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# Lipids and Liposomes in the Enhancement of Health and Treatment of Disease

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Additional information is available at the end of the chapter

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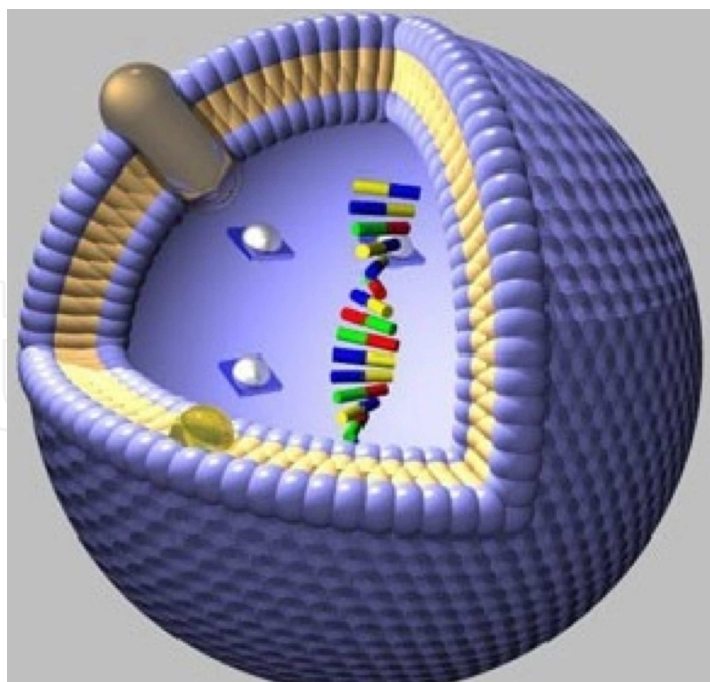
## 1. Introduction

The discovery of liposomes initially came from studies by Bangham and Horne who observed by electron microscopy the self-association of the lipid phosphatidylcholine (mixed with or without cholesterol) in water formed 'spherulites' of varying sizes which had not a recognizable lamellar shell comprising a lipid bilayer [1]. The self-assembling 'spherulites', subsequently named liposomes from the greek *lipo* (fat) and *soma* (body), were recognised to be functionally analogous to studied biological membrane systems due to the similar rates of diffusion of ions [2]. However only when an ionophore, valinomycin, was utilised demonstrating selective diffusion of  $K^+$  over  $Na^+$  from liposomes containing equal concentrations of the ions, could liposomes be confirmed as entirely sealed membrane vesicles [3]. Furthermore Papahadjopoulos and Watkins showed the differential permeability to anions and cations could be significantly altered with liposomes of different phospholipid compositions [4]. Natural liposomes have bilayers composed of phospholipids and/or cholesterol and as such are poorly antigenic, typically non-toxic and physiologically inert. Liposomes can vary in size from 25 nm to 2.5  $\mu m$  and are classified within three broad categories [5]: Multilamellar vesicles (MLV), which structurally resemble an onion with multiple concentric phospholipid bilayers separated by aqueous layers, large unilamellar vesicles (LUV) and small unilamellar vesicles (SUV) which have a single lipid bilayer surrounding the aqueous core. Typically multiple unilamellar vesicles of differing sizes can form inside of each other generating multilamellar structures.

The concept of liposomes as drug-carriers to aid in selectivity was explored in the early nineteen seventies predominantly through the work of drug-transport scientists such as Gregoriadis who initially looked at the fate of protein-containing liposomes delivered into animals [6]. The theory that liposomes stay intact and circulate in the bloodstream before

accumulating in specific tissues where they release their molecules into cells was confirmed using radiolabelled proteins entrapped in liposomes. The radioactive signal from the proteins was barely detected in the bloodstream, but predominantly in the lysosomes of cells of the liver and spleen, showing the liposomes stayed intact prior to the radiolabelled proteins being taken up by the cells. This and related studies revealed the physiological behaviour of liposomes such as their integrity and long life span in the mammalian bloodstream. It was only through the use of cell culture it was confirmed that cargo carried by liposomes was directly delivered through endocytosis into the lysosomes and thus into the intracellular environment of cells [7]. These initial studies demonstrated the huge potential for liposomes as model systems, and a number of various applications were subsequently explored as listed here: The effect of surface charge on ion permeability [8]; their susceptibility to phospholipase hydrolysis [9]; the function of integral membrane ion transporters [10]; the delivery of active enzymes to functionally deficient cells [11]; their use as immunological adjuvants [12]; as stimulants of interferon production [13]; their interaction with polyene antibiotics [14]; their incorporation of local and general anaesthetics [15]; the inclusion and presentation of virus surface proteins [16]. Since those early experiments, there has been a continued interest in the use of liposomes and currently there are applications in a wide variety of scientific fields (Figure 1).

In this chapter we will focus on the use of lipids and liposomes in the enhancement of a number of health related areas and cover the development of new synthetic molecules, which have great potential in advancing improvements in health and the treatment of disease.



**Figure 1.** A schematic representation of a liposome. The liposome can facilitate the carrying of various cargo, water-soluble drugs, DNA or RNA, in the internal hydrophilic region, water-insoluble drugs within the hydrophobic region of the bilayer, or protein linked or incorporated into the phospholipid bilayer.

## 2. Current applications in treating disease

### 2.1. Non-communicable diseases

It is evident that with their physiological attributes, liposomes are an attractive means to deliver drugs to treat a variety of communicable and non-communicable diseases. Typically, drugs for the treatment of human diseases can often have a number of biochemical and pharmacological issues such as poor stability and solubility, rapid breakdown and lack of targeted delivery. As a result there are common problems in the use of such drugs, including the lack of a strong therapeutic cure and the necessity to consume high doses, which can result in unwanted side effects. If the disease is localised to a specific body tissue, the lack of selective targeting can result in poor bioavailability of the drug at the required site, potentially resulting in toxicity in other tissues, thus restricting the dose concentration. The use of natural phospholipid based liposome formulations having minimal toxicity, extended stability in the human body, tissue selectivity and a delayed release of the active compound at the site of action suggests clear benefits for drug development and treatment. In addition multiple compounds can be distributed by the same liposomes for added therapeutic impact. Equally, compounds of varying lipophilicities can be transported, partitioning in the different hydrophobic and hydrophilic environments of the liposomes. Particle size is a critical factor both in the circulatory half-life of liposomes and also (along with the number of bilayers) dictates the amount of encapsulated drug [17]. In general, drug delivery systems are on the nanoscale, liposomes having diameters of 100 nm or less tend to have a good therapeutic index compared to conventional anticancer therapies. Typically liposomes for drug therapies approved for humans contain the neutrally charged phosphatidylcholine as the major membrane constituent, though occasionally cholesterol (up to a third of the total lipid content) is incorporated to reduce membrane instability due to serum protein binding. Thirty years ago, the application of liposomes to deliver an anti-cancer anthracycline drug doxorubicin trapped in negatively charged or neutral liposomes (called OLV-DOX) showed that it retained its antitumour activity in mice [18]. Importantly the use of the liposomal formulation reduced the accumulation of the drug in murine cardiac tissues, minimising toxicity and thus significantly improving their survival. When tested in humans however the OLV-DOX worked poorly, being rapidly cleared from the bloodstream with significant premature release of the drug, giving rise to potential cardiotoxicity [19]. These failings limited the application of liposomes in cancer treatment at that time, only resolved by the subsequent experimental trialling of polyethylene glycol (PEG) incorporation in phospholipid liposomes. The PEG was found to create a hydrophilic surface on the liposomes, reducing uptake by the reticuloendothelial system and thus increasing the circulation time of these so-called 'stealth' liposomes. This resulted in a revolution in liposome design and so in 1995 Doxil (PEGylated liposome-trapped doxorubicin) the first of the so-called 2<sup>nd</sup> generation was approved by the US Food and Drug Administration as the first liposome drug delivery system for human use [20]. Doxil was found to have extended stability in the bloodstream and reduced compound leakage resulting in increased accumulation in solid tumours (up to 22-fold) and reduced toxicity to non-target organs. Every year over 300,000 patients with ovarian cancer or Kaposi's sarcoma are now routinely intravenously treated with

