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Review Article

LIPOSOMES: VESICULAR SYSTEM AN OVERVIEW

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ABSTRACT

The conventional method of preparing liposomes is basically for the multilamellar vesicles (MLVs). However, other methods are used to reduce the size of these MLVs to small unilamellar vesicles (SUVs) so as to increase their plasma lifetime and consequently increase the possibility of achieving greater tissue localisation. Some of these methods of size reduction are sonication and high pressure extrusion. Large unilamellar vesicles (LUVs), on the other hand, are prepared mainly by detergent removal method and reverse phase extrusion technique. Novel drug delivery system aims to deliver the drug at a rate directed by the needs of the body during the period of treatment and channel the active entity to the site of action. A number of novel drug delivery system have emerged encompassing various route of administration, to achieve targeted and controlled drug delivery. The review also shows that liposomes have a lot of biomedical applications and uses. This review is mainly focused on the diseases that have attracted most attention with respect to liposomal drug delivery and have therefore yielded most progress, namely cancer, antibacterial and antifungal disorders. In addition, increased gene transfer efficiencies could be obtained by appropriate selection of the gene transfer vector and mode of delivery.

Keywords: Liposomes; Phospholipid; Clinical applications; doxorubicin; Cancer therapy; Gene Therapy; Amphotericin B

INTRODUCTION

Liposome was discovered about 40 years ago by Bangham and co-workers and was defined as microscopic spherical vesicles that form when phospholipids are hydrated or exposed to an aqueous environment[1] . Liposomes are microscopic vesicles composed of a bilayer of phospholipids or any similar amphipathic lipids. They can encapsulate and effectively deliver both hydrophilic and lipophilic substances,[2-3] and may be used as a non-toxic vehicle for insoluble drugs[4] . Weiner *et al*[5] defined liposome as a microstructure consisting of one or more concentric spheres of lipid bilayer separated by water or aqueous buffer compartments. The typical characteristic of bilayerforming lipids is their amphiphilic nature: a polar head group covalently attached to one or two hydrophobic hydrocarbon tails. When these lipids, e.g., phosphatidylcholine, phosphatidyl ethanolamine or phosphatidyl glycerol, are exposed to an aqueous environment, interactions between themselves (hydrophilic interactions between polar head groups and van der Waals interactions between hydrocarbon chains and hydrogen bonding with water molecules) lead to spontaneous formation of closed bilayers [6] .

Liposomes classification based on composition and mode of drug delivery

Type	Composition	Chracteristics
Conventional Liposomes	Neutral and or negatively charged phospholipids+Cholesterole	Subject to coated pit endocytosis contents ultimately delivered to lysosomes if they do not fuse from the endosome, useful for RES targeting, dose dependent pharmacokinetic [7-8].
PH sensitive Liposomes	Phospholipid such as phosphatidyl ethanolamine, Dioleoyl phosphatidyl ethanolamine	Subject to coated pit endocytosis at low PH fuse with cell or endosome membrane and release their contents in the cytoplasm [9].
Cationic Liposomes	Cationic Lipids	Possibly fuse with cell or endosome membrane; Suitable for delivery of negatively charged macromolecules (DNA, RNA); Ease of formation; toxic at high dose [10] .
Long circulating Liposomes or stealth Liposomes	Neutral high transisition temperature, Lipid, Cholesterol+5-10% of PEG-DSPE	Hydrophillic surface coating, Low opsonisation and thus low uptake by RES; Long circulating half life (40 hrs); dose independent pharmacokinetics upto 10 micromoles/ mouse lipid dose [11] .
Immunoliposomes	Conventional or long circulating liposomes with attached Ab or recognition sequence.	Subject to receptor mediated endocytosis; Cell specific binding; can release contents extracellulary near the target tissue and drug diffuse through plasma membrane to produce their effects [12] .
Magnetic Liposomes	Phosphatidyl choline, Cholesterol, Small amounts of linear chain aldehyde and colloidal particles of magnetic iron oxide	Liposomes that indigenously contain binding sites for attaching other molecules like antibodies on their exterior surface. Can be made use of by an external magnetic field in their deliberate, on site and rupture immediately release of their contents [13] .
Temperature or heat sensitive Liposomes	Dipalmitoyl Phosphatidylcholine	Vesicles shows maximum release at 41ºY.The phase transition temperature of dipalmitoyl phosphatidylcholine Liposomes released the entrapped contents at the target cell surface [14] .

Liposome classification by size and number of lamellae

Type	Size range	Characteristics
Multilamellar Vesicles (MLV)	0.1-0.3µm	More than one bilayer; Moderate aqueous volume to lipid ratio 1:4:1mole lipid). Greater encapsulation of lipophillic drug; Rapidly cleared by RES; Simplest to prepared by thin film lipid of hydration of lipids in presence of an organic solvents [15-16] .
1)Oligolamellar vesicles		Intermediate between LUV & MUV [17] .
2)Multivesicular vesicles		Separate compartment are present in a single MLV [18] .
3)Stable plurilamellar vesicles		Have unique physical and biological properties due to osmotic compression [19] .
Large unilamellar vesicles (LUV)	0.1-10µm	Single bilayer, High aqueous volume to lipid ratio (7:1mole lipid) useful for hydrophilic drug; High capture of macromolecules; Rapidly cleared by RES; Prepared by detergent dialysis, ether injection, reverse phase evaporation or active loading methods [20] .
Small unilamellar vesicles (SUV)	≤0.1µm	size;Thermodynamically unstable; low aqueous volume to lipid to ratio (0.2:1.5:1mole lipid); Prepared by reducing the size of MLV or LUV using probe sonication or gas extruder or by active loading or solvent injection technique [21] .

Formulation of liposomes

Liposomes are made from pure lipids or a combination of lipids. The lipids commonly employed in liposome formulations are phospholipids. Liposomes have been prepared from a variety of synthetic and naturally occurring phospholipids, they generally contain cholesterol[22] . The incorporation of cholesterol into the lipid bilayer membrane generally enhances the stability of liposomes in serum, reduces the permeability of the membranes to water soluble molecules and increases the fluidity or microviscosity of the bilayer[23-24] . The most commonly used phospholipids in liposome preparation are: egg phosphatidylcholine, synthetic dipalmitoyl–DL-α-phosphatidylcholine, brain and synthetic phosphatidylserine, sphingomyelin, phosphatidylinositol and ovolecithin. Usually, a zwitterionic or non-ionic lipid is used as the basic lipid for the preparation of liposomes. The net surface charge of liposome can be modified by the incorporation of positively charged lipids such as stearylamine, or negatively charged lipids such as diacetylphosphate, phosphatidyl glycerol or phosphatidyl serine[25]. The presence of negatively or positively charged lipids lead to a greater overall volume for aqueous entrapment and reduces the likelihood of aggregation after preparation of the liposomes [26] .

