CHAPTER 7

AN OVERVIEW OF LIPOSOME-DERIVED NANOCARRIER TECHNOLOGIES

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Abstract: Lipid-based nanocarrier systems are among the most applied encapsulation, targeting and controlled release technologies. They are being used to incorporate and protect materials with different solubilities and deliver them to the site required inside the body as well as outside the body, in vitro. Among the lipid-based encapsulation systems, liposomes and their derivatives are the most applied and further developed. There are some liposome-derived carriers approved for human use on the market, which mainly utilise oral, transdermal and parenteral delivery routes. Research for the development and optimization of liposomal systems for pulmonary and nasal applications are also ongoing. Methods of preparation of these micro- and nanocarriers have evolved to exclude utilisation of harmful substances such as toxic organic solvents and also enable preparation of safe and efficient systems on industrial scales. In this chapter, an overview of eight different liposome-derived nanocarriers with respect to their characteristics, preparation methods and application is presented

Keywords: Lipidic systems, archaeosomes, multivesicular vesicles, vesicular phospholipid gels, cochleates, virosomes, transferosomes, immunoliposomes, stealth liposomes

1. INTRODUCTION

Liposomal carrier systems are among the most promising encapsulation technologies employed in the rapidly developing field of nanobiotechnology. Liposomes and nanoliposomes are being used successfully as models of biomembranes and also as delivery and controlled release systems for drugs, diagnostics, nutraceuticals, minerals, food material and cosmetics to name but a few (Mozafari & Mortazavi 2005; Mozafari et al 2006). Due to the extra-ordinary success of liposome technology in so many fields, both in research and industry, several liposome-derived systems have been developed in recent years. These carrier systems are

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being made on micro- and nano-scales (from around 20nm to several micrometers) with different levels of complexity to meet specific applications. Some of these carriers are composed of lipids and phospholipids, while some others contain other molecules such as carbohydrates and proteins in their structure.

Compared with other encapsulation strategies such as chitosan- and alginate-based carriers (Anal et al 2003; Anal & Stevens 2005; Bhopatkar et al 2005), liposome-derived encapsulation systems have unparalleled advantages. These include the ability to entrap material with different solubilities, the possibility of being produced using natural ingredients on an industrial scale, and targetability (Mozafari 2004; Yurdugul & Mozafari 2004; Mozafari & Mortazavi 2005; Mozafari 2006). Liposomal carriers can shield an ingredient from free radicals, metal ions, pH and enzymes that might otherwise result in degradation of the bioactive compound. They impart stability to water-soluble material, particularly in high water-activity applications (Gouin 2004). They can accommodate not only water-soluble material, but also lipid-soluble agents and amphiphilic compounds simultaneously, providing a synergistic effect (Suntres & Shek 1996). Another unique property of liposome-based micro- and nano-carriers is the targeted delivery of their content both in vivo and in vitro. In general, these carriers may be targeted to the required site inside the body via active (e.g. by incorporation of antibodies) and passive (e.g. targeting based on particle size) mechanisms (Mozafari & Mortazavi 2005; Mozafari 2006). Some of the main liposome-derived carrier technologies are explained in this chapter.

2. ARCHAEOSOMES

Archaeosomes can be defined as liposomes made from one or more of the polar ether lipids extracted from the domain Archaea (Archaeobacteria). Although Archaea and Bacteria are both prokaryotes, Archaea are more closely related to the domain Eucarya than to Bacteria (Krieg 2001). Many Archaea live in environments including high salt concentrations or low pH values and high temperatures. Hence their membrane lipids are unique and enable them to survive in such hostile conditions. The core lipids (polar head groups removed) of archaea consist of archaeols (diethers) and caldarchaeols (tetraethers), wherein the regularly branched, 5-carbon repeating units forming the isoprenoid chains (usually 20 carbons per chain in archaeols, and 40 carbons per chain in caldarchaeols) are attached via ether bonds at the sn-2,3 position of the glycerol carbons. In contrast to this, the core lipids found in Bacteria and Eucarya consist of unbranched (mostly) fatty acyl chains, often unsaturated, attached via ester bonds to the sn-1,2 glycerol carbons. The polar moieties (archaeols are monopolar and caldarchaeols are bipolar) are similar to those (phospho, glyco, polyol, amino, hydroxyl groups) encountered in ester lipids, but phosphatidylcholine is rarely present in archaeal lipids (Mozafari et al 2005). Although archaeosomes are a recent technology, they have already proven to be a safe delivery system for bioactive agents including drugs and vaccines (Patel & Chen 2006).

