Asian Journal of Pharmaceutical Technology and Innovation ISSN 2347-8810

Liposome as a drug delivery system: in nano bio electronics.

Marjan Assefi *

Department of NanoScience and Nano engineering, University of North Carolina, Carolina, USA

Abstract

Nowadays, with the world wide spread of different cancers. There is an urgent need in new treatment methods, and medications. With the advent of drug delivery methods, we have come to a new era of treatment. Here, we come to the fact that the liposome will open new open new doors to the scientific experiments. Different methods in nano bioelectronics and the meaning of Nano science and Nano engineering have been discussed. Liposomes (Bottom up) are used in drug delivery system at nano bio electronic department. It has polar and nonpolar region. Drugs are going to insert into the liposomes. In this paper, they are going to tell what liposomes are, different strategies to enter liposomes to the target cells, how it can be made, and interactions between liposomes and cell membrane. I am going to give another method to syntheses liposomes.

Keywords Liposomes, Drug delivery, cell interaction, synthesis, cell membrane, Nano bio electronics.

Accepted on October 26, 2020

Introduction

Nano bio electronic and nano engineering have been introduced in recent decades. The royal society of the UK makes the distinction between nano science and nano engineering.

1) Study and manipulation of nano scale particles and systems.

2) Design, characterization, and production of structures devices, and systems.

Nano science and nanotechnology have researches and technology development at the atomic, molecular, or macromolecular level. The unique physical properties of nano scale solids in conjugation with the remarkable recognition capabilities of bio molecules could lead to miniature biological electronics and optical devices including biosensors, probes like liposomes. Sequence specific DNA detection is important because its application in the diagnosis diseases or for drug delivery used. Nano bio electronics create structures at nano scale devices. There are different methods:

Top-Down method

In top down approach they use the natural and manmade chunks or blocks that are cast, sewed or machined into formed products, small or large, ranging from integrated circuits to jumbo jets. The methods that produce nano particles from balk material include high energy, ball milling, chemistry based solution, mechanochemical processing, etching, sonication, and physical chemical vapor deposition.

Bottom up Approach

Manufacturing of nano materials by manipulating and organizing individual atoms and molecule into particular configurations in a fashion is called bottom up approach. The self-assembly lipid molecules into nano structure such as liposomes can be considered as a classical example on the bottom up approach for constructing nano materials. Lipids are amphiphilic molecules, hydrophobic and hydrophilic, one part of molecule is water hating and the other part is water loving [1]. Lipids interactions of the hydrophobic segments of the molecule with water solvent result in self-assembly of lipids; it often appears in the form of liposomes. Liposome is an aqueous core surrounded by a lipid bilayer, consists liposomes. It is like a membrane; it separates the inner aqueous core from the outside bulk. Banghan and his coworkers in 1961 discovered them and called them as swollen phospholipid bilayer structures; bangosomes and finally liposomes, which was taken from Greek words; the “Lipos” meaning fat and “Soma” meaning body. The liposome is going to make by hydration of a dry phospholipid film on the top of the main phase transition temperature. Liposomes are in different kinds which depend on their sizes and their number of lipids [2]. The liposome features are related to the preparation method. Sometimes mechanical agitation is required. For controlling over the size and structure of the formed liposomes; increase the entrapment of the desired molecules. The important aspect is the use of organic solvent which should have a freezing point above the temperature of condenser of the freeze-drying, and pay attention to rubber seals of commercial lyophilizes. Lipids are forming liposomes maybe natural or synthetic. Polymers permit a new generation of liposomes. Liposomes are biocompatible, and biodegradable, no matter if natural or synthetic lipids or polymers compose them. Because of this

property, they are suitable for biomedical research. Liposomes natural and unique feature is that they can compartmentalize and solubilize both hydrophobic and hydrophilic substances. Liposomes can couple with biodegradability and biocompatibility. This unique property makes liposomes a very interesting drug. Liposomes can be used as drug delivery vehicles. Liposomes have a great valuable role in treatment of certain tumors in the lymphatic [3].

