International Journal of Basic and Applied Biology
p-ISSN: 2394-5820, e-ISSN: 2349-2539, Volume 3, Issue 2; April-June, 2016, pp. 92-95

http://www.krishisanskriti.org/Publication.html

Application of Biotechnology: Liposome Application and Limitation an Overview

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Abstract—*Now a day with the rapid advancement in biotechnology the focus on discovering new things has moved from macroscopic to a microscopic level. Discovered by Alec Bingham in 1961 liposome, a bilayer cell organelle has many potential to be used in many places especially for drug delivery. The Liposomes are concentric bilayer vesicle like structure which used to entrap aqueous volume completely. The Liposome helps in encapsulating the biomolecules which can be easily degraded under natural environmental condition. The applications of liposomes include drug targeting, tropical drug delivery, and treatment of human immunodeficiency virus (HIV) infections, plant transport processes, gene therapy and veterinary sector and in the food industry. The limitation includes stability, sterilization, liposomal degradation, gene therapy, encapsulation efficiency*

1. INTRODUCTION

Liposomes are microscopic, spherical, concentric bilayer vesicle like structure made of up natural or synthetic phospholipid which can entrap aqueous volume completely and widely used in drug delivery. Description of liposome was 1stgivenby Alec D Bangham one British haematologist at the Babraham institute in 1961 later published in 1964. Liposome was discovered by Bhingham and R. W. Horne while testing their institute's new electron microscope by adding negative strain to the dry phospholipid.Weissmann discussed with Bangham in Cambridge pub and 1st named the structure as liposome after lysosome. The structure seen by microscope seemed resemblance to plasma lemma and it was the 1st evidence regarding bilayer lipid structure of cell membrane. Liposome found to be structurally similar with naturally occurring cell therefore found to be epitome drug carrier. Liposomal products take a long to catch up the market because it is difficult to scale-up by conventional preparation method. Phospholipid and cholesterol are main structural component of liposome. Phospholipids are known as amphipathic molecules because they have the affinity for aqueous and polar molecules due to their hydrophilic and hydrophobic tail. The head portion is polar and constitute of 2 fatty acid chains which contain 10-24 Carbon atoms and each chain contain 0-6 double bonds. Cholesterol is not helpful in forming a bilayer structure; it only functions as fluidity buffer (Sipai et al. 2012)

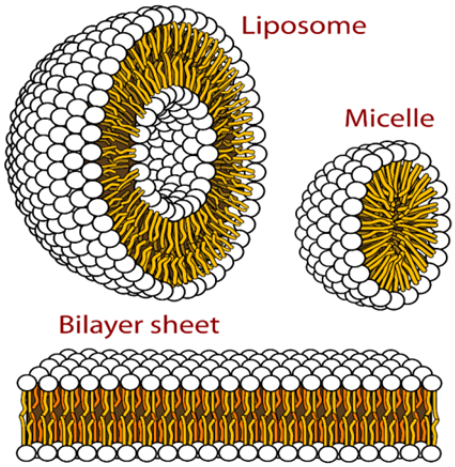

Fig. 1: Showing liposome Source: enwikipedia.org

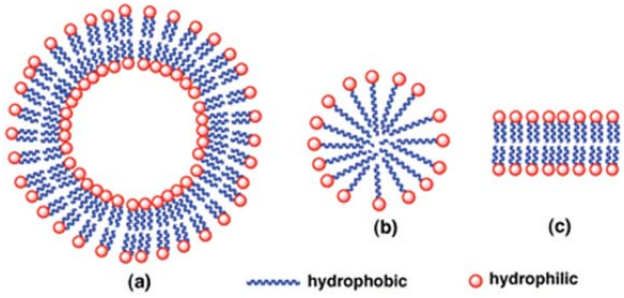

Fig. 2: Arrangement of hydrophobic and hydrophilic head; Source: pubs.rsc.org

2. APPLICATION OF LIPOSOMES

2.1. Drug Targeting and Drug delivery

Previously when drugs were given to any individual that circulates through the entire body whereas the application of drug were only required at one particular site of body and not throughout the body. This was the main reason for why drug targeting comes into mind. The drug targeting distribute drug to specific site of body thus enhance the efficiency of drugs and lowers down the level of toxicity of drugs. Liposomes can be treated as drug targeting agent for the compounds like antibodies, sugar residues, Apo proteins or hormones. Liposomes are now being created to contain specific immunogens and have been tested a number of times for treating cancer and other diseases (Lofthouse, 2002). Chemotherapeutics was dangerous before due to lack of proper tumor-to-normal tissue drug ratios but cancer treatment now can be done by the help of liposome (Thomas et al. 2005) Liposomes are not suitable for ingestion because of breaking down in the stomach and intestines. The liposomes can be used for various skin problems. It can lower the amount of drugs thus reduces the side effect. It increase skin permeability for numerous entrapped drugs .It has a number of application both in cosmetics and skin care preparations A number of antiretroviral nucleotide equivalents has been entering into market to fight against the disease AIDS. The role of antiviral drugs is to combat replication HIV and to inhibit reverse transcriptase by viral DNA synthesis. Excess use and toxicity of drugs can be reduced by encapsulating the drug by the help of liposome. Preferential up take of drugs leads to greater worth of the liposomal formulation (Oussoren et al. 1999).

