Liposomes and controlled drug release

Individual work
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Liposomes are promising and widely used carriers of bioactive substances and drugs due to their ability to biodegrade, low toxicity associated with a low incidence of side effects, and the ability to transport hydrophilic, hydrophobic and amphiphilic substances. The disadvantage is the relatively short lifetime of these particles in the bloodstream (ie approx. 200 minutes) and also the uncontrollable release of the drugs carried.

Liposome surface modification

Improvement of properties can be achieved by various modifications of liposome surfaces.

Basic methods of modification:

- enclosing a liposomal particle containing a drug in a special polymer package, so-called "scaffolding"
- binding of molecules of different nature directly to the surface of liposomes

„Scaffolding“

In general, it can be defined as the use of spatially permeable biomaterials used to improve the properties of a nanoparticle. The purpose of the scaffold is to wrap the nanoparticle, so we can call this type of surface treatment 3D. It is used to transport proteins (protein), drugs, growth factors and also liposomes in the body. Scaffolding is mainly used in tissue engineering.

The advantage of using scaffolds is longer local preservation of the substance, slower drug release and potential for long-term storage. However, the disadvantage is the necessity of repeated application of the product and the passive release of the nanoparticle content.

.Different polymers are used to create the scaffold. Their important properties include: degradability (sometimes synthetic non-degradable ones are also used), low immune response, non-toxicity, biocompatibility. Both natural and synthetic substances are suitable polymers meeting most conditions

Natural polymers are:

- proteins (collagen, fibrinogen, elastin, keratin, actin and myosin) - scaffold based on collagen - collagen has excellent properties, it is a natural polymer found in the human body. Collagen-based 3D scaffolding is used in genetic engineering and other fields.
- polysaccharides (cellulose, amylose, dextran, chitin and glycosaminoglycans),
- polynucleotides (DNA, RNA).

Synthetic polymers are:

- polyvinyl alcohol, derivatives of polyacrylic acid (carbopol) and others.

Types of liposomes according to surface layer modification

- **Conventional liposomes** – mainly contain only phospholipids in the membrane, which is why they are relatively unstable; they also have a relatively low transport capacity and high permeability. The membrane can be enriched with cholesterol, which stabilizes and extends the life of the particles in the bloodstream (up to 1000 minutes). They are the basis for the production of most other types of surface-modified liposomes.
- **Long-circulating liposomes** - also known as "long-circulating" or "stealth". In the membrane, they contain a long chain of hydrophilic polymer (most often polyethylene glycol, PEG), which forms a kind of coating on the surface of the particle preventing the triggering of an unwanted immune reaction.

- Active liposomes – derived from long-term circulating liposomes. Drug release occurs selectively and in a controlled manner, requiring a stimulus for activation.

  "Targeted" liposomes - in their structure they contain antigens, antibodies, enzymes, proteins or receptors for an affinity reaction. The affinity reaction occurs upon contact with the target area, the accumulation of liposomes and the gradual release of the transported active substance.

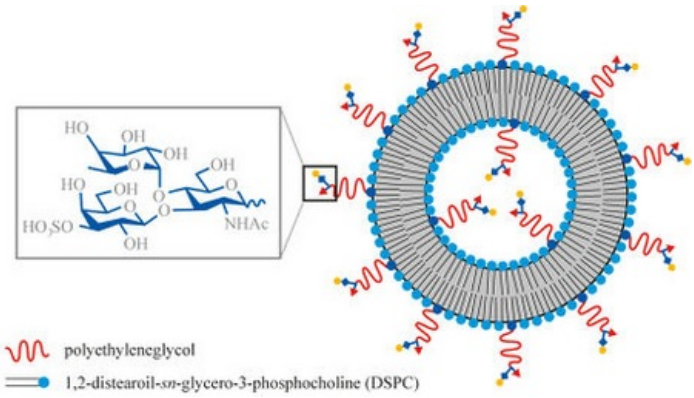
  "Triggered" liposomes – a chemical substance is built into the membrane of the liposome causing structural changes when the triggering mechanism is applied. The basic types of stimuli include: physical (temperature gradient, pressure, exposure to light), physicochemical (swelling, solvation), ionic (change in ion concentration, change in pH), enzymatic (hydrolysis) and combined.