Compared with liposomes (which are made from ester phospholipids), archaeosomes are relatively more thermostable, more resistant to oxidation and chemical and enzymatic hydrolysis. They are also more resistant to low pH and bile salts that would be encountered in the gastrointestinal tract (Patel et al 2000). Archaeosomes prepared from the total polar lipid extract or from individual purified polar lipids show promise as adjuvants that promote strong humoral and cytotoxic T-cell responses to encapsulated soluble antigens. Therefore, there is a great potential for using archaeosomes in drug, vaccine and other bioactive material delivery applications. As is the case with liposomes, it is possible to incorporate ligands such as polymers to archaeosomes. It has been shown that incorporation of polyethyleneglycol and Coenzyme Q10 into archaeosomes can alter the tissue distribution profiles of intravenously administered vesicles (Omri et al 2000). Omri et al (2003) have recently reported that intravenous and oral delivery of nanometric-sized archaeosomes to an animal model was well tolerated with no apparent toxicity. The results of these studies are very promising for the utilisation of archaeosomes in the encapsulation and delivery of different bioactive compounds.

3. MULTIVESICULAR LIPOSOMES

Multivesicular liposomes (MVL) - or multivesicular vesicles (MVV) - are composed of several small vesicles entrapped by a single lipid bilayer (Figure 1). MVLs prepared by a multiple emulsion method, possess a unique structure of multiple, nonconcentric, aqueous chambers surrounded by a network of lipid membranes (Kim et al 1983). The structure of MVL has a higher aqueous volume with

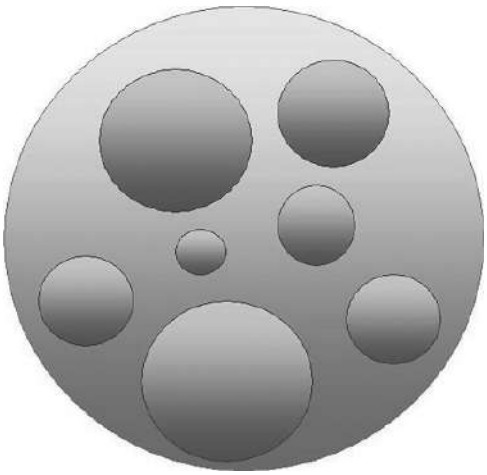

Figure 1. A multivesicular liposome in which several bilayer vesicles are encapsulated by a single bilayer vesicle, mainly composed of phospholipid molecules (From Mozafari and Mortazavi 2005, with permission)

respect to its lipid ratio and much larger particle diameter compared with multilamellar vesicles (MLVs) (Kim et al 1983; Ye et al 2000). Hence, MVLs have high capacity for loading water-soluble compounds with high encapsulation efficiencies. The bioactive agent is encapsulated within the nonconcentric internal aqueous chambers and is released over an extended period of time. The multivesicular nature of MVLs provides sustained release of encapsulated substance since, unlike unilamellar type liposomes, a single breach in the external membrane of a MVL will not result in a total release of the internal aqueous contents (Kim et al 1983; Ye et al 2000). A multivesicular liposome can be prepared by a process comprising the following steps (Kim et al 1983): (i) forming a water-in-lipid emulsion from two immiscible components, i.e. a lipid component (e.g. amphipathic lipids, one or more organic solvents, and a neutral oil such as triolein or trioctanoin) and an aqueous component containing the material to be encapsulated in MVLs; (ii) dispersing the water-in-lipid emulsion into a second aqueous component to form solvent spherules; and then (iii) removing the organic solvent from the solvent spherules to form the multivesicular liposomes suspended in the second aqueous component.