Doxil [21] and it is occasionally utilised in cases of breast cancer and also in combination with bortezomib for multiple myeloma.

Alongside Doxil, to date five other liposomal formulations are approved for human cancer treatment (Table 1). Myocet, a related non-PEGylated liposome formulation of doxorubicin is used in combination with cyclophosphamide for breast cancer [22]. DaunoXome, a liposome formulation of daunorubicin is similarly used to treat Kaposi's sarcoma [23]. DepoCyt, a formulation of unusually large liposomes containing cytarabine is active against malignant lymphomatous meningitis [24], while Marqibo is a more typical nanoscale formulation of liposomes of vincristine utilised for acute lymphoblastic leukemia [25]. Recently, more success has come from the trial use of such liposome formulations in combination with standard anti-cancer drugs, one example being a Doxil and carboplatin composition which shows a better therapeutic index and less toxicity than the standard paclitaxel/carboplatin mixture used to treat ovarian cancer in the elderly [26]. Similarly in comparison to a standard treatment, a combination of Doxil, bortezomib and dexamethasone showed a strong therapeutic response and improved tolerability in patients with multiple myeloma [27].

| Market Product           | Drug used                          | Target diseases                        | Company                                      |
|--------------------------|------------------------------------|--|--|
| Doxil or Caelyx          | Doxorubicin                        | Kaposi's sarcoma                       | SEQUUS, USA                                  |
| DaunoXome                | Daunorubicin                       | Kaposi's sarcoma, breast & lung cancer | NeXstar, USA                                 |
| Amphotec                 | Amphotericin-B                     | Fungal infections, Leishmaniasis       | SEQUUS, USA                                  |
| Ventus                   | Prostaglandin-E1                   | Systemic inflammatory diseases         | The liposome company, USA                    |
| Alec                     | Dry protein free powder of DPPC-PG | Expanding lung diseases in babies      | Britannia Pharm, UK                          |
| Epaxal-Berna Vaccine     | Inactivated hepatitis A virions    | Hepatitis A                            | Swiss serum & vaccine institute, Switzerland |
| Avian retrovirus vaccine | Killed avian retrovirus            | Chicken pox                            | Vineland lab, USA                            |
| Novasome                 | Smallpox vaccine                   | Smallpox                               | Novavax, USA                                 |
| Depocyt                  | Cytarabine                         | Cancer therapy                         | Skye Pharm, USA                              |
| Topex-Br                 | Terbutaline sulphate               | Asthma                                 | Ozone, USA                                   |

**Table 1.** Current products utilising liposomes

A major issue in cancer treatments is the failure of many forms of chemotherapy due to the phenomenon of multidrug resistance. As a result, the tactic of using drug combinations has become widely adopted due to the greater therapeutic index and efficacy in reversing the multidrug resistant phenotype [28]. In a combination treatment drugs can have one of three



effects, synergistic, additive or antagonistic and this can be shaped by their specific molar ratios in the formulation [29]. Unfortunately in an *in vivo* setting, the combinatorial effect can be weakened due to disrupted pharmacokinetics of the drugs in the system, leading to incorrect dose ratios at the site of action [30]. Based on prior research, it was clear that the pharmacokinetic issues of combination therapies can be eliminated by the use of liposome formulations, resulting in delivery of the drugs to their site of action at the correct effective ratio [31]. Equally important in the enhancement of health is the use of liposomes in the treatment of cardiovascular disease, the leading cause of deaths worldwide. Again relatively early in liposome research, it was observed that liposomes carrying the MRI contrast agent <sup>99m</sup>Tc-DTPA accumulated in regions of the heart experimentally induced to undergo myocardial infarction (MI), a common cause of death [32]. In MI, during the ischemic phase, the exhaustion of nucleotide pyrophosphates causes extensive myocardial cell damage [33]. The obvious solution, the infusion of adenosine triphosphate (ATP) intravenously to increase myocardial cell energy levels is sub-optimal due to the molecule's short circulatory half-life and strong charge. The relatively unstable ATP could however be protected and successfully delivered using liposomes and clearly accumulated in canine myocardia damaged by ischemia [34]. Subsequently it was discovered that targeted ATP-containing liposomes can significantly protect against the subsequent effects of ischemia in an *ex vivo* rat heart model [35]. When translated into an animal model, ATP-loaded liposomes reduced the amount of irreversible myocardial damage by greater than 50% compared to control treated rabbits [36].

Another significant agent in the prevention and treatment of ischemic injury and indeed heart failure, coronary artery disease and hypertension in general, is Coenzyme Q10 (CoQ10) [37]. Evaluation of CoQ10 loaded liposomes again in the aforementioned rabbit model revealed that only 30% of the affected myocardia was at risk of irreversible damage compared to the control, indicative of significant protection and great potential in this approach [38]. In humans, the use of large-scale trials of adenosine on clinical patients with acute MI has shown some promise but again is a poor compound with a short half-life and hypotensive and bradycardic inducing properties [39]. These issues may be overcome based on an experimental study using PEGylated liposomes of adenosine which generated non-toxic and cardioprotective effects against MI in rats [40]. In treating another common cause of ischemic damage, thrombus formation, a range of thrombolytic drugs have been developed. Due to its need for constant infusion and potential to cause haemorrhage, one of the first thrombolytic drugs, heparin was quickly assessed in a liposomal formulation [41]. Liposomal heparin was much more effective in its thrombolytic effects, being retained in the plasma longer and generated a prolonged activity due to a gradual release of the agent. Ultimately an inhalable formulation successfully replaced the intravenous version in rat models of deep vein thrombosis and pulmonary embolism giving promise to future clinical trials [42].