- Lipoplexes - so-called "charged liposomes" contain positively charged phospholipids. Their covalent interaction with oppositely charged macromolecules (DNA, RNA and proteins) occurs. Due to the possibility of DNA transport, they are widely used in genetic engineering, they also have potential for the treatment of cancer, sickle cell anemia, cystic fibrosis, hemophilia and other genetic diseases.

Replenishment of Medicines

Medicines with small hydrophilic molecules are widely used in clinical practice, despite the fact that treatment with them is often associated with a number of complications and side effects, e.g. low intracellular absorption, cytotoxicity, rapid elimination from the body or suboptimal biodistribution.

These problems can be avoided to a certain extent if a liposome is used to transport the active substance to the target tissue. Liposomes are artificially prepared spherical vesicles formed by a lipid bilayer. They have specific ligands, markers or lipid chains on their surface, thanks to which binding to cell receptors is mediated.



Long-term circulation liposomes

Thanks to the selective permeability of the liposome membrane, the encapsulated substance is protected from the influence of ions and large molecules with a dipole (saccharides, proteins). Another indisputable advantage of the liposome is its biocompatibility with the patient's organism and the possibility of regulating the release of the active substance.

Liposomes can be used to treat a wide range of diseases. However, the success of the chosen medical procedure does not depend only on the administration of a certain medicinal substance, but also on the chosen method of preparation of liposomes.

Liposome preparation

Parameters when choosing a procedure

- properties of the material with which the liposome is to be filled and their possible interaction with the lipid membrane
- the effects of the internal environment to which the vesicles will be exposed
- effective concentration of the captured substance and its cytotoxicity
- other processes that take place in the body during the application of liposomal drugs
- optimal vesicle size and durability.

Liposomes are able to transport not only hydrophilic but also hydrophobic substances. Depending on the affinity of the transferred material to water, the method of preparation of the liposome differs.

Filling with a hydrophilic substance

The preparation of a liposome with a hydrophilic filling takes place using the so-called film method, which means that a lipid bilayer is first prepared, from which spherical structures filled with the active substance are then created.

- In the first step, it is therefore necessary to mix a homogeneous solution of lipids with the help of an organic solvent. The solvent in this step is pure chloroform or its mixture with methanol.
- In some workplaces, polysorbate 80 is added to this mixture, which acts as an emulsifier and in the last stage of preparation increases the effectiveness of encapsulation, as well as cholesterol, which is an integral part of biological membranes in the body, as it increases their flexibility.
- Once the lipids are dissolved, the chloroform must be removed from the mixture, which can be done in a fume hood with a stream of dry nitrogen or argon for smaller volumes of solvent (<1 mL). Larger volumes of solvent are removed in a rotary evaporator at a temperature of 50 °C. However, even after this procedure, a small amount of chloroform remains in the obtained lipid film. Residual solvent is removed by thorough drying and freezing.
- Subsequently, the obtained film is hydrated with a phosphate buffer of a physiological solution of pH 6.5 and filled with an active substance. The product of hydration is multilamellar vesicles with an onion-like structure. The individual layers are electrostatically charged and because of this they repel each other, which is undesirable for their future use. Therefore, the resulting liposomes are sonicated in the final phase of their production, i.e. reduced and adjusted to unilamellar formations with the help of ultrasound. Nowadays, however, ultrasound is considered a somewhat outdated method, because its application can damage the structure of the drug. For this reason, the Mozafari method or the extrusion method, which is more gentle on medicinal substances, is preferred in the production of medicines.

Filling with a hydrophobic substance

.As already mentioned above, liposomes can also carry hydrophobic substances. However, this transfer is specific in that these substances must be taken into the lipid bilayer. Differences in production also arise from this fact.

Application of liposomes as drugs

.Despite the differences in production, the application of liposomes is very similar in different cases. Oral administration of such drugs would not be very effective, as liposomes are very little resistant to low pH in the stomach, enzymes of the gastrointestinal tract and salts of bile acids. For this reason, they are applied parenterally or topically. In the organism, the membrane of the liposome merges with the membrane of the target cell and the liposomal contents are poured into the cell (see picture).

Although drugs using liposomes as active substance carriers have already been introduced to the market, more than half a century after their discovery, intensive research is still ongoing in the field of targeted organ distribution and efforts to produce such a liposome that would release the active substance only on the basis of a certain stimulus and be usable in clinical practice.