Synthesize of Liposomes

To prevent leakage from liposomes; there are different methods: For choosing the method, some steps should be considered;

The physicochemical characteristics of the entrapped liposomal ingredients

The nature of the medium

The effective concentration of the encapsulated material and its toxicity

Additional processes (delivery of liposome)

Shelf-life of liposomes, optimum size, polydispersity for the intended application

batch to batch reproducibility, and possibility of large scale production of safe and efficient liposomal products [1].

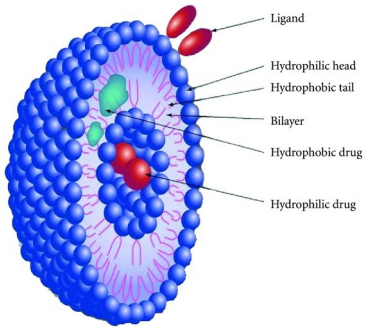

Figure 1. Liposomal drug delivery

To determine the circulation half-life of liposomes in drug delivery, the liposome size is a crucial factor; the amount of encapsulated drug is related with the size and number of bilayers of the prepared liposome, and different liposome preparation methods can be employed, regarding the desired formation; the main difference in these methods is to overcome the low and solubility of lipids in water, so This method can be classified as mechanical agitation, solvent evaporation [1].

Mechanical agitation

Lipids are directly solubilized in water, through the use of probe sonication. It is on the simplest methods to prepare liposome, but small liposome which are unstable because of their size, and have drawn back potentials, it is impossible to remove completely the risk of lipid degradation by contact with the hot probe [1]. The advantage is the exclusion of use of organic solvents as described in the method. With mechanical agitation prepared liposomes are not suitable because of their size instability and high leakage of encapsulated drugs.

Solvent evaporation

This method consists of four major steps; 1) The solubilization of the lipid (Hydrophobic compound) in an organic solvent, 2) Solvent evaporation, 3) hydration with a buffer (Hydrophilic Compound), 4) If needed, getting unilamellar liposomes from the obtained multi lamellar one. The aqueous volume within inside these lipid membranes is a very small proportional of total volume used for preparation [1]. During the preparation, large amount of water soluble drug is wasted. Lipid soluble drug can be encapsulated with 100% efficiency. In the presence of neutral lipids, the effect of charging lipids in the membrane can also be achieved by freezing and thawing repeatedly the obtained liposomes. 30% volume can be achieved. It can be increased at higher lipid concentration. The freeze-thaw procedure shows a dramatic change in liposome morphology which is followed by freeze- fracture electron micrographs [1]. Before freeze- thawing, there are “onions skin” arrangements of concentric bilayers. New structures are observed after a few freeze-thaw steps where interlamellar spaces are increased.

Solubilization of the lipid

Preparing an organize solution of membrane lipids to ensure complete and homogenous mixing of all the components [1]. Compounds (Lipid soluble) should be added to the organic solution. Compounds should be entrapped in the aqueous compartment of liposomes should be dissolved in the aqueous environment. In this method, first we dissolve phospholipids in an organic solvent with lipid soluble compounds.

Solvent evaporation

Let the solvent to evaporate in a glass- container; or the best using a rotary evaporator connecting to a vacuum pump. In this way, we get a thin film of the lipid on the walls of a round bottom flask. To increase encapsulation, we should start with a large volume round bottom flask [1]. Lipids will dry down onto a large surface area possible to form vacuum pump and introduce it to nitrogen. Then we remove the container, and fix a lyophilized, or we can expose it to high vacuum overnight to remove the residual solvent [1].