## 2.2. Communicable diseases

As significant as the development of liposomes in the treatment of non-communicable human diseases has been, the field of anti-parasitic drug development generated the first liposomal formulation to be mass marketed. With no vaccines effective against any of the primary

parasitic infections, anti-parasitic drug treatment remains the main approach. Many current anti-parasitic compounds were developed over 50 years ago and though effective, are hardly comparable to the modern view of the biochemical and clinical properties of an ideal drug. Due to the fact that many anti-parasitic drugs have solubility issues, low bioavailability and poor absorption by the gastrointestinal tract, it was an obvious choice to test the potential of liposomes for anti-parasitic drug delivery. AmBisome, a natural liposome configuration of the potent anti-leishmanial amphotericin B was the first liposome drug based formulation to be commercialised in 1990 (Table 2) [43]. A sterol biosynthesis inhibitor, amphotericin B is the standard second-line treatment for visceral leishmaniasis (caused by *Leishmania donovani*) and is essential in endemic disease areas in India due to the extensive development of resistance against the standard pentavalent antimonial compounds [44]. AmBisome is particularly effective against *Leishmania* (2 to 5-fold more potent than the free drug) due to the fact that the parasite infects the very macrophages which clear the liposomes from the bloodstream, increasing the therapeutic effect and additionally reducing the nephrotoxicity of amphotericin B. Initially liposomes containing antimonial compounds were tested in a hamster model of visceral leishmaniasis and were greater than 700 fold more active than the free drug version showing the significant potential of liposome use [45]. Interestingly antimonial encapsulated liposomes were also shown to be potent against cutaneous leishmaniasis where the parasites alternatively reside in peripheral tissues [46].

| Drug           | Route of Administration                 | Targeted Diseases                 |
|----------------|---|-----------------------------------|
| Amphotericin-B | Oral                                    | Mycotic infection, Leishmaniasis  |
| Insulin        | Oral, Ocular, Pulmonary and Transdermal | Diabetic mellitus                 |
| Ketoprofen     | Ocular                                  | Pain muscle condition             |
| Pentoxifylline | Pulmonary                               | Asthma                            |
| Salbutamol     | Pulmonary                               | Asthma                            |
| Tobramycin     | Pulmonary                               | Pseudomonas infection, aeruginosa |
| Benzocain      | Transdermal                             | Ulcer on mucous surface with pain |
| Ibuprofen      | Oral                                    | Rheumatoid arthritis              |
| Adrenaline     | Ocular                                  | Glucoma, Conjunctivitis           |
| Penicillin G   | Pulmonary                               | Meningococcal, staphylococcal     |
| Methotrexate   | Transdermal                             | Cancer                            |

**Table 2.** Therapeutic applications utilising liposomes

While AmBisome remains the only liposomal antiparasitic agent on the market, other liposome formulations have been developed showing potency against *Leishmania spp.* Liposomes modified with sugars improved the targeting of antileishmanial pentamidine to infected macrophages with increased potency as a result [47]. Similarly, delivery of the alkylphospholipid miltefosine (hexadecylphosphocholine) in a liposomal form proved twice as active against *L. donovani* and actually even increased the susceptibility of a miltefosine-resistant parasite line [48]. Far less exploration has been done to assess the value of liposomal agents against the causative agents of Human African Trypanosomiasis (HAT) and Chagas' disease,

*Trypanosoma brucei* and *Trypanosoma cruzi* respectively. Both species of parasite have disseminating infections and localise to tissues of the body where there is limited interaction with liposomes. However a number of *in vitro* and *in vivo* studies using liposomes have evaluated potential anti-trypanosomal effects. Two related investigations demonstrated that phosphatidylcholine/stearylamine only liposomes at low concentrations (100  $\mu$ M) non-toxic to erythrocytes, rapidly killed both *T. cruzi* [49] and *T. brucei* [50] through destabilization of their plasma membranes. Notably this effect was not seen with identical concentrations of the individual lipid components, suggesting the vesicle structure was important for activity. The surprising failure of liposomes containing benznidazole to improve on the potency of this classical anti-*T. cruzi* treatment was hypothesised to be due to a lack of drug delivery [51], though the anti-leishmanial AmBisome demonstrated some success in suppressing *T. cruzi* infections *in vivo* [52].

With so many anti-malarial drugs commercially available and in development, the focus of liposome development in this field is on the protection of drugs from premature metabolism, to generate a slow release to improve the therapeutic index and reduce toxicity. To this end, liposomes of Artesunate, a semi-synthetic derivative of artemisinin, were found to release only 30% of the drug in 24 h in an *in vitro* test, giving promise to this method as a means to reduce the dosing frequency of antimalarial drugs [53]. In a rabbit model, Arteether directed for chloroquine resistant *Plasmodium falciparum*, when trapped in dipalmitoylphosphatidylcholine, dibehynoylphosphatidylcholine, cholesterol liposomes persisted longer with greater bioavailability in the gastrointestinal tract when compared to the aqueous suspension [54]. Similarly, liposomes of chloroquine, the widely used 4-aminoquinoline antimalarial, modified with an antibody to selectively deliver to infected erythrocytes were found to cure the majority of chloroquine-resistant *Plasmodium berghei*-malarial infections in mice [55], proving that targeted liposomes can be very efficient in overcoming drug resistance. In the treatment of systemic mycoses such as aspergillosis, there are few antifungal agents available, but due to its broad spectrum of action amphotericin-B is potent against a wide range of fungi. In the form of AmBisome and other related formulations (e.g. Abelcet and Amphocil) they are even more effective in treatment of infections [56]. These formulations have also proved valuable in treating *Candida albicans* infections in immunocompromised patients, eradicating an efficient pathogen that is able to form fungal biofilms [57].

Understandably as the second most lethal infectious disease, many therapeutic cures for tuberculosis (TB) have been available for over 50 years, but there are often patient issues with the length of treatment and dose burden. As a result, treatment failures are common and can promote the development of multi-drug resistant strains. Due to their potential to overcome these problems, the development of drug carrying liposomes has become an important focus in anti-tubercular studies. A ground-breaking study demonstrated that gentamicin loaded liposomes had significantly greater antibacterial activity than the free drug, reducing the bacterial load in the spleen and liver in a mouse model of *Mycobacterium avium* [58]. As well as similar results utilising second-line antibiotics, lung-targeted liposomes were created comprised of a mixture of phosphatidylcholine, cholesterol, dicetylphosphate, O-steroyl amylopectin and monosialogangliosides, distearylphosphatidylethanolamine-poly(ethylene



glycol) 2000 to deliver with less toxic effects, isoniazid and rifampicin for more efficient chemotherapy [59]. As the predominant site of infection for TB is the respiratory system, many efforts are now being made to develop aerosolised liposome formulations to successfully deliver anti-tubercular drugs to the lungs by inhalation [60].

