist of the luteinizing hormone used in the treatment of hormone dependent prostate carcinoma, endometriosis and precocious puberty. Smarticles are charge reversible liposomes for the therapeutic delivery of siRNA directly into cells. Smarticles are negatively charged under physiological conditions; however, upon endocytosis as the pH decreases to 5 or 4, the liposome becomes positively charged resulting in fusion between the liposome and endosome membranes, allowing the escape of the therapeutic agent into the cytoplasm of the cell. Micromethason is a liposome-based delivery system for Glucocorticoids, a commonly used anti-inflammatory medication which currently suffers from high dosage costs and opportunistic infections arising due to their immunosuppressive action.
- **LiPlasome Pharma AS** was founded in 2001 to commercialise LiPlasomes® that were initially developed at the Technical University of Denmark. The tumour activated liposome drug delivery system (LiPlasomes) was developed to deliver anticancer drugs such as 5-fluorouracil, Ara-C, doxorubicin and cisplatin that do not readily diffuse across the liposome membrane upon entering a tumour site. LiPlasomes® are PEG stabilised liposomes incorporating anticancer etherlipids (AELs)

that are both toxic to cancer cells as well as enhancing drug permeability across the cancer cell membrane. Delivery of the anticancer etherlipids to the tumours occurs due to the site specific activation and conversion of inactive proAELs to cytotoxic AELs due to the elevated level of secretory phospholipase A2 enzymes, which have been clinically shown (Abe *et al.*, 1997) to be specifically upregulated in a range of tumours. LiPlasome's technology is anticipated to enter full clinical trials in 2006.

Examples of liposomal chemotherapeutics in at least Phase II development are included in Table 1.

Conclusions

The age of targeted drug delivery is at hand, embraced by both academic and industrial researchers, with the full potential of liposome-based delivery systems of therapeutic agents yet to be fully realised. Over the next decade the number of liposome-based pharmaceutical products will continue to expand, justifying the investment in research and development.

Cancer type	Company	Therapeutic agent	Study Phase
Breast	NeoPharm/Pharmacia	Liposomal doxorubicin	III
Breast	Liposome Co.	Liposomal doxorubicin	III
Breast	NeoPharm/Pharmacia	Liposome encapsulated Paclitaxel	II/III
Leukaemia (AML)	Aronex Pharmaceuticals/Abbott Laboratories	Liposomal nystatin	II
Lung	Aronex Pharmaceuticals	Liposomal platinum	II
Lung (NSCLC)	Biomira	Liposomal vaccine (BLP25)	IIb
Lung (SCLC)	OSI Pharmaceuticals	Liposomal topoisomerase inhibitor	II
Lymphoma (NHL)	Antigenics, Inc.	Liposomal all-trans retinoic acid	II
Lymphoma (NHL)	NeXstar	Liposomal daunorubicin	II
relapsed NHL	Inex Pharmaceuticals	Liposomal vincristine (Onco-TCS)	III
Ovarian	Gilead Sciences	Liposomal topoisomerase inhibitor	II
Prostate	NeoPharm/Pharmacia	Liposomal doxorubicin	III
Skin	AGI Dermatics	T4 endonuclease V (T4N5)	Application submitted

Table 1 – Examples of liposomal chemotherapeutics in at least Phase I development (Source: <http://www.cancerconsultants.com>).

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