Strategies for liposomal targeting to tumors

We have active and passive targeting. Passive Targeting: Tumor associated blood vessels have a leaky nature. The mechanisms that are used by PEG are; 1) Preventing opsonization [4]. 2) Enhancing the repulsive interaction between polymers coated liposomes and blood components. 3)Increasing surface hydrophilicity and forming a polymeric layer over the liposome surface in order to hand in them to large molecules of opsonins. Binding a PEG like polymers on the surface of liposomes reduces uptake by the macrophages of mononuclear phagocytic system and prolongs another important parameter. That impacts passive targeting through

EPR effect is the size of the liposomes. The gathering and accumulation of liposome in the tumor depends on the size of the endothelial gaps in the capillary vasculature for a particular cancer. To have better use of EPR effect, the liposomes should be smaller than 400 nm in size [4]. The threshold vesicle size of approximately 400 nm for extravasation into tumors. Other factors that influence passive targeting are the composition and charge on the surface of liposomes. Their positive surface charge leads to non-specific interactions with the anionic species in the blood. A) Active Targeting: Actively targeted liposomes are designed with the goal of reducing off target effects. Certain receptors such as folate and transferrin receptors are over expressed on many cancer cells. Long circulate actively targeted liposomes in the tumor vasculature through passive targeting; it results in endocytosis into the cells through the targeting moiety, enhancing uptake and the therapeutic effect. PH sensitive liposomes are more effectively than non PH sensitive liposomes [5]. They have high affinity to adhere cell membrane [Figure 1]. As the PH decreases, the carboxylic group of the amphiphilic reduces their stabilizing effect, so PH sensitive liposomes can lead to invert to hexagonal phase. Most of the Liposomes contain the derivatives of “PE”, “C-DopE”, “C-Dops”. Liposomes with these qualifications exhibit the ability to form non-bilayer phase. PH sensitive liposomes that contain AcylPhosphatidylethanolamine can not only release their encapsulated contents at low PH values, but also they can promote the fusion of the cell membrane. When we add PEG to the surface of the liposomes [Figure 1], it reduces the RES and prolongs the liposomes circulation duration in the system. When we insert the PH sensitive peptide such as “GALA”, “INF” a kind of novel PH sensitive liposomes is developed. The peptide or protein remains inactive in the neutral PH environment. In acidic environment, the conformation changes, and will cause promotion of the fusion between liposomal membrane and cell membrane. The PH sensitive liposomes release the encapsulated items. An experiment in mouse reported if we have direct intra tumor injection into the solid tumors of B16 melanoma, the PH sensitive liposomes can improve the control of the tumor growth rates.