A recent application of multivesicular liposomes was for the encapsulation and release of the antineoplastic agent cisplatin in mice inoculated with a murine carcinoma tumor (Xiao et al 2004). The authors found out that cisplatin-MVLs exhibit high encapsulation efficiency, prolonged sustained release and higher drug accumulation in tumor regions when compared to the un-encapsulated form of the drug (Xiao et al 2004).

4. VIROSOMES

Virosomes (Kara et al 1971; Almeida et al 1975), or artificial viruses, are one type of liposome that contain reconstituted viral proteins in their structure. Unlike viruses, virosomes are not able to replicate but are pure fusion-active vesicles. Due to the presence of the specialized viral proteins on the surface of virosomes, they can be used in active targeting (Mozafari 2006) and delivery/controlled release of their content at the target site. Viruses have developed the ability to fuse with cells during the course of evolution, thus, allowing for release of their contents directly into the cell. This is due to the presence of fusogenic proteins on the viral surface that facilitate this fusion. If these fusogenic viral proteins are reconstituted on the surface of a liposome then the liposome also acquires the ability to fuse with cells. This is an extremely useful tool in active transport because it allows the direct release of the liposomal contents into the cell. As there is no diffusion of the bioactive material involved, it results in a more effective delivery. The most common viruses used in the construction of virosomes are the Sendai, Semliki Forest, influenza, herpes simplex, and vesicular stomatitis viruses. The presence of virus proteins not only allows the liposome to target a particular cell but also allows it to fuse with the cell ensuring direct delivery of the incorporated material (Lasic 1993).

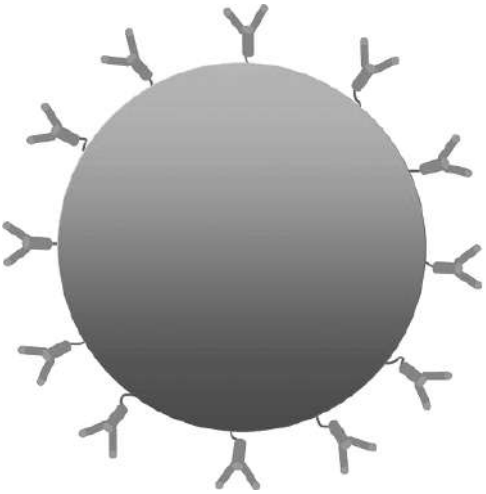

Figure 2. Schematic presentation of an immunoliposome containing antibody molecules on its surface (From Mozafari and Mortazavi 2005, with permission)

5. IMMUNOLIPOSOMES

Another class of lipid vesicles designed for active targeting of their encapsulated/entrapped material inside the body is known as immunoliposomes. The immunoliposomes (Huang et al 1981; Mizoue et al 2002) possess moieties such as antibodies, carbohydrates, and hormones on the outer surface of their membrane (Figure 2). The various ligands can be attached to the outer surface of the lipid vesicles by either insertion into the membrane, adsorption to the surface, via biotin-avidin pair or through the most preferable method, covalent binding (Lasic 1993). These ligands attached to the immunoliposome have a complementary binding site on the target cell. Therefore when the liposome arrives within the area of the target cell it will bind to this cell. Consequently the drug will be released into the surrounding region of the target cell minimising harm and side-effects to healthy cells and tissues. In a recent study, immunoliposomes have been used for gene targeting to human brain cancer cells, which has resulted in a 70-80% inhibition in cancer cell growth (Zhang et al 2002).