Technology of liposome production

Since the early 1970s many hundreds of drugs, including anti-tumour and antimicrobial agents, chelating agents, peptide hormones, enzymes, other proteins, vaccines and genetic materials, have been incorporated into the aqueous or lipid phases of liposomes of various sizes, compositions and other characteristics by an ever-increasing number of techniques. Liposomes have evolved from mere experimental tools of research to industrially manufactured products for clinical and veterinary use. This success depends on advanced techniques to obtain efficient drug entrapment and increased stability of the products. The conventional method and the advanced techniques based on this method are discussed as follows.

Conventional method

The Convectional method was first described in detail by Bangham et al[27] for the preparation of MLVs. In the procedure; the phospholipids are dissolved in an organic solvent (usually a chloroform/methanol mixture) and deposited from the solvents as a thin film on the wall of a round bottom flask by use of rotary evaporation under reduced pressure. MLVs form spontaneously when an excess volume of aqueous buffer containing the drug is added to the dried lipid film. Drug containing liposomes can be separated from nonsequestered drug by centrifugation of the liposomes or by gel filtration. The time allowed for hydration of the dried film and conditions of agitation are critical in determining the amount of the aqueous buffer (or drug solution) that will be entrapped within the internal compartments of the MLVs. For instance, it is reported that more of the aqueous phase can be sequestered when the lipid is hydrated for 20 hours with gentle shaking, compared with a hydration period of two hours, with vigorous shaking of the flask, even though size distribution of the MLVs was unaffected[28] . This means that slow hydration is associated with greater entrapment of aqueous volume.

sonication method

This method is used in the preparation of SUVs[29] and it involves the subsequent sonication of MLVs prepared by the convectional method[30] either with a bath type or a probe type sonicator under an inert atmosphere, usually nitrogen or argon. The principle of sonication involves the use of pulsed, high frequency sound waves (sonic energy) to agitate a suspension of the MLVs. Such disruption of the MLVs produces SUVs with diameter in the range of 15–50nm. The purpose of sonication, therefore, is to produce a homogenous dispersion of small vesicles with a potential for greater tissue penetration. The commonly used sonicators are of the bath and probe tip type. A probe tip sonicator delivers high sonic energy to the lipid suspension but has the disadvantage of overheating the lipid suspension to cause degradation. The probe tip also tends to release titanium particles into lipid suspension, which must be removed by centrifugation. For this reason, bath sonications are the more widely used. By this technique, a test tube containing the suspension is placed in the bath sonicator and sonicating for 5–10 minutes above the transitional temperature of the lipid (i.e., the temperature at which the lipid melts). The major drawbacks in the preparation of liposomes by sonication include oxidation of unsaturated bonds in the fatty acid chains of phospholipids and hydrolysis to lysophospholipids and free fatty acids. Another drawback is the denaturation or inactivation of some thermolabile substances (e.g., DNA, certain proteins, etc) to be entrapped.

solubilisation and detergent removal method

This method is used in the preparation of LUVs[31] and it involves the use of detergent (surfactant) for the solubilisation of the lipids. Detergents used include the non-ionic surfactants e.g., n-octyl-bete-D-glucopyranose (octyl gluside), anionic surfactants (e.g., dodecyl sulphate) and cationic surfactants (e.g., hexadecyltrimethyl

ammoniumbromide).The procedure involves the solubilisation of the lipids in an aqueous solution of the detergent and the protein(s) to be encapsulated. The detergent should have a high critical micelle concentration (CMC), so that it is easily removed. The detergent is subsequently removed by dialysis or column chromatography[32]. During detergent removal, LUVs of diameter 0.08–0.2μm are produced.

Reverse phase evaporation technique

Szoka and Papahadjopoulos[33] pioneered the preparation of lipid vesicles by reversed phase evaporation technique. It consists of a rapid injection of aqueous solution of the drug into an organic solvent, which contains the lipid dissolved with simultaneous bath sonication of the mixture leading to the formation of water droplets in the organic solvent (i.e., a "water-in-oil" emulsion). The resulting emulsion is dried down to a semi solid gel in a rotary evaporator. The next step is to subject the gel to vigorous mechanical agitation to induce a phase reversal from water-in-oil to oil-in-water dispersion (i.e., an aqueous suspension of the vesicles). During the agitation, some of the water droplets collapse to form the external phase while the remaining portion forms the entrapped aqueous volume. Large unilamellar vesicles (diameter 0.1–1μm) are formed in the process.

Drug targeting

The need for "drug targeting" arises from a problem situation whereby a drug administered (iv for example) enters the blood stream and is distributed to varying extents throughout the body when the actual desire is to deliver or direct the drug selectively to its site of action. This site could be an organ structure, a cell subset, or even an intracellular region. In such a case pumping the drug throughout the whole body is not only wasteful but, more fundamentally, it is also likely to lead to undesirable side effects. On the other hand, restricting the distribution of the drug to the specific target site should allow for an increase in efficacy at low dose with attendant decrease in toxicity[34-35] . Hence, the benefits of drug targeting include reduced drug waste, and it is possible to deliver a drug to a tissue or cell region not normally accessible to the free or untargeted drug. The approach for drug targeting via liposomes involves the use of ligands (e.g., antibodies, sugar residues, apoproteins or hormones), which are tagged on the lipid vesicles. The ligand recognises specific receptor sites and, thus, causes the lipid vesicles to concentrate at such target sites. By this approach the otherwise preferential distribution of liposomes into the reticuloendeothelial system RES (liver, spleen and bone marrow) is averted or minimised. The preferential distribution of liposomes into the RES can be modified by the incorporation in the liposome membrane of protein or carbohydrates possessing specific affinity toward a target tissue or organ[36-38] . A ligand selection is based on its recognition by, and specificity for, the target site. In cancer treatment, for example, one of the approaches is to target the drug to tumour cells via receptor specific ligands, which may be specific antibodies for antigens produced by the tumour cells. The first step, therefore, is to determine the antigens that are produced by the tumour cells. Also, molecules bearing oligosaccharide chains have been used as ligands for direction, and specific attachment, to ganglion sites in cells [39] .