Liposome opening

From the point of view of the application of encapsulated (contained) substances, the process of degradation (destabilization) of the phospholipid membrane is very important. This leads either to the complete destruction of the liposome itself, or to the formation of a hole in the membrane. Subsequently, the encapsulated substance is released.

It is important that the degradation of liposomes occurs in the "right place". For example, if they contain drugs, destabilization of the phospholipid membrane must not occur before reaching the target. If the release occurred prematurely, it would reduce the effectiveness of the therapy, or it could even lead to intoxication of the organism. If liposomes are used as vectors for plasmid DNA, they must remain stable until crossing the nuclear membrane.

Opening trigger mechanisms

There are many triggering mechanisms for liposome opening, the most common being, for example:

- pH change,
- change in redox potential,
- chemical changes caused by the action of various enzymes.

Much attention is paid especially to liposomes, which are sensitive to pH. They began to be used mainly due to the fact that in the case of a large number of pathological cell processes (e.g. tumors, inflammation), the pH decreases in the affected area. Although this change is relatively small (around 0.4 to 0.8 units), liposomes have also been proposed that are able to react to such a small difference.

Liposomes sensitive to pH often act on the principle of neutralizing the surface charge of the membrane. During neutralization, the charge of the "polar head" of phospholipids is lost, which leads to a decrease in surface area and the subsequent collapse of the entire membrane.

The fate of liposomes in the body also depends very much on the way in which they were applied. For example, when administered intravenously, it is retained by the lungs. From them, they continue further into the bloodstream.

Opening by ultrasound

Since the mid-90s of the last century. scientific studies have been published showing that ultrasound helps facilitate the transport of membrane-impermeable compounds into living cells. Nowadays, its effects are being investigated in particular in connection with biological substance carriers, such as liposomes.

The opening of liposomes using ultrasound and the subsequent release of drugs into the cells is based on a process called sonoporation. This is a temporary permeabilization of the cell membrane that can occur as a result of exposure to ultrasound. There are two mechanisms of **sonoporation** that relate to different biophysical processes. However, scientific studies from recent years indicate that, in addition to the sonoporation process, other biological mechanisms, such as endocytosis, are also triggered by the ultrasound action and the subsequent release of drugs.

Mechanisms involved in ultrasound-induced sonoporation

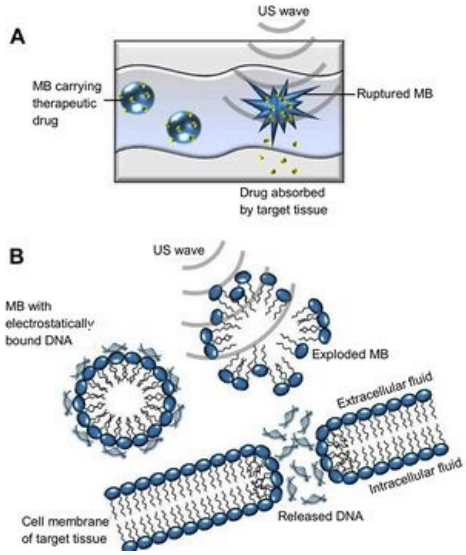
- non-collapsing cavitation also known as stable *cavitation* ;

biophysical aspects : non-collapsing cavitation occurs at very low ultrasound intensities (at low acoustic pressure), when the bubbles oscillate symmetrically, linearly, i.e. their expansion and compression corresponds to the acting acoustic pressure. In symmetrical oscillation [1], the increase in gas volume during one cycle of expansion/compression is equal to 0 (the bubble does not increase in size). In this case, the uptake of drugs by the cell takes place in two ways:

- by the formation of pores in the cell membrane (this is used more for small particles, with a small diameter). Oscillations inside the bubbles and their vibrating surface induce fluid swirling and flow, *micro-flows that induce* shear stress in the liquid near them. Assuming that the bubbles are located closely adjacent to the cell

membrane, even a small amplitude of oscillation is sufficient, therefore even lower ultrasound intensities are sufficient to cause cell poration. On average, the pores range in size from a few tens to a few hundred nanometers. However, the bubbles must be in direct contact with the cell. After interrupting or turning off the ultrasonic radiation, the pores re-closed in the order of milliseconds to seconds, which can be understood as the fact that the pores exist only as long as the oscillating bubbles are present.
- the second way is endocytosis (most often for larger particles; with a larger diameter and higher molecular weight).