The strength of liposomes in supporting in the treatment of disease goes beyond purely as drug delivery vehicles as they can be powerful tools to deliver vaccines, notably against viral infections (Table 1). Significantly, liposomes can be engineered to deliver a variety of immunogenic molecules, whether protein, nucleic acid or carbohydrate to stimulate a strong protective response. A notable commercially available preparation, Epaxal is a vaccine based on inactivated intact Hepatitis A virus adsorbed on to liposomes (thus named virosomes) which in a single dose are well tolerated and highly immunogenic giving good seroprotection [61]. Marketed nearly twenty years ago, Inflexal V, a vaccine preparation against Influenza virus consists of the viral haemagglutinin and neuraminidase surface proteins displayed in phosphatidylcholine based liposomes [62]. In particular, Inflexal V mimicking a natural influenza infection is a paradigm for liposome based vaccines with its safe but strong immunogenicity covering a wide range of ages and health conditions.

### 2.3. Other medical conditions

In addition to the treatment of disease, liposomes have the capability to aid in many other medical-related conditions (Table 2). Notable examples of their use include in analgesia, alleviation of macular degeneration, and as surfactants for pulmonary diseases. A variety of liposome preparations have been marketed for use in analgesia or post-surgical pain-relief. DepoDur and EXPAREL are liposome preparations of morphine and bupivacaine respectively and when intravenously injected, demonstrate stability and extended release properties to give prolonged anaesthesia or analgesia [63,64]. The typical therapy for neovascular age-related macular degeneration requires repeated intravitreal injections of an anti-vascular endothelial growth factor drug, effective in stabilising vision but an encumbrance for patients. The creation of Visudyne, a liposome based formulation of the photosensitiser Verteporfin which requires only intravenous injection has simplified patient treatment with relative success [65]. A common problem in pulmonary diseases such as respiratory distress syndrome is a lack of pulmonary surfactant, the phospholipid-protein complex needed to contribute a functional respiratory surface at the mammalian lungs. Curosurf, a modified natural surfactant isolated from pig lungs contains the essential phospholipid-associated surfactant proteins B and C, and is widely used successfully in clinical treatment [66].

## 3. New approaches utilising synthetic lipids and fatty acids

### 3.1. Non-natural lipids and fatty acids

While having many advantages, natural liposomes utilised for the treatment of disease have some drawbacks. Typically they are difficult to produce and are inherently unstable reducing the potential storage time. In recent years a new generation of liposomes have been developed

with altered biochemical properties designed to improve stability, functionalization and drug release in addition to altered immunogenic and selective targeting properties. Construction of these liposomes was only possible due to the use of non-natural fatty acids and lipids in the particles. As an alternative to the standard inclusion of cholesterol in liposomes for increased stability, a series of sterol-modified phospholipids were constructed [67]. These involved the covalent attachment of cholesterol to the glycerol backbone of phosphatidylcholine, replacing a fatty acid chain and resulting in sterol modified liposomes (SML). Other hydrophobic molecules such as porphyrins and photosensitive agents can similarly replace a fatty acid chain, [68]. In generating synthetic lipids, there are also many simple changes to the lipid headgroup possible, vastly changing their chemical and biophysical properties [69]. Common modifications can include the addition of a polymer, nucleic acid, carbohydrate, amino acid or an assortment of functional chemical moieties for the downstream covalent attachment of ligands.

### 3.2. Advantages of using synthetic lipids in liposomes

As mentioned above, liposomes incorporating synthetic lipids can have three main advantages, extended stability of the liposome, directed cell targeting and controlled release of the cargo and examples of the modifications are discussed here:

Sterol modified liposomes carrying doxorubicin had similar therapeutic efficacy to the standard Doxil in a colon carcinoma model, but with greater overall stability in circulation, improved uptake into the liver and spleen [70]. This and other studies show the potential of SMLs as drug delivery systems that are easy to synthesise from commercially available molecules. Similarly the use of synthetic lipids incorporating porphyrins or photosensitive agents to generate liposomes known as porphysomes which have applications in photodynamic therapy and diagnostics. Importantly these porphysomes demonstrate good pharmacokinetics in mice, are safe at high doses, accumulate in tumours and can be imaged for diagnostic purposes [68]. The use of the synthetic polyethylene glycol (PEG-2000) modified 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine in liposomes greatly altered the surface hydrophilicity and by decreasing cell uptake, extended the circulatory half-life as mentioned previously for Doxil [20].

Utilising liposomes constructed with synthetic lipids can create a number of novel functions. In addition to the aforementioned polymer coating, modified headgroups are useful for the attachment of cargo or specific ligand targeting. Often these modifications result in improved targeting and biodistribution of liposomes by interacting with ligands present on specific cells or tissue types. One notable example is nucleic acid modified lipids, which can result in the physical interaction of the liposomes with single-stranded nucleic acids via base pairing. This has proved biologically important in the efficient targeting of the nucleic acid binding drug cisplatin to its site of action overcoming previous limitations in drug delivery and showing potency against a number of sensitive and resistant cancer cell lines [71]. Liposomes have also been generated using lipids synthesised with a range of functional groups to bind a number of ligands. Most common is a maleimide lipid, although others with ester, ether, avidin, thiol, hydrazine and carboxylic acid moieties in the headgroup have also been constructed [72]. The

maleimide group can react with a free thiol and thus can allow liposomes to couple to any exposed cysteine on a protein such as a single chain antibody. This approach has proved useful in the modification of amyloid- $\beta$ -targeting liposomes, made of sphingomyelin/cholesterol/phosphatidic acid and functionalised through a terminal maleimide group on PEG-phosphatidylethanolamine to display an anti-transferrin receptor antibody. This design gave the liposomes the ability to cross an *in vitro* blood brain barrier model of human brain capillary endothelial cells and thus hold huge potential for the successful delivery of therapeutics to the central nervous system targeting amyloid- $\beta$  and other defective proteins in Alzheimer's disease [73].

In an ideal scenario in the liposomal drug delivery system, the liposomes should be stable in the circulation till they reach their destination and rapidly release their contents to have the desired effect. In optimising the release of cargo from liposomes, a number of methods are possible. Some such as altering the liposome formulation to destabilise the membrane or increasing the hydrophilicity of the cargo are applicable to natural liposomes. However, through the use of synthetic lipids, it is possible to control the release of liposome content with various environmental cues, either external such as heat, light or ultrasound; or internal e.g. pH or redox environment. This relies on the use of lipids to create liposomes that are sensitive to specific stimuli to trigger the delivery of material at the appropriate time and place. This is particularly useful in the delivery of small interfering RNAs (siRNA) where the use of pH responsive ionisable lipids containing amine headgroups means that liposomes release the nucleic acid only into the cytosol of the cell [74].