Topical drug delivery

The application of liposomes on the skin surface has been proven to be effective in drug delivery into the skin[40-43] . Liposomes increase the permeability of skin for various entrapped drugs and at the same time diminish the side effect of these drugs because lower doses are now required[44-45] . Liposomes have also found an important application in cosmetics for skin care preparations [46] . In this regard, the liposomes are applied to the skin in the form of solution or in hydrogels[47] . Hydrophilic polymers are suitable thickening agents for the gels. However, the liposomes may in certain instances be trapped in the polymeric network of the hydrogels and, hence, impair bioavailability into the skin[48-49] . Nevertheless, Gabrijelcic et al[50] found enhanced transport of liposome-entrapped substances into the skin from hydrogels prepared from xanthan gum. The enhanced drug transport into the skin is attributed to the lipid nature of the vesicles, which serve as carriers for the drug.

Treatment of human immunodeficiency virus infection

Several antiretroviral nucleotide analogues have been developed for the treatment of patients suffering from the acquired immunodeficiency syndromes (AIDS). These include antisense oligonucleotide, which is a new antiviral agent that has shown potential therapeutic application against HIV-1[51]. These antiviral agents are able to combat replication of the HIV by inhibiting reverse transcriptase and, thereby, viral DNA synthesis[52]. However, these agents display a dose-related toxicity. The effective dose can be reduced by encapsulation of such drugs in liposomes, thus reducing the incidence of toxicity. The greater efficacy of the liposomal formulation relates to the preferential uptake of the liposomes into the virus compared with the host tissue[53] .

Enhanced antimicrobial efficacy/safety

Antimicrobial agents have been encapsulated in liposomes for two reasons. First, they protect the entrapped drug against enzymatic degradation. For instance, the penicillins and cephalosporins are sensitive to the degradative action of β-lactamase, which is produced by certain microorganisms. Secondly, the lipid nature of the vesicles promotes enhanced cellular uptake of the antibiotics into the microorganisms, thus reducing the effective dose and the incidence of toxicity as exemplified by the liposomal formulation of amphotericin B[54].

Applications of liposomes in drug delivery

The Pharmaceutical Industry is one of the most potent industries all over the world, facing high risks and important challenges. Both, the cost of bringing a New Molecular Entity (NME) to market is between 500-1000 million Euros and the time required to launch a NME is 10-15 years. One of the challenges to rationalise and further improve these NME will be the development of appropriate drug delivery systems, namely Liposomes.The two aspects of the utilization of Liposomes: as carriers either for macromolecules, for the treatment of cancer and inflammatory disorders or for small molecular weight drugs for the treatment of intracellular infectious disease. Intracellular infections, namely those localized in mononuclear phagocytic system (MPS) are very difficult to eradicate due to the low access of drugs to the sites of infection resulting in sub- therapeutic local drug concentration. Liposomes are ideal carriers to transport drugs to infected macrophages, as they have the tendency to accumulate in MPS cells by passive targeting. This capability was exploited for the treatment of leismaniasis and tuberculosis, with liposomes incorporating dinitroanilines and rifamycins, respectively. Dinitroanilines are herbicides that also showed antiparasitic properties, namely against different strains of *Leishmania*. Nevertheless, its use as an antiparasitic agent by parenteral and oral routes has been limited, due mainly to its low water solubility and unusual low vapour pressure Liposomal formulations of Dinitroanilines were developed, acting Liposomes as drug solvent, a stabilizing system for their storage during a substantiate time after production and as carriers able to transport drugs to the sites of infection.The biological activity of Dinitroaniline liposomes was assayed both in a murine visceral model. (*L. donovani*) and in a murine cutaneous model (*L. major*) of Leismanial infection.A significant reduction of parasite loads in both animals models, after treatment of liposomal formulations, in comparison with the non-treated group and with the standard drugs, were observed. No signs of toxicity after dinitroaniline Liposome treatment were found[55-56]. Superoxide dismutase (SOD) incorporated in Liposomes also presented advantages over the free from of the enzyme in the treatment of rat adjuvant arthritis. Liposomes increased the short half-life of SOD, targeted the enzyme to the inflamed sites and increased its anti-inflammatory activity. Long circulating liposomes incorporating SOD are superior to conventional Liposomes, in terms of anti-inflammatory activity [57-58].

Clinical applications

New drug delivery systems such as Liposomes are developed when an existing formulation is not satisfactory and reformulation in Liposomes offers clear benefits with respect to targetability, therapeutic efficacy and safety compared to the existing

formulations. Lack of specificity in pharmacologically active agents is an obstacle to their effective use in biological research and medicine. It follows that any approach enabling an agent to reach its target selectively and in a controlled fashion would contribute to the elimination of problems inherent in conventional methods. One such approach was the development of a non-toxic and biodegradable carrier capable of containing a variety of substances of biological interest that the carrier could, upon contact with the leaving entity, direct to the site of action and subsequently allow to perform their task. It has been well established that liposomes can meet many of these requirements, *i.e.,* Liposome formulations of some drugs have shown a significant increase in therapeutic efficacy and/or therapeutic indices in preclinical models and in humans compared to conventional formulations. Encouraging results of Liposomal drugs in the treatment or prevention of a wide spectrum of diseases in experimental animals and in human, indicate that more Liposome-based products for clinical and veternary applications may be forthcoming[59]. These could include treatment of skin and eye diseases, antimicrobial and anticancer therapy, metal chelation, enzyme and hormone replacement therapy, vaccine and diagnostic imaging, *etc.*some of the Liposome applications with realistic prospects of being developed for clinical use.