2.2. Liposomes and Gene Therapy

Liposomes can entrap and deliver genetic material. Cationic liposomes are most suitable for gene therapy. The key obstacle that is to be overcome is that the entrapped genetic material should continue its function and simultaneously lowering its susceptibility of DNA & RNA to nucleases and design of liposomes. They should fuse on recognition of the target cell as they have longer shelf life (Chonn et al., 1998). It allows DNA plasmid condensation and leads to formation of highly structured DNA and protects against degradation. The diseases like cystic fibrosis, viral diseases, and some cancers could be effectively treated when DNA trapped in SUVs fused when the target cell was encountered(Chen et al., 2000).Liposomes were tagged with viral envelope protein so that they can recognize and get attached to the target cell membrane. Genetic material encapsulated within liposome can be up taken easily and thereby on fusion ensures high therapeutic delivery to cell.

2.3. Non-Food Agricultural Uses of Liposomes

Studies has been conducted on the application of liposomes in agriculture i.e. plants and animals. Liposomes are primarily used as a model or control membrane system. But reagent and drug delivery system had also been developed.

2.4. Liposomes for diagnostic imaging

Liposomes are used in diagnostic imaging such as MRI (magnetic resonance imaging), CTI (computed tomography imaging) and sonography. Diagnostic agents can be incorporated in liposome by combining metal with suitable chelating compound. The metal incorporation enhances the contrast proprieties and improves diagnostic imaging technique (Torchilin et al. 2000).

2.5. Application of Liposome on Plant cell

Plant aging and drying tolerance properties of plant can be improved through the application of liposome. Liposome application can be done in plant for freeze tolerance. Antifreeze proteins protect the cells from cold stress by retarding the ice recrystallization upon thawing of frozen solutes. These antifreeze proteins are produced by many plants. Antifreeze proteins induce membrane-lipid dependent fusion and these can be entrapped in liposome. Liposome can be prepared by varying di galactosyl di glyceride (DGDG), mono galactosyl di glyceride (MGDG) and PC composition. Liposome helps in plant transport process. The applications include studying the transport of solutes across cellular membranes, explicate the mechanism of toxin and antimicrobial activity, study the mechanism of action in pesticides, and deliver therapeutic substances to farm. (Taylor et al. 2005)

2.6. Liposomes as vaccines

Liposome based vaccines are capturing the clinical market and human trials are also done now a days. Liposomes are used to carry antigens and it makes hypersensitivity reaction. Components of liposome prevent their DNA from attack of deoxyribonuclease**.** For vaccine development it is required to deliver antigen and other required molecules into the cytosol of target cells. It can be done through the application of liposome based model. It has been observed that yeast-lipid when fused with macrophages it leads effective deliver of the entrapped solutes into the cytoplasmic compartment. It has been observed from antigen ovalbumin model entrapped in the yeast lipid liposome can facilitate $CD8^+$ T cell responses which are antigen reactive (M.Owais et al. 2000)

2.7. Liposomes in Veterinary application

Liposomes are used with suitable agents to cure cat disease like mammary adenocarcinoma, squamous cell carcinoma, chronic rhinitis. Analgesia, malignant histiocytosis, Leishmaniosis, atopic dermatitis, blastmycosis, oral melanoma, osteosarcoma like dog diseases are cured with the help of liposome. Cattle disease like brucellosis, leukaemia, anti-inflammatory and analgesic, Birds disease like arthritis can be cured with the application of liposome (C. Underwood et al. 2012)

2.8. Liposomes in the Food Industry

In food industries liposomes are used for encapsulation purpose. Encapsulation of sugar, protein, minerals, vitamins and anti-oxidant is done in micro or nano-encapsulation form. In food sector acids, bases, flavors, nutrient like compounds are encapsulated by using liposomes (Gibbs et al., 1999). Liposome is now used in making of cheese and food emulsion like spreads, margarine and mayonnaise Controlled release of food material can be done by using liposome. Sensitive components of food can be encapsulated by using liposome. By encapsulation shelf life and stability of sensitive food components can be enhanced. (Mozafari et al. 2008)

3. LIMITATION OF LIPOSOMES

There are some of the limitations of liposome enlisted below.