## 4. The use of lipids and liposomes as molecular tools

### 4.1. Molecules for imaging

The routine application of modified or synthetic lipids in liposomes and their subsequent biocompatibility *in vivo* or *ex vivo* demonstrated that such particles could also be visualised through the incorporation of fluorescently tagged lipids. This methodology effectively replaces the original inconsistent approach of using liposomes carrying fluorescein as cargo for imaging studies [75]. There are a number of commercially available synthetic lipid species which have a fluorophore replacing a lipid fatty acid chain or alternatively replacing or conjugated to a phospholipid headgroup. Common fluorophores attached to lipids include non-polar 4,4-difluoro-4-bora-3 $\alpha$ ,4 $\alpha$ -diazas-indacene (BODIPY), polar nitrobenzo-2-oxa-1,3-diazole (NBD) and dansyl groups, hydrophobic pyrene and the highly fluorescent rhodamine dyes [76]. Importantly, in selecting a fluorophore to use in labelling lipids and liposomes, it is common to use dyes that emit light in the 650-1100nm far red/near infrared region to avoid the conflict with the UV responsive autofluorescence of most eukaryotic tissues. When fluorescent lipids are constructed into liposomes, the simple visualisation of the labelled particles has aided in the study of a number of areas of research such as drug delivery, disease diagnosis and membrane fusion events. A recent study using fluorescence microscopy specifically revealed that carbocyanine dye modified liposomes contain-

ing the antileishmanial agent meglumine antimoniate were taken up faster by *Leishmania major* infected macrophages compared with non-infected cells, most likely due to parasite-modified phagocytosis [77]. The attachment of the fluorescent curcumin molecule to 1,2-dipalmitoyl-3-(2-(1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohept-6-enylthio)ethyl phospho)-*sn*-glycerol (DPS) has a clinically relevant application for the diagnosis of Alzheimer's disease. As curcumin targets the A $\beta$  peptide, injection of DPS-curcumin containing liposomes into the brains of mice revealed the nanoparticles could successfully target and stain the pathology causing A $\beta$  deposits *in vivo* [78]. These types of imaging based studies may be further extended in a dual approach, for example in the efficient bimodal imaging of tumour angiogenesis through the use of rhodamine conjugated phosphatidylethanolamine and gadolinium-DTPA-bis(stearylamide) lipids for optical imaging and magnetic resonance imaging studies respectively [79]. These liposomes were additionally constructed with RGD cyclic peptide moieties conjugated to maleimide-PEG-DSPE, specifically to target the  $\alpha v \beta 3$  integrin highly expressed in angiogenesis. This and other studies [reviewed in 80] have strongly validated the use of these modified lipids in an effective streamlined approach for the *in vivo* visualisation and treatment of angiogenesis, a critical process in metastatic tumour biology.

#### 4.2. Immune system modulators

Due to their biophysical properties, cell targeting and entering abilities, liposomes were candidate adjuvants to aid in the modulation of the human immune system. Initially it was observed that negatively charged liposomes of a certain composition of natural phospholipids carrying diphtheria toxin could induce an enhanced antibody response prior to release of the true antigen [81]. Subsequently this use was more deliberate, for example in the delivery of *Shigella flexneri* lipid A containing liposomes to stimulate an immune reaction [82]. To date, the wide use of liposomes containing monophosphoryl lipid A has proved highly effective in safely enhancing immune responses to candidate vaccines to HIV-1, malaria and a number of cancers [reviewed in 83]. Significantly, liposomes have been developed to act as primary adjuvants, incorporating lipids whose headgroup is covalently bound to antigens. Through the use of synthetic lipids like 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(*p*-maleimidophenyl)butyramide], a range of peptide, carbohydrate, lipid and even antibody-like molecules can be attached [84]. These modified liposomes can be administered via oral or nasal routes rather than injection and in addition to little or no toxicity have significant capability to be both prophylactic and therapeutic vaccines. One caveat however is that even early in liposome research it was clear that in certain circumstances any headgroup modified lipids could be adjuvants, and based on the evidence to date, it is likely that most synthetic lipids will induce some sort of immune response [69]. While the use of liposomes to stimulate the immune system to exert a seroprotective effect as discussed is desirable, there are situations where an immune suppressive effect may be desired. A directed suppression of the immune system is a desirable goal in the treatment of autoimmune disease, allergies and preventing the rejection



of organ transplants. Administering liposomes coated in the bisphosphonate aldrionate successfully caused an anti-inflammatory effect in a rabbit model through the systemic inactivation and depletion of macrophages and monocytes [85], similarly seen in models of tissue graft rejection [86] and arthritis [87]. These and other related liposomes based strategies have great potential to deliver therapeutic success in a safe manner for many immune-related conditions [88].

#### 4.3. Nucleic acid carriers

It is clear liposomes have an enormous ability to transport an assortment of molecules to a variety of cells and tissue types and a rapidly expanding field is the delivery of nucleic acid into cells. In genetic modification, the delivery of genetic material to augment the existing genes or alternatively silence and/or remove genes has become essential. Some current approaches to deliver genetic material into cells and tissues include chemical-based and viral based methods and can be inefficient with membrane permeability issues and potentially cytotoxic effects. Particularly in exploring the concept of gene therapy, replacing a defective copy with a functional wild-type copy, the development of non-viral based vectors to deliver nucleic acid into cells has focused on liposomes due to their ability to carry large fragments of DNA and their low toxicity and immunogenicity. In the development of liposome based nucleic acid delivery systems, it was discovered that a cationic synthetic lipid N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) would form liposomes that would readily interact with negatively charged DNA and stably hold it in the aqueous interior [89]. Typically cationic lipids have a positively charged polar amino head group on top of a lipid-like hydrophobic domain. In contrast, neutral or negatively charged lipid based liposomes can't form electrostatic interactions to bind and hold any negatively charged molecules. This ground-breaking research opened up the field of using cationic liposomes to deliver material and there are now a wide range of synthetic phospholipid and cholesterol analogues that generally form positively charged liposomes [90]. Cationic liposomes have great promise as nucleic acid delivery agents as they are highly efficient, readily interacting with negatively charged membranes for uptake into cells to deliver their cargo. Since the initial discovery, many cationic liposomes have been used to deliver nucleic acids not just into cells in culture, but also animals and even in patients in phase I and II clinical trials though with some dose-dependent toxicity issues [90].

More recently, a revolution in the use of liposomes in nucleic acid delivery has come about through the discovery of small interfering RNAs (siRNA). These siRNA are molecules which are designed to bind the messenger RNA of a specific gene and thus silence its expression. This approach could potentially revolutionise the treatment of diseases such as cancer where suppression of gene expression is of paramount importance. However, the use of siRNA in a clinical setting has been restricted because the molecules have a short half-life, show poor uptake into cells and are rapidly cleared from the system. Again the application of liposome technology has resurrected this form of therapy with the poten-



tial of liposomes specifically targeting the siRNA to the appropriate tissue in high concentrations, preventing degradation of the molecule and therefore providing a safe non-toxic delivery system in humans and animals. Typically by using phosphatidylcholine based neutral liposomes, an efficient and stable targeted delivery of siRNAs into tumour tissues was observed in a variety of animal models, significantly with a concomitant inhibition of tumour growth [reviewed in 91].