Liposomal Cell Interaction

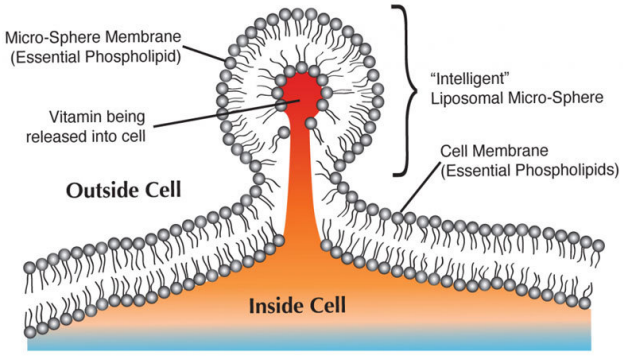

Figure 2. Interaction of Liposomes and cell membranes

Hydrophobic drugs penetrate inside the bilayer of liposome and hydrophilic drugs. Then they get entrapped inside the aqueous core at the bilayer link up [1]. The drugs which bind liposome into or onto their membranes are anticipated [Figure 2] to be transported without rapid degradation and minimum side effects to the recipient. Liposomes are generally composed of biodegrable biologically inert and non-immunogenic lipids. In the presence of hydrophilic drugs, lipids are hydrated, a portion of it gets entrapped inside the liposome and another remains in bulk, outside the aqueous core of liposome; the drug in bulk should be removed [1]. To purify usually get filtration column chromatography and analysis on the basis of size differences between the liposomes and the non-capsulated material. Techniques such as centrifugation can be used where DNA or proteins are being capsulated, or where we are concerned about non-encapsulated materials that may form large aggregates. A hydrophilic drug may not be encapsulated with high efficiency because the drug molecules can diffuse in and out of the lipid membrane, so it is difficult for the drug to retain inside liposomes. Compounds with Ionized groups and those that are both water and liquid soluble can be encapsulated with high efficiency 90%. The PH of the interior part of the liposome is such that unionized drug which enters the liposome by passive loading is ionized inside the liposome, then the ionized drug molecules lose their ability to diffuse, so high concentration of the ionized drug is obtained inside the liposome. Example; Doxorubicin and Epirubicin can be entrapped in performed SUV with high efficiency through active loading. Hydrophobic drugs are solubilized in the phospholipid bilayer of the liposomes. When trapped, they remain in the liposome bilayer; during the preparation of liposomes hydrophobic drugs are solubilized in the organic solvent with the phospholipids, so they stay entrapped in the hydrophobic bilayer region during the hydration phase. There is a hydrophobic drug in the liposomal photosensitizer verteporfin which rapidly transfers to blood proteins in vivo, and if we activate the drug by targeting laser light to blood which flows through the eye causes its site-specific activity in wet molecular degeneration treatment. Encapsulating a non-permeating buffer ion such as glutamate inside the liposomes at low pH and replacing the extra liposomal buffer with this is iso-osmolar at pH=7.00 [1]. They do not produce pyrogenic or antigenic reactions, so they possess limited toxicity. So these properties make liposomes more attractive candidates in order to be used as drug delivery vehicles than other drug carrying systems like Nanoparticles, and micro emulsions. Both hydrophilic and hydrophobic, entrapment of the drugs into liposomes causes by passing the generic toxicity which happens frequently; toxicity with the drug is often seen in cancer drugs, and the modification of liposomes allows passive or active targeting tumor site. In this way, we enable an efficient drug to be pay load into the malignant cell of tumors, while the non-malignant cells minimally impacted or unaffected. Liposomes represent advanced Nano delivery systems for a great number of biologically active compounds; the last amount of encapsulated drug is affected by a selected

preparation method, and it provides a preparation of liposomes based on their physiochemical Lamelarity properties and on their different sizes. Nano particles are colloidal particles, and they are delivering devices to liposome or viral vectors [6]. A new field that is rapidly in progress is Nano medicine; it has shown some promises in delivering drugs. Researchers can deliver low molecular mass compounds, proteins and recombinant DNAs to local areas of disease or to tumors. These Nano Medicines are also expected to improve early diagnosis by molecular imaging technique. The benefits and limitations of liposome drug carriers depend on physicochemical and colloidal characteristics such as site, composition, loading efficiency and stability, as well as their biological interaction with the cell membranes. Four major interactions can be seen between liposomes and cells [1]. One of the problems with Liposomes is that how to enter intracellular sites [7]. Drugs such as doxorubicin, meaning weak base drug can enter to the cells, but small hydrophilic drugs can enter cells via nucleoside transporter. The quint essential feature is their ability of site specific delivery, not only to the aimed organ, but also to targeted sub cellular compartments [6]. The predominant interaction among them is subsequent endocytosis or simple adsorption. Adsorption takes place when the attractive forces exceed the repulsive forces. It is clear that this type of interaction relies on the surface properties of liposomes. When delivered liposome and its contents place themselves indirectly of the liposomal content directly into the cell via the emerge of liposome lipids into the membrane is much rare, the lipid exchange is the last possible interaction. It is a long- range interaction. It involves the exchange of bilayer constituents like lipids, cholesterol, and membrane bound molecules. As soon as it enters the body, the delivered liposomes trigger the response of the immune system. Then the en capsulated material might become in active. So, more accurate researches have been gone through to carry out the development of the biocompatible and non-recognizable liposomal surfaces. Encapsulation ability of both hydrophobic and hydrophilic drugs is one of its advantages. Stability, storage and sterilization are the physical properties of liposomes. There are vast areas of research related to cancer therapy according to liposomes.