- **collapsing cavitation**, also known as transient cavitation (*inertial cavitation*, *transient cavitation*);

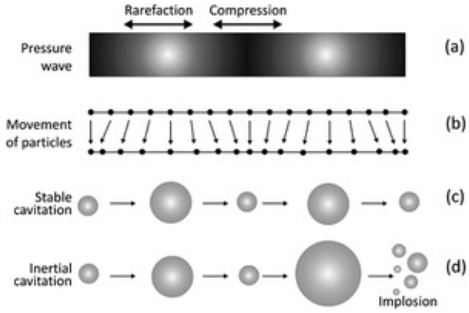


Mechanisms of ultrasound on microbubble

biophysical aspects : collapsing cavitation occurs at higher acoustic intensities, when the amplitude increases and its constant increase occurs, due to the increasing volume of the bubble, because it "inflates" in the expansion phase. After reaching a size called the **bubble resonant radius (R r**, *bubble resonant radius*), it suddenly increases and then collapses drastically, i.e. it *implodes* [2]. The resonant radius of the bubble is determined by the type of gas/liquid inside the bubble, the medium in which the bubble is located, and the parameters of the ultrasonic wave. For an air bubble in water, the resonant magnitude can be estimated by the equation:

$$R_r \sim \frac{3{,}28}{\mathrm{f}} \qquad (1)$$

where f is the ultrasound frequency (in kHz). *Implosion* results in fragmentation of the bubble into many smaller microbubbles. During this decay, shock waves and strong currents ("jets") can form in the surrounding liquid. These create very significant forces that can perforate the cell membrane if the imploding bubble is in its vicinity.



a) pressure wave (acoustic) b) particle movement c) non-collapsing cavitation d) collapsing cavitation

Pores created by collapsing cavitation are larger than those created by stable cavitation. Their size varies on average from hundreds of nanometers to micrometers, and at the same time correlates with the acoustic pressure: the higher the acoustic pressure, the larger the oscillation amplitude and the larger the pores. This type of cavitation is therefore suitable for the transport of larger particles.

However, high-intensity ultrasound waves pose a risk of damage to the cell and surrounding tissue, so it is necessary to proceed with increased caution. It has been shown that in the presence of already formed cavitation nuclei, such as ultrasound contrast agents, the cavitation threshold can be lowered, thus lower ultrasound intensities are sufficient to deliver drugs or genes to cells, such as rat fibroblasts and chondroblasts, human cancer cells HeLa, NIH/3T3, C127I, DU145 prostate cancer cells into tissues such as muscle, liver, lung, brain, or tumors. Small gas microbubbles covered with surfactant (serum albumin, polymer, phospholipid) are used as ultrasound contrast agents.

Bubbles are most often particles of gases (e.g. polyfluorocarbons) or liquids that are attached to a drug-filled liposome or encapsulated inside it together with the drug.

While stably cavitating bubbles need direct contact with the cell membrane, transiently cavitating bubbles can influence the cell membrane by the reactions they cause, even from a greater distance.

We distinguish three basic types of ultrasound action, which cause different biological reactions:

- low intensity ultrasound ("low intensity ultrasound")
- high intensity ultrasound ("high intensity ultrasound")
- application of ultrasound without the presence of microbubbles

Modes of degradation

Degradation of own liposomes can take place in several ways, but the exact sequence of events that take place after contact with the cell is still unclear. The known ways of degradation are:

- fusion with the cell membrane
- exocytosis
- phagocytosis
- insufficient membrane strength

The first of the putative ways of degradation of liposomes is **fusion with the cell membrane**. In this case, fusion occurs, which is the joining of membranes.

Furthermore, it was found that degradation can also occur by so-called exocytosis, which in this case would mean the complete exclusion of the liposome from the intracellular space of the given cell in which it was located.

Phagocytosis is also one possible cause of degradation. During phagocytosis, the liposome is absorbed by a cell other than the one into which it was targeted.

Another degradation factor is the insufficient strength of the membrane surrounding the given liposome, which can subsequently lead to a decrease in the efficiency of the liposome's function due to spontaneous leakage of the transported substance. The researchers believe that these spontaneous escapes may have two explanations. The first explanation for the leakage of the substance out of the liposome is the small pores in the membrane surrounding the liposome itself. The second explanation is the leakage of the substance based on the principle of diffusion.