#### 4.4. Decoys for pathogens

Possibly the most unusual application for liposomes comes from the development of particles that when administered into an individual would impersonate the host cell type recognised by invading pathogens, trapping the infectious agent and thus reducing the potential for disease. A recent example is the use of liposomes bearing the glycan sialylneo-lacto-N-tetraose c (LSTc), an analogue of the influenza virus targeting sialic acid molecule found on the surface of respiratory tract cells. Decoy liposomes containing LSTc conjugated to 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine successfully bind influenza virus particles in competition assays in culture and in a mouse model prevent virus spread and increase the survival time even under challenge of a lethal dose [92]. This is significantly better than using free sialic acid analogues, which have shown some success, but are not suitable due to toxicity and solubility issues. Importantly due to their mode of action, the decoy liposomes should have the ability to successfully target both newly emerging and established drug resistant influenza strains without discrimination. This approach has the possibility to become a key preventative treatment against a wide range of pathogens that target specific cell surface receptors.

## 5. Lipid analogues as cytotoxic molecules

### 5.1. Alkyllysophospholipids

Of all the lipid analogues that have been synthesised to date, the alkyllysophospholipids (ALPs) are probably the most studied for their toxicity to cells. This series of ether lipids was born out of the observation that the natural lipid lysophosphatidylcholine (lysoPC) possessed potent immunomodulatory properties but was rapidly metabolised, reducing its effectiveness [93]. To increase the stability but retain the activity of this molecule, lysoPC analogues were synthesised that incidentally had inhibitory effects on tumour growth [94]. With a chemical structure containing a long alkyl chain, ALPs insert into the lipid bilayer of cell membranes and act similarly to a detergent at high concentrations causing cell lysis. At more physiologically relevant concentrations, ALPs have a number of biological effects relating to the disruption of cell membranes, including influencing membrane domains, phospholipid turnover and lipid associated signalling pathways [95]. The consequences of these diverse modes of action include growth inhibition, cell stress, cell cycle arrest and

apoptosis. The most commonly studied ALPs are listed in table 3 showing the timeline of their development and primary publications. For their chemical structures see [95].

| Alkyllysophospholipid              | Year | Targeted disease                                     | Ref. |
|------------------------------------|------|--|------|
| Edelfosine (ET-O-CH <sub>3</sub> ) | 1967 | Cancer, Leishmaniasis, Human African Trypanosomiasis | 96   |
| Miltefosine (HePC)                 | 1983 | Cancer, Leishmaniasis, Human African Trypanosomiasis | 97   |
| Ilmofofinsine                      | 1984 | Cancer, Leishmaniasis, Human African Trypanosomiasis | 98   |
| Erucylphosphocholine (ErPC)        | 1992 | Cancer   | 99   |
| Perifosine (D-21266)               | 1997 | Cancer, Leishmaniasis                                | 100  |
| Erufosine (ErPC3)                  | 2002 | Cancer   | 101  |

**Table 3.** Common alkyllysophospholipids used for disease treatment.

## 5.2. Anticancer

The first ALP to be studied in detail, edelfosine was demonstrated to have a cytotoxic effect on a wide range of cell types, both tumour derived and normal [96]. However it was apparent that edelfosine demonstrated a high selectivity towards tumour cells, strongly stimulating apoptosis by an unknown mechanism. Over the next forty years a number of analogues of edelfosine were similarly investigated for their cytotoxic anti-cancer properties. Although the most potent of the ALPs, the clinical use of edelfosine has remained limited to the treatment of acute leukaemia patients in the purging of bone marrow prior to autologous tissue transplantation [102]. Miltefosine, even though it is metabolised in cells unlike the other ALPs, still has potent anti-tumour activity in some animal models [103]. Unfortunately due to its haemolytic properties, its clinical use is restricted as a topical formulation, promising in phase II trials in the treatment of cutaneous metastases of breast cancer [104]. The most recent ALPs, the homologous erucylphosphocholine and erufosine are suitable for intravenous injection having longer 22 carbon chains and a double bond which causes them to associate in aqueous environments as non-haemolytic lamellar rather than micellar structures. They are valued ALPs in the development of cancer treatments as they have the ability to cross the blood-brain barrier, accumulate in the brain and show anti-tumour effects both *in vitro* [105] and *in vivo* [106]. Perhaps the ALP with the most therapeutic potential, perifosine, created by replacing the choline in miltefosine with a heterocyclic piperidine group, demonstrated good pharmacokinetics and strong cytotoxicity against a wide variety of tumours [107]. Its poor performance in single agent phase II trials however, has stimulated its use in successful application in combination with various anti-tumour treatments that affect other pathways in the cell. Perifosine has showed highly promising anticancer therapy in combination with inhibitors of the anti-apoptotic mTOR signalling network. Individually, drugs targeting mTOR are less effective as often the inhibition is overcome through induction of a positive feedback loop by

the protein kinase Akt to upregulate mTOR [108]. The additive effect generated with the combinational approach is due to perifosine inhibition of Akt causing suppression of the positive feedback loop.

### 5.3. Antifungal

In the treatment of invasive mycoses, the use of miltefosine has demonstrated some broad spectrum fungicidal activity *in vitro* and in a mouse model of cryptococcosis [109]. In general however the application of ALPs to treat fungal infections in humans has been restricted by the limited therapeutic effect against cryptococcal infections in animals [110]. This may be in part due to their apparent mode of action in inhibiting cytochrome C oxidase in *Saccharomyces cerevisiae* and phospholipase B in *Cryptococcus neoformans*, two non-essential yeast proteins [109,111]. The use of ALPs as potent antifungal agents might be resurrected in part by two recent developments. The synthesis of new analogues based on existing structure-antifungal activity relationship (SAR) information of ALPs has given hope that this class of compounds can have benefit in the treatment of invasive or device-related fungal infections [112]. Furthermore, in a study of combinational therapeutics, some synergy was observed in a number of fungal strains with miltefosine and the broad-spectrum drug voriconazole which may develop with further research into clinical relevance [113].