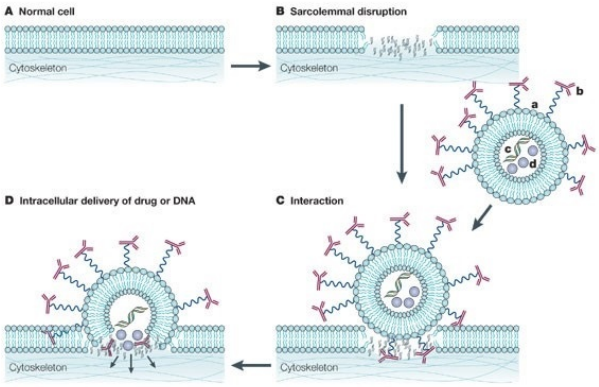

Figure 3. Four interactions can be seen between liposomes and cell membrane

Physio chemical characteristics should be entrapped [Figure 3]; the nature of medium that the liposomes are scattered; the effective concentration of the encapsulated material and its potential toxicity; during the delivery of liposomes, additional process is needed; process for optimum size, poly disparity and shelf life of liposomes is needed; possibility of large scale production of safe and efficient liposomal product and batch to batch reproducibility [1]. Liposomes containing combination of drugs, and these advances led to delivery of anti-cancer, anti-fungal, and anti-biotic drugs [7]. Cagdas stated that liposome size is a vital parameter if we want to find out about the circulation of the size and the number of bilayers of the prepared liposome; as soon as lipids are hydrated in the presence of hydrophilic drugs, a portion of the drug gets entrapped inside the liposome and another portion stays in bulk, meaning outside of the aqueous core of the liposome. We can explain liposome stability by physical chemical and biological means. Physical and chemical stability determine the shelf life liposomes. Liposomes can be used in many cases in order to increase the stability of administered drugs [8]. They decrease side effects and they improve their efficiency [1]. The performance of the phospholipids bilayers hydrolysis of the outer bonds which link the fatty acids to glycerol backbone cause chemical degradation. The level of oxidation should be kept to minimum. A solution to the oxidation problem is to reduce the level of oxidizible lipids in the membrane. We should use saturated lipids instead of unsaturated ones. Permeability of liposome has high dependency toward the membrane lipid composition. Ionic or large polar molecules are more efficient than low molecular weight lipophilic compounds. Stability of liposomes is dependent on their charge. In various stages of drug delivery, biological liposome stability plays an important role [1]. From composition of phosphatidyl choline, cholesterol, Phosphatidic acid, and stearylamine, liposomes were prepared [3]. Liposomal entrapment has some effects with methotrexate it may have some therapeutic advantages in localized release of anti-cancer drugs. Liposomes have demonstrated one of the most established Nano platforms with several FDA approved formulations for cancer treatment. They had had the greatest impact on oncology so far. They have low toxicity and immunogenicity. Physicochemical and colloidal characteristics such as composition, size, loading efficiency and stability of the carrier, is related to liposome applications in drug delivery. Conventional liposomes are referred to liposomes made up commonly used ester phospholipids such as phosphatidyl choline. They are very attractive for encapsulation and drug delivery applications. They entrap both hydrophobic and hydrophilic materials. It is because the lipid bilayer and aqueous core are present [1]. Liposomal formulations with one or more lipids are being called Archaeosomes. They contain diether and tetroether linkage which are found in archaeobacterial membrane. They show highly stabilities to several conditions like (temperature, acidic media, and anaerobic atmosphere.) In the last decades a lot of studies have been done on liposomes, and many conventional methods involve use of either water miscible organic solvent or detergent molecules [1]. The benefit for targeted drug delivery

is to reduce the toxic agents as well as to enhance drug accumulation at the target site [9]. To target drugs to specific tissue systems inside the body; drug molecules can be directly attached to a targeting agent [9,10]. This paper is going to continue on how to prepare liposomes, load drugs in liposomes, Function of liposomes, and targets of liposomes.