Cancer therapy

Cytotoxic drugs can distribute non-specifically throughout the body, lead to death of normal as well as malignant cells, thereby giving rise to a variety of toxic side effects. Entrapment of these drugs into liposomes resulted in increased circulation lifetime, enhanced deposition in the infected tissues, Protection from the drug metabolic degradation, altered tissue distribution of the drug, with its enhanced uptake in organs rich in mononuclear phagocytic cells (liver, spleen and bone marrow) and decreased uptake in the kidney, myocardium and brain. To target tumors, liposomes must be capable of leaving the blood and accessing the tumor. However, because of their size liposomes cannot normally undergo transcapillary passage. In spite of this, various studies have demonstrated accumulation of liposomes in certain tumors in a higher concentration than found in normal tissues[60-61]. Many research efforts have been directed towards improving the safety profile of the anthracycline cytotoxics, doxorubicin (DXR) and daunorubicin (DNR), along with vincristine (VCR), which are associated with severe cardiotoxic side effects, although acute gastrointestinal effects and other toxicities may also occur. Liposomal entrapment of these drugs showed reduced cardiotoxicity, dermal toxicity and better survival of experimental animals compared to the controls receiving free drugs[62]. Such beneficial effects of liposomal anthracyclines have been observed with a variety of liposomal formulations regardless of their lipid composition provided that lipids used _high cholesterol (Cho) concentration or phospholipids with high phase transition temperature (Tc)_ are conducive to drug retention by the vesicles in the systemic circulation[63] . DXR is a potent antineoplastic agent active against a wide range of human cancer including lymphomas, leukemia and solid tumors. However, administration of this drug produces acute toxicity in the form of bone marrow depression, alopecia and oral ulceration[64-66] . DXR entrapped in liposomes shows reduced non-specific toxicity and maintains or enhances anticancer effect. DXR hydrochloride constitutes the first liposomal product (DoxilTM) to be licensed in the United States. Surface grafted methoxypolyethylene glycol (MPEG) provides the hydrophilic stealth coating, which allows the DoxilTM liposomes to circulate in the blood stream for prolonged periods. The lipid matrix and an internal buffer system combine to keep virtually all the DXR encapsulated during liposome residence in the circulation. This means that the drug is not free to exert its toxic effects[67-68]. Liposome association alters the drug pharmacokinetics and thus the liposome has a half-life of approximately 55 hours in humans, whereas the free drug distributes to the tissues within a few minutes and is entirely cleared from circulation within 24 hours[69]. Liposomal formulation showed decreased toxic effects of DXR; a dose higher than the *LD*50 could be administered without acute toxicity, which suggests that these liposomes extravasate from the endothelium of tumor tissues and reside around tumor cells where they release the drug into the interstitial fluid. Therefore, the therapeutic effect was achieved by a slow and sustained release of the drug at the target site. Furthermore, liposomal DXR has substantial activity against ovarian cancer in patients that failed to respond to platinum and paclitaxel-based regimens. The responses achieved with liposomal DXR were durable and maintained with minimal toxicit[70]. Encapsulation of DXR in stealth liposomes showed significant accumulation, enhanced therapeutic efficacy and reduced toxic effect against human pancreatic carcinoma AsPC-1, implanted into nude Swiss mice compared to DXR suspension.Increased penetration into the tumor and a long presence with a slow drug release from liposomes in the tumor account for the enhanced therapeutic effects[71]. DXR also plays an important role in the treatment of breast cancer, both in the adjuvant and metastatic settings. However, the benefits of conventional DXR in terms of antitumor activity are limited by its therapeutic index. Pegylated liposomal DXR provides tumor-targeted efficacy without many of the toxicities associated with conventional DXR, including myelosuppression, alopecia, nausea and vomiting, and most importantly, cardiac toxicity. It has also demonstrated efficacy in combination with other agents or modalities, including cyclophosphamide, paclitaxel, docetaxel, gemcitabine, vinorelbine, and hyperthermia. Owing to its comparable efficacy and favorable safety profile, pegylated liposomal DXR may be a useful alternative to conventional DXR, as well as other agents commonly used in the treatment of breast cancer[72-73]. Sterically stabilized liposomes derived from the antitumor agent hexadecyl phosphocholine showed reduced uptake by the mononuclear phagocyte system (MPS) and improved antitumor activity in breast carcinoma in nude mice compared to conventional hexadecyl phosphocholine liposomes or free hexadecyl phosphocholine[74]. Cisplatin when encapsulated in polyethyleneglycol-coated long-circulating liposomes results in prolonged circulation time and enhanced tumor uptake in different mouse tumor models. In spite of these results, due to the extremely slow release rate, no superior antitumor activity is seen for liposomal cisplatin over plain cisplatin. Results demonstrated that improvement in release kinetics of the prepared liposomes would lead to higher therapeutic efficacy of entrapped cisplatin[75].

Antimicrobial therapy

Antibiotics can only act against intracellular infections if they can penetrate the phagocytic cells. It is a well known fact that liposomes are able to localize in the liver and spleen, especially the RES component, where many pathogenic microorganisms reside; they can be therefore used for targeting of antibiotics on these organs. In a simple *in vitro* culture, liposomal neomycin[76]. And penicillin[77] were found to be active against bacteria, whereas liposome entrapment markedly reduced the antimicrobial activity of chloramphenicol[78]. Liposome encapsulation alters the tissue distribution of gentamicin when given by intravenous route to rabbits[79]. However, when administered by intramuscular route, it resulted in sustained release from the injection site, providing prolonged plasma concentrations of the drug[80]. Moreover, Lutwyche *et al*[81] demonstrated that encapsulation of membrane-impermeative antibiotics such as gentamicin in appropriately designed lipid-based delivery systems can enable their use in treating intracellular infections. Rifampin delivered twice weekly for two weeks in tuftsin-bearing liposomes was at least 2,000 times more effective than the free drug in lowering the load of lung bacilli in infected animals[82]. Liposome encapsulated clarithromycin may be more effective than the free form against *Mycobacterium avium* intracellular (MAI) infections *in vivo*, and the use of a combination therapy with ethambutol could further enhance the efficacy[83] . Furthermore, when the activity of TLC G-65 (liposomal gentamicin preparation), alone and in combination with rifapentine, clarithromycin, clofazimine and ethambutol, was evaluated in the beige mouse model of disseminated *Mycobacterium avium* infection showed that the combination of rifapentine and TLC G-65 was more active than either agent alone. The activity of clarithromycin in combination with TLC G-65 was similar to that of either agent alone. Clofazimine improved the activity of TLC G-65 with respect to the spleen, while ethambutol improved the activity with respect to the liver[84]. Entrapment of ciprofloxacin in liposomes increases the circulation