6. STEALTH LIPOSOMES

Considerable amount of research and studies have been devoted to develope carrier systems that can avoid phagocytosis and thus circulate longer in the blood. As a result of these studies the so-called “Stealth” particles have emerged. Stealth carriers can be made by covering the surface of the bioactive delivery vehicle with hydrophilic chains which prevent opsonisation. Grafting of poly (ethylene glycol) (PEG) is the most effective method and has been applied

to nanoparticles (Gref et al 1994) and liposomes (Woodle and Lasic 1992) to produce sterically stabilised carriers. Other polymers such as poly (hydroxyethyl L-asparagine) (PHEA) have also been considered to increase liposome circulation time (Metselaar 2003). The sterically stabilised liposomes are involved in passive targeting (Mozafari 2006) of the material they carry.

When sterically stabilised liposomes are injected into an individual, who for instance has either a solid tumour or an internal infection, the vesicles will migrate and accumulate in the tumorous or infected area. As the stealth liposomes become degraded, they will release their drugs into the surrounding area (Allen 1994). This is an example of passive targeting because the stealth liposomes are left to their own devices and yet they migrate and treat the injured area. It has been reported that stealth liposomes with diameters between 70 and 200 nm have longer circulation times (Litzinger et al 1994). Another important consideration when using sterically stabilized liposomes is the size of the coating polymer. If it is too large it may interfere with the ligand-receptor binding of the stealth liposome and the target cell.

7. TRANSFEROSOMES

Delivery of various materials through the skin is highly important in different areas particularly in cosmetics and skin care. For transdermal delivery of bioactive agents using carrier systems, the bioactive compounds must be associated with specifically designed vehicles, in the form of highly deformable particles, and applied on the skin non-occlusively. To meet this end, another type of optimised liposome-based carrier system, called transferosome, has been developed (Cevc and Blume 1992; Cevc 1996). Transferosomes consist of phospholipids, cholesterol and additional surfactant molecules such as sodium cholate. The inventors claim that transferosomes are ultradeformable and squeeze through pores less than one-tenth of their diameter. Therefore 200 to 300nm-sized transferesomes are claimed to penetrate intact skin (Figure 3). Penetration of these particles works best under in vivo conditions and requires a hydration gradient from the skin surface towards the viable tissues.

Insulin-loaded transfersomes, for example, were reported to deliver the drug through the non-compromised skin barrier with a reproducible drug effect that resembles closely that of the ultralente insulin (a long acting insulin used in the treatment of diabetes mellitus) injected under the skin with comparable pharmacokinetic and pharmacodynamic properties (Cevc 2003). It has been suggested that transfersomes can respond to external stresses by rapid shape transformations requiring low energy. This high deformability allows them to deliver drugs across barriers, including skin (Cevc et al 1995). To prepare these vesicles, the so called ‘edge activators’ were incorporated into the vesicular membranes. Surfactants were suggested as examples of such edge activators (Cevc et al 1993), and also sodium cholate or sodium deoxycholate have been used for this purpose (Planas et al 1992; Cevc et al 1995; Paul et al 1995; Lee et al 2005).

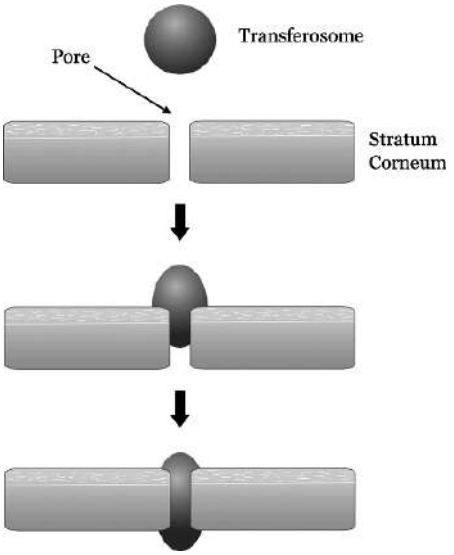

Figure 3. Transferosome penetration through the pores in stratum corneum, the outermost layer of the skin (From Mozafari and Mortazavi 2005, with permission)