Research Plan

The process of liposomes formation has been discussed. They use it as nano bio electronic devices for different strategies for entering drugs in to the targeting cells. The beneficial of using liposomes is more than using other conventional techniques. For synthesizing Liposomes, in the mechanical agitation methods: It is not appropriate for small liposomes because it will be unstable because of the size. In the Solvent evaporation method, lots of amount of water is going to be wasted. So, I have thought to make another method to synthesize liposomes: I am going to mix Mechanical agitation and solvent evaporation method to overcome the challenge of wasting water, and limiting size; under the name of Mechanical Evaporation method. Mechanical Evaporation method: Liposomes are manufactured in the water-soluble material with mixed lipid composition the lipid must first be dissolved and mixed in an organic solvent to assure a homogenous mixture of lipids. In this process we should use chloroform with methanol mixture. Once the lipids are thoroughly mixed in the organic solvent, the solvent is removed to yield a lipid film. We should use nitrogen gas, for small amount of lipids. Let the solvent to evaporate in a glass- container; or use a rotary evaporator connecting to a vacuum pump. In this way, we get a thin film of the lipid on the walls of a round bottom flask. To increase encapsulation, we should start with a large volume round bottom flask. Lipids will dry down onto a large surface area possible to form vacuum pump and introduce it to nitrogen. Then we remove the container. The aqueous volume within inside these lipid membranes is a very small proportional of total volume used for preparation. During the preparation, large amount of water soluble drug is wasted. But to increase encapsulation, we should start with a large volume round bottom flask. These are our challenges in this experiment. With Mechanical evaporation method, we will have liposomes in all different sizes without any size limitation and without any leakage of content from inside of the liposomes. We should work 6 months on this experiment, and with approximately 6500 $ grant on it.

References

1. Cagdas M, Ali DS, Bucak S. Liposomes as Potential Drug Carrier Systems for Drug Delivery. 2014.
2. Pinheiro M, Marlene L, José LFC, et al. Liposomes as Drug Delivery Systems for the Treatment of TB. Nanomedicine (Lond) 2011; 6: 1413-1428.
3. Richardson VJ, Ryman BE. Effect of liposomally trapped antitumour drugs on a drug-resistant mouse lymphoma in vivo. British Journal of Cancer. 1982; 45: 552–558.
4. Deshpande, Pranali P, Vladimir PT. Current trends in the use of liposomes for tumor targeting. Nanomedicine (Lond) 2013; 8: 1509-1528.
5. Liu x, Guihua H. Formation strategies, mechanism of intracellular delivery and potential clinical applications of pH-sensitive liposomes. j.ajps. 2013; 8: 319-328.
6. Rajendran l, Hans Jk, Kai s. Subcellular targeting strategies for drug design and delivery. Nat Rev Drug Discov.2010; 9: 29-42.
7. Theresa MA, Pieter RC. Liposomal drug delivery systems: From concept to clinical applications. Advanced drug delivery reviews. 2013; 65: 36-48.
8. Vladimir PT. Recent approaches to intracellular delivery of drugs and DNA and organelle targeting. Annual Review of Biomedical Engineering. 2006; 8: 343-375.
9. Bareford ML, Peter WS. Endocytic mechanisms for targeted drug delivery. Adv Drug Deliv Rev. 2007; 59: 748–758.
10. Immunoliposomes: Different strategies for conjugating antibodies to liposomes. Liposome: Encapsula's Scientific Blog 2011.

*Correspondence to

Dr. Marjan Assefi

University of North Carolina at Greensboro

North Carolina

USA

Email: m_assefi@uncg.edu