half-life of the drug when given by intravenous route in mice, which is associated with enhanced delivery of the drug to the liver, spleen, kidneys, and lungs. Furthermore, liposomal entrapment was associated with increased therapeutic efficacy against the *Salmonella typhimurium* infection model in mice[85]. Stevenson and coworkers[86] showed enhanced activity of streptomycin and chloramphenicol against *Escherichia coli* in the cells of the J774 murine macrophage line mediated by liposome delivery. The apparent intracellular antibacterial activity of antibiotics was increased more than tenfold by entrapment in liposomes. Khalil *et al*[87] demonstrated a higher accumulation of streptomycin sulfate in the liver and spleen when encapsulated in liposomes than that exhibited by the free drug. Furthermore, streptomycin liposomes, when administered in two intravenous injections, caused greater reduction of the colony forming unit in the spleen, lungs and liver, when compared with the free drug, which was given in a much higher dose by intramuscular route[88]. Moreover, long circulating liposomes and conventional liposomes encapsulating streptomycin, when given twice weekly, showed bactericidal activity against *Mycobacterium avium* complex (MAC) strain 101 in the spleen when the level of infection after treatment was compared to that before treatment[89] .

Gene therapy

A number of systemic diseases are caused by lack of enzymes, factors due to missing or defective genes. Gene delivery systems are designed to control the location of administered therapeutic genes within the patient's body. Successful *in vivo* gene transfer may require: *(i)* condensation of the plasmid and its protection from nuclease degradation, *(ii)* cellular interaction and internalization of condensed plasmid, *(iii)* escape of the plasmid from endosomes (if endocytosis is involved), and *(iv)* plasmid entry into cell nuclei 90 . Gene therapy methods involve introduction of genetic material into the patient's cells to synthesize the therapeutic protein. Direct administration of genes to patients may be virally or non-virally mediated. As viruses represent a highly suitable vector for gene transfer, several viruses including retrovirus, adenovirus, adeno-associated-virus and herpes virus have been investigated for their potentials in gene delivery.

However, there is always the potential risk that the viruses woned become replication competent and therefore infectious, immune and inflammatory responses have also been reported in clinical trials. In recent years, several attempts have been made to restore the gene expression by delivery of relevant exogenous DNA or genes to cells[90-91]. Cationic liposomes are considered to be a potential non-viral human gene delivery system[92-98]. These liposomes are usually composed of cationic lipid derivatives and a neutral phospholipid such as dioleoylphosphatidyl ethanolamine (DOPE). Widely accepted cationic liposome formulations are lipofectin, lipofectamine, transfectace, transfectam and DC-Cho _3BN-(*N*_,*N*_-dimethylaminoethane) carbamoyl-cholesterol_. Cationic liposomes based on dioleyloxypropyl trimethylammonium chloride (DOTMA), such as lipofectin, and several other types have demonstrated success both in *in vitro* and *in vivo* gene delivery[99-100]. The negatively charged genetic material, for example, plasmid, is not encapsulated in liposomes but complexed with cationic lipids by electrostatic interactions. Plasmid liposome complexes are thought to enter the cells through fusion with the plasma or endosome membrane[101]. These liposomes were generally more effective in transfecting genes than micelles of the same lipid composition, thus suggesting a role for the bilayer structure in facilitating transfection. In addition, the transfection efficiency of liposome-delivered genes was highly dependent upon the lipid composition, lipid/DNA ratio, particle size of the liposome-DNA complex, and cell lines used[102]. They can be prepared with defined physicochemical properties, such as size, shape and surface charge, which in turn control the stability, distribution and uptake of DNA *in vivo*. Liposomes have been shown to potentiate DNA mediated vaccination. Intramuscular immunization of mice with DNA encoding the S region of hepatitis B antigen entrapped into cationic liposomes has led to improved humoral and cell mediated immunity, as compared to the naked DNA or DNA complexed with preformed similar liposomes. It is assumed that immunization with liposomes entrapped plasmid DNA involves antigenpresenting cells (APC), either locally or in the regional draining lymph nodes[103].

CONCLUSION

This review showed that liposomes have been prepared from a variety of synthetic and naturally occurring phospholipids and generally contain cholesterol as membrane stabiliser. Also shows the formulation and techniques of Liposomes. Furthermore, liposomes are tools for drug targeting in certain biomedical situations (e.g., cancer) and for reducing the incidence of dose-related drug toxicity. Thirty-four years long research in liposomal drug delivery has led to vast improvement of the technology in terms of drug entrapment efficiency, vesicle stability in storage and in the body, design of vesicles for controlled release, site specific targeting and scale up production. In parallel, noteworthy advances have been made in understanding and controlling liposomal behavior *in vivo*. This has facilitated the application of a wide range of liposomal drugs in the treatment and prevention of diseases in experimental animals and clinically. Commercial introduction of the various liposomal formulations represents a milestone in the history of liposomal drug delivery. Many more liposome-based drug formulations can be expected in the near future both for delivery of conventional drugs and for new biotechnology therapeutics such as recombinant proteins, antisense oligonucleotides and cloned genes. With the recent development in the field, several companies are already actively engaged in expansion and evaluation of liposome products for anticancer, antifungal therapy and for prophylaxis. The future of drug therapeutics may not lie in the development of new chemical entities but in the modification of the existing drug molecules using suitable carriers to eliminate toxicity and improve activity, the principle of new lives for old drugs.