### 5.4. Antiparasitic

It is in the field of parasitology where the ALPs have shown great promise. Initially, alongside their anti-cancer effects, a range of ALP analogues were found to have strong anti-protozoal activity against the free-living ciliate *Tetrahymena pyriformis* [114] and *Leishmania donovani*, the causative agent of visceral leishmaniasis [115]. Subsequent research demonstrated that different ALPs had varying potency against different protozoan species and lifecycle stage. In general however a range of *Leishmania* species, *Trypanosoma brucei* and *T. cruzi* parasites have showed significant susceptibility to these ALPs with effective dose killing responses in the low micromolar range [reviewed in 116]. The development of ALPs as potential anti-parasitic drugs in a clinical setting came from the discovery that miltefosine completely prevented *L. donovani* infection in mice with very little side effects [117]. Ultimately this research led to the development of a clinically approved formulation of miltefosine, Impavido in 2000, still to date the only approved oral drug for leishmaniasis [118]. Impavido is approved for the treatment of cutaneous and mucosal leishmaniasis but is particularly utilised for the first-line treatment of endemic visceral leishmaniasis in Asia. In addition, the use of miltefosine has been shown to be effective in curing patients infected with *L. donovani* parasites unresponsive or resistant to antimony treatment [119]. Similar to the anti-cancer effects of ALPs, the mode of action of miltefosine against *Leishmania spp.* is not entirely clear. There is a notable structural damage to the plasma membrane of most ALP treated parasites, suggestive of alterations to the lipid content [116]. Recent modern metabolomic approaches have defined cellular changes in miltefosine action against *Leishmania* and indicate disruption of the lipid metabolism of the cells as a primary target [120]. Issues with the development of resistance to miltefosine (partly due to weak therapeutics and rapid metabolism) have led to the development of ALP loaded

liposomes. These have proven to be more active than the ALP alone in the treatment of animal models and as in other studies they have shown efficacy against the drug-resistant cell lines [121]. It is clear that the combined use of liposomes and ALPs is a powerful tool, giving a potential dual target approach to combat parasite infection and drug-resistance.

## 6. Lipids and liposomes in health and nutrition

### 6.1. Fatty acids and lipids

It is well documented that omega-3/omega-6 polyunsaturated fatty acids (PUFA) are essential for normal growth and development, especially for visual and neurological development in infants [122, 123]. These fatty acids have also been shown to have numerous beneficial effects on various aspects of human health, and as such the recommended minimal daily intake is set at 250 mg [124]. As humans we are unable to *de novo* synthesise omega-3 PUFA, as we do not have the necessary fatty acid desaturase enzyme(s) and thus rely solely upon dietary intake of these PUFA. Two crucial PUFAs are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), these are primarily accessed via marine sources, i.e. fish or krill oil, while  $\alpha$ -linolenic acid (ALA), is found in numerous plant sources such as nuts and seeds. The biological activities of omega-3/omega-6 PUFA have been under extensive study for several decades and their beneficial effects on several diseases have been well documented, some of which will be now be discussed.

Dietary changes in fatty acid composition have been shown to change the proportion of different types of PUFA in inflammatory and immune cells and thus influencing their function, this is thought to be because they can act as precursors to lipid mediators (eicosanoids/docosanoids) or as ligands for transcription factors [125]. Various omega-3 supplements also seem to boost the effectiveness of anti-inflammatory drugs, several clinical studies have reported that fish oil supplementation has beneficial effects in rheumatoid arthritis, inflammatory bowel disease, and among some asthmatics, supporting the idea that omega-3 PUFA trigger anti-inflammatory and immunomodulatory activities [126]. Along the same lines the PUFA arachidonic acid (AA) is a precursor for prostaglandins, leukotrienes, and related compounds acting as secondary messengers, modulating various roles in inflammation and immunity. Even gamma-linolenic acid a non-essential fatty acid, present in high levels in borage oil has been shown to have several beneficial effects in the treatment of rheumatoid arthritis, atopic eczema and diabetic neuropathy, as well as in the reduction of cholesterol levels [127].

The changing diet of Western societies since industrialisation has been argued to have promoted the pathogenesis of many inflammatory-related diseases, including depressive disorders. Researchers have found a correlation between cultures that eat foods with high levels of omega-3 PUFA also have lower levels of depression [128]. Several epidemiological studies have also shown a significant inverse correlation between intake of oily fish and depression and bipolar disorders [128, 129]. However it has been suggested that the preventive role of omega-3 PUFA may depend on other factors, such as overall diet quality

and the social environment. Accordingly, some research suggests that omega-3 PUFAs may be effective in several ways in protecting people against Alzheimer's disease and dementia by reducing the rate of gradual memory loss linked to aging and enhancing the effectiveness of antidepressants [130].

It is common knowledge that fish and fish oil consumption (high in omega-3 PUFA) can significantly reduce the risk of cardiovascular diseases (CVDs) and slow the formation of plaque in the arteries [131]. It is now becoming clear that it is increasingly important to know which fatty acids especially EPA and DHA are attached to which lipid species. This has recently become apparent as no significant statistically correlation is observed between omega-3 PUFAs and the reduced risk of cardiovascular diseases (CVDs) when 20 studies on 68680 patients were re-evaluated. The latest evidence suggests the true biologically active component, or parent lipid species, has a direct influence on its delivery and subsequent usage/*in vivo* activity [132 and references therein]. Despite the various and numerous biological activities of omega-3 PUFA and their corresponding health benefits being extensively studied for several decades. However, the potential different forms in which these could be delivered via dietary intake such as triglycerides (TGs) versus ethyl esters or phospholipids (PLs), has largely been neglected. The fatty acid chain length and unsaturation on the lipids affects their intestinal absorption efficiency, whereas the chemical structure of the lipids (TGs *versus* PLs) determines their digestion products prior to absorption. The enrichment of essential fatty acids in particular phospholipids for increased dietary uptake and nutritional activity in the body is important. Several studies show evidence that dietary PLs have a positive impact in several diseases and potentially reduce side effects of some drugs [133, 134].

Modern diets are often depleted of complex and diverse mixtures of PLs due to increased use of refined oils and purified raw materials, which had led to an overall reduction in the uptake of PLs. Hence, the supplementation of marine PLs may serve three important functions within the functional food segment: (a) emulsifying properties, (b) supplementation of omega-3 PUFAs and (c) beneficial nutritional effects of the PLs themselves [135]. A better understanding of the impact of PL supplementation and its health benefits is required.

The potential anti-obesity effect of conjugated linoleic acid, and its mode of action lowering body fat mass has recently been reviewed by Kennedy *et al* [136]. Conjugated linoleic acid (CLA), a group of conjugated cis and trans isomers of octadecadienoic acid that have been converted from linoleic acid by microbes in the gastrointestinal tract of ruminant animals and often found in beef, dairy foods, and dietary supplements, reduces adiposity in several animal models of obesity and in some humans. CLA was discovered by Pariza and colleagues in 1987, and was first identified as an anti-carcinogen, but subsequently shown to exhibit anti-atherosclerotic and more recently anti-obesity properties [137]. Interest in CLA as an alternative treatment to conventional diabetic and weight loss therapies has increased over the past decade. Supplementation with a mixture of CLA isomers decreased the body fat mass in many animal and some human studies. The major 10,12 isomer of CLA, seems to be responsible for the antiobesity effects. Commercial preparations of CLA are now made from the linoleic acid



of safflower or sunflower oils under alkaline conditions. Kennedy *et al* have summarised the recent *in vivo* and *in vitro* findings and propose potential mechanisms by which CLA reduces adiposity including its impact on energy metabolism, adipogenesis, inflammation, lipid metabolism, and apoptosis [136].