8. VESICULAR PHOSPHOLIPID GELS

Vesicular phospholipid gels (VPGs) are highly concentrated phospholipid dispersions of semisolid consistency and vesicular morphology (Brandl et al 1994; Tardi et al 2001). They are under investigation as potential implantable depots for sustained release of bioactive agents (Grohganz et al 2005). VPGs can be prepared by high-pressure homogenisation of high concentrations of phospholipid molecules. Vesicular phospholipid gels can also be prepared by the heating method (Mozafari 2006) without using toxic volatile organic solvents or detergents. Upon dilution, VPGs constitute normal diluted liposome dispersions. During in vitro release tests, Tardi and co-workers found that the incorporated hydrophilic marker (calcein) was released in a sustained manner within periods ranging from several hours up to several days depending on the concentration and composition of the lipids within the matrices (Tardi et al 1998). It appears that vesicular phospholipid gels could be useful as parenteral depot formulations. Alternatively, by mixing with excess buffer, VPGs may be converted to unconcentrated liposome suspensions with small and homogeneous particle sizes possessing high encapsulation efficiencies (Brandl et al 1998). Consequently, VPGs are also useful as intermediates for liposome dispersions, especially those with drugs with high leakage rates and poor storage stabilities such as gemcitabine (Moog 1998). By virtue of the in vitro drug release and the entrapment investigations of VPGs containing bioactive agents such as 5-fluorouracil (Kaiser et al 2003) and chlorhexidine (Farkas et al 2004), good applicability of these carriers is expected as implantable gels or as redispersed liposomes.

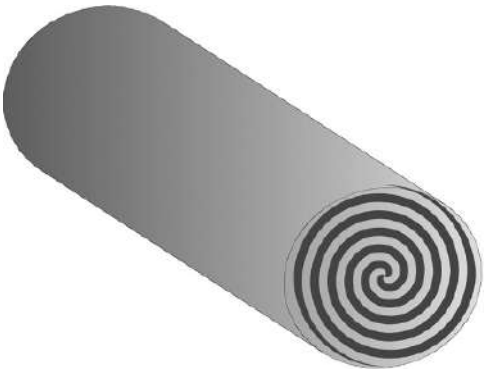

Figure 4. Schematic representation of typical structure of a cochleate

9. COCHLEATES

Cochleates are small-sized and stable lipid-based carriers comprised mainly of a negatively charged lipid (e.g. phosphatidylserine) and a divalent cation such as calcium (Zarif et al 2000; Zarif 2003). They have a cigar-shaped multilayered structure consisting of a continuous, solid, lipid bilayer sheet rolled up in a spiral fashion with little or no internal aqueous space (Figure 4). Hydrophobic, amphiphilic, negatively or positively charged molecules can be delivered by cochleates. Cochleates and their sub-micron versions (i.e. nanocochleates) have been used to deliver proteins, peptides and DNA for vaccine and gene therapy applications (Mannino & Gould-fogerite 1997; Zarif & Mannino 2000). Due to their nanometric size, stability and resistance to degradation in the gastrointestinal tract nanocochleates have revealed great potential to deliver bioactive agents both orally and parenterally (Mannino & Gould-fogerite 1997; Zarif & Mannino 2000; Zarif et al 2000; Zarif 2003). Cochleates containing amphotericin B (AmB) are now in development to enter Phase I clinical trials, for both the oral and parenteral treatment of fungal infections (Zarif 2003). The unique structure and properties of cochleates make them an ideal candidate for oral and systemic delivery of sensitive material including peptide and nucleic acid drugs.

10. SUMMARY

Several liposome-derived bioactive delivery systems have been developed for specialized applications as described in this chapter. Some of these carriers can be employed for active delivery of encapsulant, while others are suitable for passive bioactive delivery. These systems provide a choice of optimized encapsulation and delivery for various applications including systemic and transdermal delivery as well as the choice of short or long-term release. The commercialization of these encapsulation systems is progressing, as is the development of their preparation methods. Safe and reproducible manufacture of these carriers on industrial scales is

now possible. The development of these encapsulation technologies and associated products, for pharmaceutical, cosmetics and food industries, continues to be pursued actively by a number of groups globally. Accordingly, it is reasonable to project that this field will experience steady growth for the foreseeable future.

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