REFERENCES

1. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol* 1965; 13:238–252.
2. Fielding MR. Liposomal drug delivery: advantages and limitations from a clinical pharmacokinetics and therapeutic perpective. *Clin Pharmacokinet* 1991; 21:155–164.
3. Akbarieh M, Besner JG, Galal A, Tawashi R. Liposomal delivery system for the targeting and controlled release of praziquantel. *Drug Dev Ind Pharm* 1992; 18:303–317.
4. Lidgate DM, Felgner PL, Fleitman JS, Whatley J. Invitro and in vivo studies evaluating a liposome system for drug solubilisation. *Pharm Res 1988; 5:*759–764.
5. Weiner N, Martin F, Riox M. Liposomes as drug delivery system. *Drug Dev Ind Pharm.*1989; 15(10):1523–1554.
6. Frezard F, Liposomes: from biophysics to the design of peptide vaccines. *Braz J Biol Res 1999;* 32(2):181–189.
7. Gregoriadis G, Ryman BE. Liposomes research in drug delivery. Eur. J Biochem 1972a; 24: 485.
8. Gregoriadis G, Ryman BE. Liposome research in drug delivery. Eur. J Biochem. 1972a; 24: 485.
9. Mathiowitz E et al, eds, Encyclopedia of controlled drug delivery, John Wiley & sons, New York, 1999; 461.
10. Felgnu PL, Ringold GM. Vesicular system: an overview. Nature 1994; 337: 387.
11. Gregoriadis G, Mccormack B. Targeting of drugs:Strategies for stealth therapeutic system. 1998, Plenum,New York.
12. Plantz GE. Antitumour effect of intratumoral injection of Liposomes. Proc Natl, Acad. Sci. USA, 1993; 90: 4645.
13. Elimi MM, Sarbolonki MN. Vesicular system an overview. Int. J. Pharm. 2001; 45: 215.
14. Sullivan SM, Huang L. Enhanced delivery to target cells by heat sensitive immunoliposomes. Proc. Natl. Acad. Sci. 1986; 83: 6117.
15. Bonte F, Puisieux F, Couvreur P, Delattre J, Devissagnet JP. Liposomes New system and new Trends in their applications. 1995; 75.
16. Alving CR. Vesicular System an overview. Proc. Natl. Acad. Sci. 1978; 75:2959.

17. Mathiowitz E et al, eds, Encyclopedia of controlled drug delivery, John Wiley & sons, New York, 1999; 461
18. Kim S. Vesicular System an overview. Biochem Biophys. Acta. 1983; 728: 338.
19. Gruner SM, Lenk RP, Janoff AS, Ostro MJ. Liposomes as carriers for polypeptides. Biochemistry 1985; 24: 2833.
20. Reevs JP, Dowben RM. Prepration of multivesicular Liposomes. J. Cell Physiol. 1969; 73: 49.
21. Sharma A, Sharma US. Encapsulation in Cationic Liposomes enhances antitumor activity. Int.J. Pharm 1997; 154: 123.
22. Rogers JA, Aderson KE. The Potential of Liposomes in oral drug delivery. *Crit Rev Ther Drug Carrier System 1998;* 13(5): 421–480.
23. Weiner N, Martin F, Riox M, Liposomes as drug delivery system. *Drug Dev Ind Pharm, 1989;* 15(10): 1523–1554.
24. Senior J, Gregoriadis G. Stability of small unilamellar liposomes in serum and clearance from the circulation: the effect of the phospholipid and cholesterol com-ponents. *Life Sci 1982;* 30: 2123–2136
25. Frezard F. Liposomes: from biophysics to the design of peptide vaccines. *Braz J Biol Res 1999;* 32(2): 181–189
26. Goldbach P, Herve B, Pascal W, Andre S. Sterile filtration of liposomes: retention of encapsulated carboxyfluorescein *Int J Pharm* 1995; 117: 225–230.
27. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol* 1965; 13: 238–252.
28. Olson F, Hunt CA, Szoka FC, Vail WJ, Papahadjopoulos D. Preparation of Liposoms of defined size distribution by extrusion through polycarbonate membranes. *Biochim Biophys Acta 1976;* 557: 9–23.
29. Johnson SM, Bangham AD, Hill MW, Korn ED. Single bilayer liposomes. *Biochim Biophys Acta 1971;* 233: 820–826.
30. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol* 1965; 13: 238–252.
31. Brunner J, Skrabai P, Hauser H. Single bilayer vesicles prepared without sonication, Physicochemical properties. *Biochim Biophys Acta 1976;* 455: 322–331.
32. Frezard F. Liposomes: from biophysics to the design of peptide vaccines. *Braz J Biol Res.* 1999; 32(2): 181–189.
33. Szoka F, Papahadjopoulos D. Procedure for preparation of Liposomes with large internal aqueous space and high capture by reverse phase. *Proceeding to the National Academy of Sciences 1978;* 75: 4194–4198.
34. Fielding MR. Liposomal drug delivery: advantages and limitations from a clinical pharmacokinetics and therapeutic perpective. *Clin Pharmacokinet 1991;* 21:155–164.
35. Vadiei K, Perez-Soler R, Lopez-Bernstein G, Luke DR. Pharmacokinetic and pharmacodynamic evaluation of liposomal cyclosporine. *Int J Pharm 1989;* 57: 125–131.
36. Hwang KJ. *Liposomes: From Biophysics to Therapeutics.* New York: Marcel Dekker Inc. 1987; 109
37. Al-Angary AA, Bayomi MA, Khidir SH, Al-Meshal MA, Al-Dardiri M. Characterisation, stability and invivo targeting of liposomal formulations containing cyclosporine. *Int J Pharm 1995;* 144: 221–225.