3.1. Stability

Stability is one of the major problems associated with liposomes. It is both physically and chemically unstable therefore they have shorter shelf lives. It is chemically unstable due to hydrolysis of ester bonds or unsaturated acyl chains oxidation or both may be the cause. Drug leakage and fusion of vesicles are the prime cause of physical instability. To enhance shelf life and improve its stability lyophilisation process is done and material is preserved under stable dry state. Liposome products are entering into market for clinical trial as a lyophilized powder. AmBisome TM is one of the examples of lyophilized powder of liposome (Sharma et al. 1997). Liposome multi-layered made up of chitosan and sodium alginate prevents it from environmental stress like pH, and storage condition (liu et al, 2016). In addition to above control release of drugs from liposome can be improved by inserting stability anchor like bola type copolymers of pH sensitive kind into liposome (Hao et al. 2015).

3.2. Sterilization

To find a suitable method for sterilisation of liposome is one of the major challenges faced in the field of liposome. The available method for sterilisation of liposome is filtration of liposome through 0.22 µm sterilized membranes. It cannot be used for vesicles of larger diameter (>0.2 µm).Sterilisation cannot be done by irradiation method or by using any chemical agents because it leaves trashes of toxic contaminants. Sometimes liposomes are sterilised by autoclave method but it leads to phospholipid degradation and loss of content (Zuidam et al. 1995). In addition to above sterilisation techniques gamma-irradiation sterilisation, saturated steam sterilisation, dry heat sterilisation, Ethylene oxide sterilisation, Aseptic manufacturing, Dense gas techniques are employed for sterilisation of liposomes. But they have their own limitations for sterilising liposomes. Now days it was thought that combination of filtration, aseptic manufacturing, and dense gas technique to produce liposome can solve many problems but it may be time consuming. Hence still there are search for techniques, which is efficient, maintain physicochemical stability, and cost effective (Toh et al. 2013).

3.3. Encapsulation efficiency

Researches are still going on to improve encapsulation efficiency. Active loading sometimes used to enhance the encapsulation efficiency. Whereas it cannot be applied for hydrophobic drugs like paclitaxel whose encapsulation efficiency is less than 3 mole %. This happens due to the lower affinity of drugs for the lipid bilayers (Straubinger et al. 1995). Now day's micro-encapsulation vesicle method is employed to increase the efficiency of encapsulation of drugs (Nii et al. 2005)

3.4. Lysosomal degradation

It is not possible always for the cells to uptake the drug at the site of action. If the immune liposome arriving the cells cannot release the entrapped drug then it will be ultimately ruined in lysosomes (Gregoriadis et al. 1995).

3.5. Gene therapy

Transfection ability is more in case of viral vectors than liposomes. Again the DNA-lipid compounds are unstable due to their particle size over a long time. Liposomes lack in vivo targeting therefore the toxicity of cationic lipids confines the directed dose of DNA lipid complex. It has been noticed that plasmid-liposomes complexes are more suitable for distribution of genetic material by supervision (Gao et al. 1995)

3.6. Active Targeting

The efficacy is determined by the amount of drugs present in liposome and the volume reaching the target molecules. Immuno liposomes can convey the drug to the selective site of cells but the diffusion into the cytoplasm in required amount is not guaranteed which is the prime requirement for pharmaceutical operation (Amarnath et al. 1997). Active targeting shows marginal improvement in therapeutic application compared to passive targeting liposome system due to poor penetration, obstruction of binding sites (Caracciolo et al. 2015).

4. CONCLUSION

Application of Liposome is greatly increasing day by day. The active target property of liposome makes it unique and helping it in drug delivery. It was first used for drug delivery but now in gene therapy, diagnostic imaging, food sector, everywhere liposome is used. Stability and encapsulation efficiency is the major limitations of liposome research are going on to improve application of liposome.

REFERENCE

[1] Sipai Altaf Bhai M.,Vandana Yadav., Mamatha.Y., Prasanth V.V., "Liposomes: An Overview",1(1),*Journal of Pharmaceutical and Scientific Innovation,*2012, pp.13-21.

[2] Lofthouse, S. "Immunological aspects of controlled antigen delivery".54 *Advanced Drug Delivery Reviews,* 2002, pp.863–870.

[3]Thomas,L,Andresen.,Simon,S,Jensen.,Kent,Jørgensen., "Advanced strategies in liposomal cancer therapy: Problems and prospects of active and tumor specific drug release", 44, *Progress in Lipid Research* ,2005, pp. 68–97.