## 6.2. Liposomes

Liposomal encapsulation technology used by medical researchers to deliver drugs effectively to specific areas or organs in the body, as discussed earlier, is also being used to target delivery of a number of poorly soluble and high molecular weight bioactive dietary components including natural products such as carotenoids, phytosterol, omega-3 PUFAs, vitamins and other antioxidants to the body. The liposomes provide a number of advantages to other delivery systems and this is why a number of nutritional companies are now utilizing this technique in the oral delivery of dietary supplements and nutrients that are not prematurely decomposed and are pinpointed to specific tissues and organs. This approach has the added bonus that the doses can be reduced by 5 to 15 times less than normal supplement intake, i.e. tablets and capsules. The beneficial action of liposomes in oral delivery of nutrients is due to several modes of their action, including improved nutrient solubilisation and protection against environmental conditions such as moisture, oxygen and degradation by the presence of enzymes in oral and esophageal digestive juices prior to being absorbed into the body [138-140]. The phospholipids of the liposomes are able to repel undesirable activities of the digestive juices of the gastrointestinal tract, until the contents have reached the target tissue and are endocytosed, delivering their cargo into the intra-cellular space. It is important to note that more conventional delivery routes for nutrients, such as tablets, capsules etc., offer different and complementary forms of nutrient bio-availability, however the various food additives used in tablets and capsules, such as binders, fillers, gelatins and sugar affect the absorption process and may cause incomplete disintegration, hence reducing bioavailability of the active components.

More than 50 products and product combinations have been formulated using liposomal delivery systems, some of them are listed in Table 4. A key example of the full potential of using oral liposomal encapsulation is vitamin C, which causes a ~10-fold increase of vitamin C into cellular systems compared to oral tablet/capsule formulations, with no negative effects, such as gastric distress, urinary output or extra load on the liver [141].

| Vitamin/herb/botanical | Active compound(s) | Health benefits   |
|------------------------|--------------------|---|
| Vitamin A              | Retinol            | Retina function, Epithelial tissue growth, Bone growth, Embryonic development   |
| Vitamin B2             | Riboflavin         | Essential for metabolizing carbohydrates, fats, and lipids<br>Necessary for the function of vitamins B6, folic acid, and niacin |
| Vitamin B12            | Cyanocobalamin     | Deficiency causes pernicious anemia, muscle and nerve paralysis   |
| Vitamin C              | Ascorbic acid      | Antioxidant   |

| Vitamin/herb/botanical       | Active compound(s)         | Health benefits   |
|------------------------------|----------------------------|---|
| Vitamin E                    | alpha-Tocopherol           | Detoxifies free radicals, Prevents damage to cell membranes.<br>Enhances immune response,<br>Antioxidant  |
| CoEnzyme Q10                 | Ubiquinone-50              | Increased mitochondria function, cofactor in oxidative respiration  |
| DHEA                         | Dihydroepiandrosteindione  | Increased libido, Feelings of well being Decreased viral load   |
| Echinacea                    | Echinacosides              | Immuno-stimulation  |
| Gingko biloba extract        | Gingolides                 | Dementia, Equilibrium disorders, Intermittent claudication  |
| Glucosamine sulfate          | Glucosamine                | Osteoarthritis, Bone, cartilage, and Muscle growth  |
| Grape seed extract           | Procyanidines              | Antioxidant, Inhibits tooth decay, Source of essential oils   |
| IGF-1                        | Insulin, Growth Factor-1   | Anti-aging  |
| Kava kava                    | Kava lactones              | Anxiolytic, insomnia  |
| Ma huang                     | Ephedra                    | Cough, Bronchitis, Appetite suppression   |
| Melatonin                    | Melatonin                  | Insomnia, Jet lag   |
| Milk thistle                 | Silymarin                  | Hepatoprotection, Cirrhosis, Hepatitis, Immunomodulation  |
| St. Johns wort               | Hypericin, pseudohypericin | Anxiolytic, Depression, Topical inflammation, Wound healing   |
| Zinc gluconate, zinc sulfate | Zinc ion                   | Essential part of more than 200 enzymes involved in digestion, metabolism, reproduction (sperm formation), and wound healing.<br>Involved in sense of taste. Role in function and structure of cell membranes. Major part of the immune system. Component of insulin deficiency |

**Table 4.** Liposomal nutritional products on the market

Dietary polyphenols, including flavonoids, have long been recognized as a source of important molecules involved in the prevention of several diseases, including cancer. However, due to their poor bioavailability, polyphenols remain difficult to be employed clinically. The recent use of liposomes, as a means of improving their pharmacokinetics and pharmacodynamics, hence their bioavailability means there is a renewed interest into the therapeutic benefits of wide range of polyphenols [142, 143].

## 7. Future perspectives

Liposomes are being used in a wide range of applications from drug and gene delivery to diagnostics, cosmetics and food nanotechnology being able to be administered orally, parenterally or topically. Liposome formation and entrapment of various different types of cargo is now a well established methodology, allowing them to stabilise the encapsulated materials against a range of environmental and chemical changes, including enzymatic and chemical modification, as well as buffering against extreme pH and temperature [144].

There are a rapidly increasing number of new applications for liposomes in the drug and food industry, due to their biocompatibility and biodegradability. The natural composition of the liposomes, which helps in overcoming regulatory hurdles, and if required newly developed formulations can quickly be implemented. However, use of multiple lipid sources (e.g. animal, plant, synthetic sources) often requires additional characterisation and comparability studies. The quality and purity of the lipid starting materials for liposome formulations are essential to maintain the quality of the later drug or encapsulated product. Therefore the appropriate characterization and specification of the lipid starting material is considered as vital as the product being delivered, as laid out by EU directive 2001/83/EC, along with guidance on process validation CHMP/QWP/848/99 and EMEA/CVMP/598/99 and marketing authorisation of a medicinal product (EMA/CHMP/QWP/396951/2006).

The remarkable biocompatibility of liposomes probably stems from the fact that they are closely analogous to both naturally occurring endosomes that circulate in the bloodstream before accumulating in specific tissues, and lamellar bodies known to lubricate and protect tissue surfaces and serve in specialist functions, i.e. act a surfactant in the lungs to allow oxygen to pass from the air into the bloodstream. The depletion of lamellar bodies is also implicated in a range of diseases, including serious progressive respiratory conditions, including Cystic Fibrosis and Chronic Obstructive Pulmonary Disease [144]. These are obvious areas of research where liposome technology could be a game changer.

In order to extend and take full advantage of this highly biocompatible and safe biodegradable delivery system, future research should focus on the production of the lipid vesicles through safe, scalable methods, as well as accessing the required high quality/purity of lipids in a cheap and sustainable manner.

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