38. Wolff B, Gregoriadis G. The use of monoclonal anti-Thy1-IgG1 for the targeting of liposomes to AKR-A cells invitro and invivo. *Biochim Biophys Acta 1984;* 802: 259–273.
39. Torchilin V. Liposomes as targetable drug carriers. *CRC Crit Rev Ther Drug Carr Syst.* 1985; 2: 65–115.
40. Schafer-Korting M, korting HC, Braun-Falco O. Liposome preparations: A step forward in tropical drug therapy for skin disease? *J Am Acad Dermatol 1989;* 21:1271–1275.
41. Guo J, Ping Q, Sun G, Jioa C. Lecithin vesiclar carriers for transdermal delivery of cyclosporin A. *Int J Pharm 2000;* 194: 201–207.
42. Gamal MM, Maghraby E, Adrian C, Williams W. Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes in-vitro *J Pharm Pharmacol* 2001; 53: 1069–1077.
43. Gamal MM, Maghraby E, Adrian C, Williams Brain W. Skin hydration and possible shunt route preparation in controlled estradiol delivery from ultradeformable and standard liposomes. *J Pharm Pharmacol* 2001; 53: 1311–1322.
44. Lasch J, Wholrab W. Liposome-bound cortisol: a new approach to cutaneous therapy. *Biomed Biochim Acta 1986;* 45: 1295–1299.
45. Wholrab W, Lasch J, Taube KM, Woznak KK. Hautpermeation von liposomal incorporirtem hydrocortisone. *Pharmazie 1989;* 44: 333–335.
46. Wendel A, Ghyczy M. Liposomal cosmetics. *Soap Cosmet Chem Spectrosc 1990;* 6: 33–37.
47. Gabrijelcic V, Sentjurc M. Influence of hydrogels on liposome stability and on the transport of liposome entrapped substances into the skin. *Int J Pharm 1995;* 118: 209–212.
48. Cevc G, Blume G. Lipid vesicles penetration into intact skin owing to the transdermal Osmotic gradients and hydration force. *Biochim Biophys Acta 1992;* 1104: 226–232.
49. Cevc G, Schatzlein A, Blume G.Transdermal drug carriers: basic properties, optimization and transfer efficiency in the case of epicutaneously applied peptides. *J Control Rel* 1995; 36; 3–16.
50. Gabrijelcic V, Sentjurc M, Kristl J. Evaluation of liposomes as drug carriers into the skin by one-dimensional EPR imaging. *Int J Pharm* 1990; 62: 75–79.
51. Lavigne C, Yelle J, Sauve G, Thierry AR. Lipid base delivery of combinations of antisense oligodeoxynucleotides for the in vitro inhibition of HIV-1 replication. *Pharm Sci Tech 2003;* 3(1).
52. Oussoren C, Magnani M, Fraternale A, Casabianca A, Chiarantini L, Ingebrigsten R, Underberg WJM, Storm G. Liposomes as carriers of antiretroviral agent dideoxycytidine-5'-triphosphate. *Int J Pharm 1999;* 180: 261–270.
53. Senior J. Fate and behaviour of liposomes in vivo: a review of controlling factors. *CRC Crit Rev Ther Drug Carr Syst 1987;* 3: 123–192.
54. Gorin NC, Prentice HG, Meunier F, Ringden O, Tura S. Amphotericin B-liposome for confirmed opportunistic deep mycoses in patients with leukemia. 1990.
55. European Patent nº 1.030.653 B1, Cruz MEM, Carvalheiro MC, Jorge JCS, 2005.
56. Cruz E, Carvalheiro M, Jorge J, Eleutério C, Sousa A, Croft S. *Parassitologia 2005;* 47 (Suppl.1) 81.
57. Corvo ML, Jorge JC, van't Hof R, Cruz MEM, Crommelin DJA, Storm G. *Biochim Biophys Acta 2002;* 1564: 227-236.
58. Cruz MEM, Gaspar MM, Martins MB, Corvo ML. *Methods in Enzymology,* Liposomes, Part E 2005; 391: 395-413.
59. Gregoriadis G. *Fate of Injected Liposomes: Observations on Entrapped Solute Retention, Vesicle Clearance and Tissue Distribution in vivo*, in *Liposomes as Drug Carrier: Recent Trends and Progres* Wiley Chichester 1988; 3–18.
60. Gregoriadis G. Overview of liposomes. *J. Antimicrob. Chemother 1991;*28: 39–48.
61. Gregoriadis G. Florence AT. Liposomes and cancer therapy. *Cancer Cells 1991;* 4: 144–146.
62. Gregoriadis G. Overview of liposomes, *J. Antimicrob. Chemother1991:* 28: 39–48.
63. Mayer LD, Tai LC, Bally MB, Mitilenes GN, Ginsberg RS, Cullis PR. Characterization of liposomal system containing doxorubicin entrapped in response to pH gradients. *Biochim. Biophys Acta 1990;* 1025**:** 143–151.
64. Olson F, Mayhew E, Maslow D, Rustum Y, Szoka F. Characterization, toxicity and therapeutic efficacy of adriamycin encapsulated in liposomes. *Eur. J. Cancer Clin. Oncol 1982;*18**:** 167–176.
65. Herman EH, Rahman A, Ferrans VJ, Visk JA, Schien PS. Prevention of chronic doxorubicin cardiotoxicity in beagles by liposome encapsulation. *Cancer Res 1983;* 43**:** 5427–5432.
66. Van Hoesel CM, Steerenberg PA, Crommelin DJA, Van Dijk A, Wild DJ, Hillen FC. Reduced cardiotoxicity and nephrotoxicity with preservation of antitumor activity of doxorubicin entrapped in stable liposomes in the LOU/M Wsl rat. *Cancer Res 1984;* 44: 3698–3705.
67. Lasic DD, Frederic PM, Stuart MCA, Berenholz Y, McIntosh IT. Gelation of liposomes interior: novel method of drug encapsulation. *FEBS Lett.1992;* 312: 255–258.