[4] Oussoren,C.,Magnani M., Fraternale A., Casabianca A., Chiarantini L., Ingebrigsten R., Underberg, WJM., and Storm, G., "Liposomes as carriers of antiretroviral agent dideoxycytidine- 5'-triphosphate",180, *Int J Pharm*, 1999, pp.261–270.

[5] Chen, D., Cole, D.L., and Srivatsa, G.S., "Determination of free and encapsulated oligonucleotides in liposomes formulated drug product", 22, *Journal of Pharmacology and Biomedical Analysis,* 2000 pp.791–801.

[6] Chonn, A., and Cullis, P.R., "Recent advances in liposome technologies and their applications for systemic gene delivery",30, *Advanced Drug Delivery Reviews*, 1998, pp.73–83.

[7] Torchilin VP. "Polymeric contrast agents for medical imaging", 1, *Curr Pharm Biotechnol*, 2000,pp.183–215.

[8] T. Matthew, Taylor., and P. Michael, Davidson., "Liposomal Nanocapsules in Food Science and Agriculture", 45, *Critical Reviews in Food Science and Nutrition,* 2005 pp.587–605.

[9] Owasis,M., and Gupta,C.M., "Liposome-mediated cytosolic delivery of macromolecules and its possible use in vaccine development", 267(13),*European Journal of Biochemistry*, 2000, pp.3346-3956

[10] Underwood,C., Van Eps, A.W., "Nanomedicine and veterinary science: The reality and the practicality", 193, *The Veterinary Journal*, 2012, pp.12-23.

[11] Gibbs, B.F., Kermasha, S., Alii, I., and Mulligan, C.N. "Encapsulation in the food industry: A review",50, *International Journal of Food Science and Nutrition*, 1999, pp. 213–224.

[12] M, Reza, Mozafari., Chad, Johnson., Sophia, Hatziantoniau., and Costas, Demetzos.,"Nano liposomes and Their Applications in Food Nanotechnology", 18(4), *Journal of liposome research*,2008.

[13] Sharma, A., Mayhew, E., Bolcsak, L., Cavanaugh, C., Harmon, P., Janoff, A., Bernacki, R.J., "Activity of paclitaxel liposome formulations against human ovarian tumor xenografts", 71, *Int. J. Cancer,*1997, pp.103- 107.

[14] Weilin, Liu., Wei Liu., Aiqian Ye., Shengfeng Peng., Fuqiang, Wei., Chengmei, Liu., Jianzhong, Han., "Environmental stress stability of microencapsules based on liposomes decorated with chitosan and sodium alginate", 196, *Food Chemistry,* 2016, pp.396-404.

[15] Weiju, Hao., Xia, Han., Yazhuo, Shang., Shouhong, Xu., Honglai, Liu., "Insertion of pH-sensitive bola-type copolymer into liposome as a "stability anchor" for control of drug release" 136, *Colloid and surfaces B: Biointerfaces,* 2015,pp. 809-816.

[16]Zuidam, N.J., Lee, S.S., Crommelin, D.J., "Gamma irradiation of non-frozen, frozen, and freeze-dried liposomes",12, *Pharm Res,*1995 pp.1761-1768.

[17] Ming-Ren, Toh., Gigi, N.C. Chiu., "Liposomes as sterile preparations and limitations of sterilisation techniques in liposomal manufacturing", 8, *Asian Journal of Pharmaceutical sciences,*2013,pp.88-95.

[18] Straubinger, R., Sharma, A., Mayhew, E., "Taxol Formulation", US Patent 5415869, 16 May, 1995.

[19] Tomoko, Nii., Fumiyoshi, Ishii.,"Encapsulation efficiency of water-soluble and insoluble drugs in liposomes prepared by the microencapsulation vesicle method", 298 (1) , *International Journal of Pharmaceutics,*2005,pp.198-205.

[20] Gregoriadis, G. "Engineering liposomes for drug delivery: progress and problems", 13 *Trends Biotechnol*, 1995, pp.527-537.

[21] Gao, X., Huang, L.,"Cationic liposome-mediated gene transfer",2,*GeneTherapy*,1995,pp.710-722.

[22] Amarnath, Sharma., Uma, S. Sharma., "Liposomes In Drug Delivery: Progress and Limitation", 154,.*Int.J. Pharm*, 1997, pp.123-140.

[23] Giulio, Caracciolo. "Liposome–protein corona in a physiological environment: Challenges and opportunities for targeted delivery of nanomedicines", 11, *Nanomedicine: Nanotechnology, Biology, and Medicine*, 2015, pp.543-557.