68. Barenholz Y, Haran G. Loading and controlled-release of amphiphilic pharmaceuticals to and from liposomes. Eur. Pat. *Chem. Abstr.1990;* 114: 12-18.
69. Gabizon A, Catane R, Uziely B. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene glycol-coated liposomes. *Cancer Res 1994;* 54: 987–992.
70. Muggia FM, Hainsworth JD, Jeffers S, Miller P, Groshen S, Tan M, Roman B, Uziely Burnett A, Greco FA, Morrow CP, Paradiso LP, Liang LJ. Phase II study of liposomal doxorubicin in refractory ovarian cancer: antitumor activity and toxicity modification by liposomal encapsulation. *J. Clin. Oncol 1997;* 15: 987–993.
71. Vaage J, Donovan D, Uster P, Working P. Tumor uptake of doxorubicin in polyethylene glycol-coated Liposomes and therapeutic effect against a xenografted human pancreatic carcinoma. *Br. J.Cancer 1997;* 75: 482–486.
72. O'Shaughnessy JA. Pegylated Liposomal Doxorubicin in the treatment of breast cancer, *Clin. Breast Cancer 2003;* 4: 318–328.
73. Chao TC, Wang WS, Yen CC, Liu JH, Chen PM. A dose-escalating pilot study of sterically stabilized, pegylated liposomal doxorubicin (Lipo-Dox) in patients with metastatic breast cancer. *Cancer Invest 2003;* 21: 837–847.
74. Arndt D, Zeisig R, Eue I, Sternberg B, Fichtner I. Antineoplastic activity of sterically stabilized alkyl phosphocholine liposomes in human breast carcinomas. *Breast Cancer Res. Treat 1997;* 43: 237–246.
75. 75)Horowitz A, Tzemach D, Gabizon A. Pharmacological studies of cisplatin encapsulated in long-circulating liposomes in mouse tumor models. *Anticancer Drugs* 1999; 10: 911–920.
76. Hodges NA, Mounajed R, Olliff CJ, Padfield JM. The enhancement of neomycin activity on Escherichia coli by entrapment in liposomes. *J. Pharm. Pharmacol 1979;* 31: 85.
77. Chowdhury MKR, Goswami R, Chakbarti P. Liposomes trapped penicillins in growth inhibition of some penicillin resistant bacteria. *J. Appl. Microbio 1981;* 51: 223–226.
78. Stevenson M, Baillie AJ, Richards RME. Antimicrobial activity of liposome entrapped Chloramphenicol. *J. Pharm. Pharmacol 1981;* 33: 31–36.
79. 79).Morgan JR, Williams KE. Preparation and properties of liposome associated gentamicin, *Antimicrob.Agents Chemother 1980;* 17**:**544–548.
80. Cabanes A, Reig F, Garcia-Anton JM, Arboix M. Evaluation of free and liposome-encapsulated gentamycin for intramuscular sustained release in rabbits. *Res. Vet. Sci 1998;*64: 213– 217.
81. Lutwyche P, Cordeiro C, Wiseman DJ, St-Louis M, Uh M, Hope MJ, Webb MS, Finlay B.B.. Intracellular delivery and antibacterial activity of gentamicin encapsulated in pH-sensitive liposomes. *Antimicrob Agents Chemother 1998;* 42: 2511–2520.
82. Agarwal A, Kandpal H, Gupta HP, Singh NB, Gupta CM. Tuftsin-bearing liposomes as rifampin vehicles in treatment of tuberculosis in mice. *Antimicrob. Agents Chemother1994;* 38: 588–593.
83. Onyeji CO, Nightingale CH, Nicolau DP, Quintiliani R. Efficacies of liposome-encapsulated clarithromycin and ofloxacin against Mycobacterium avium-M intracellular complex in human macrophages. *Antimicrob. Agents Chemother 1994:* 38:523–527.
84. Cynamon MH, Klemens SP, Swenson CE. TLC G-65 in combination with other agents in the therapy of Mycobacterium avium infection in beige mice. *J. Antimicrob. Chemother 1992;* 29: 693–699.
85. Webb MS, Boman NL, Wiseman DJ, Saxon D, Sutton K, Wong KF, Logan P, Hope MJ. Antibacterial efficacy against an *in vivo* Salmonella typhimurium infection model and pharmacokinetics of a liposomal ciprofloxacin formulation. *Antimicrob Agents Chemother1998;* 42: 45–52.
86. Stevenson M, Baillie AJ, Richards RM. Enhanced activity of streptomycin and chloramphenicol against intracellular Escherichia coli in the J774 macrophage cell line mediated by liposome delivery. *Antimicrob. Agents Chemother 1983;* 24: 742–749.
87. Khalil RM, Murad FE, Yehia SA, El-Ridy MS, Salama HA. Free versus liposome-entrapped streptomycin sulfate in treatment of infections caused by Salmonella enteritidis. *Pharmazie 1996;* 51: 182–184.
88. 88)Gangadharam PR, Ashtekar DA, Ghori N, Goldstein JA, Debs RJ, Duzgunes N. Chemotherapeutic potential of free and liposome encapsulated streptomycin against experimental Mycobacterium avium complex infections in beige mice. *J. Antimicrob. Chemother 1991;* 28: 425–435.
89. Gangadharam PR, Ashtekar DR, Flasher DL, Duzgunes N. Therapy of Mycobacterium avium complex infections in beige mice with streptomycin encapsulated in sterically stabilized liposomes. *Antimicrob. Agents Chemother 1995;* 39: 725–730.
90. Mahato RI, Rolland A, Tomlinson E. Cationic lipid-based gene delivery systems: Pharmaceutical perspectives. *Pharm. Res.1997;* 14: 853–859.
91. Crystal RG. Transfer of genes to humans: early lesions and obstacles to success. *Science 1995;* 270: 404–410.
92. 92)Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, Northrop JP, Ringold GM, Danielsen M. Lipofectin: a highly efficient, lipid mediated DNA transfection procedures. *Proc. Natl. Acad. Sci. USA 1987;* 84: 7413–7417.
93. Felgner PL, Zangg RH. Norman JA Synthetic recombinant DNA delivery for cancer therapeutics. *Cancer Gene Ther 1995;* 2: 61–65.
94. 94)Felgner PL, Ringold GM. Cationic Liposome-mediated transfection. *Nature 1989;* 377: 387–388.
95. Gao X, Huang L. Cationic liposome mediated gene transfer. *Gene Ther 1995;* 2: 710–722.
96. Ishiwata H, Suzuki N, Ando S, Kikuchi H, Kitagawa T. Characteristics and biodistribution of cationic liposomes and their DNA complexes. *J. Contr. Rel 2000;* 69: 139–148.
97. Kim A, Lee ES, Choi SH Kim CK. *In vitro* and *in vivo* transfection efficiency of a novel ultradeformable Cationic Liposome. *Biomaterials 2004;* 25: 305–313.
98. Yoshida J, Mizuno M, Fujii M, Kajita Y, Nakahara N, Hatano M, Saito RM, Nobayashi M, Wakabayashi T. Human gene therapy for malignant gliomas (glioblastoma multiforme and anaplastic astrocytoma) by *in vivo* transduction with human interferon beta gene using cationic liposomes. *Human Gene Ther 2004;* 15: 77–86.
99. Lasic D. Liposomes – an industrial view. *Chem. Ind;* 1996: 210–213.
100. Tomlinson E, Rolland AP. Controllable gene therapy: pharmaceutics of non viral gene delivery systems. *J. Contr. Rel 1996;* 39: 357–372.
101. Sharma A, Sharma US. Liposomes in drug delivery: progress and limitations. *Int. J. Pharm 1997;* 154: 123–140.
102. ZouY, Zong G, Ling YH, Perez-Soler R. Development of Cationic liposome formulations for intratracheal Gene therapy of early lung cancer. *Cancer Gene Ther 2000;* 7: 683–696.
103. Gregoriadis G, Saffie R, De Souza JB. Liposome-mediated DNA vaccination. *FEBS Lett*.1997; 402: 107–110.