Since the investigation of the function of liposomes is among the newer topics that are still being investigated, no other factors and methods have been known to explain the degradation of liposomes.

Therapeutic applications

Liposomes are one of the unique drug delivery systems that can be potentially useful in controlling and targeting their delivery. They can carry a wide variety of therapeutic agents, including hydrophilic and lipophilic drugs, oligonucleotides, proteins, peptides, and genes. It is also possible to use them in thrombolysis, in the treatment of atherosclerosis, in gene therapy or in the delivery of bioactive gases (xenon, hydrogen sulphide, nitric oxide, carbon monoxide) to tissues.

Cosmetics

Few ingredients in cosmetic products have had as much hope as liposomes. Today, they are usually added to gels and emulsions. Liposomes can improve the penetration of cosmetic and medicinal active substances. During their disintegration, they cause "liquefaction" (so-called fluidization) of the lipids of the stratum corneum, which thus becomes more permeable. Liposomes act in a similar way to urea, which is used more and more frequently and in higher concentrations as a moisturizing factor in cosmetics. However, according to several surveys, there is also a significant negative associated with improved penetration, namely that not only active substances penetrate the skin from cosmetic products, but allergenic substances - for example, preservatives such as parabens, alcohol, technical substances including trace residues of solvents, catalysts and other substances that may appear in the preparation during chemical production. The same applies to all substances from our environment that come into contact with the skin - the original function of the skin to limit and create a barrier against the penetration of the surrounding world into the human organism is thus impaired.narušena.

The main area of use of the liposome in cosmetics

- hydration
- cellulite
- stretch marks
- protection against the effect of sunlight
- hair care
- so-called Anti-Aging preparations or anti-aging preparations (not only for the skin)

Liposomes as nutritional food supplements

Until recently, the use of liposomes was focused primarily on targeted drug delivery. Now they are also used for the oral application of some dietary and nutritional supplements. Water-soluble vitamins are absorbed only in small amounts (absorption through the intestinal wall depends on transport mechanisms) and have so-called low bioavailability. In contrast, liposome encapsulation offers a very effective method of bypassing the destruction of nutrients in the digestive tract by digestive enzymes and aids the absorption of nutrients into cells and tissues.

An example of nutritional supplements using a liposomal form

- Vitamin C - reduces oxidative stress, improves the quality of fibrous tissues, protects against infection
- Vitamin B - improves the metabolism of nerve cells
- Glutathione - protects against aging, reduces oxidative stress

Treatment of patients with atopic dry skin

Atopic eczema is accompanied by itching and dry skin. Patients have increased transmendal water loss and reduced water binding capacity. Liposomes made from epidermal lipids could significantly help in the treatment of this disease by replacing the existing epidermal lipids in the stratum corneum (the outermost layer of the epidermis).

Treatment of patients with Kaposi's sarcoma (associated with the HIV virus)

Treatment with the liposomal technique was far more effective than treatment with the combination method of bleomycin and vincristine (BV). Patients receiving BV treatment have been associated with peripheral neuropathy (damage to the peripheral nerves). The second group of patients who underwent liposomal treatment was

associated with neutropenia (an impairment of granulocytes, more precisely a very low number of the neutrophil segment in the blood). The result of the treatment was the finding that the treatment with the liposomal technique was far more effective than the BV treatment and also well tolerated, but more myelosuppressive (bone marrow depression).

Liposomes and cancer

Another interesting property of liposomes is their natural ability called EPR (enhanced permeability and retention effect), thanks to which they are able to target cancer. This principle is that a tumor cannot grow beyond a certain size without forming new blood vessels. Thanks to the high rate of blood vessel formation, they are "porous" and therefore permeable to molecules and particles usually up to 200 nm in size. Vessels elsewhere in the body do not allow liposomes into the tissues, or only to a limited extent. The liposome can therefore enter the tumor and begin to act.

Method of application

There are certain types of liposomes that are administered orally. In general, however, the majority of manufactured drug forms of liposomes are not resistant to the acidic environment of the stomach, digestive enzymes and bile acid salts. Therefore, the more common methods of administration are intravenous or topical.

Future perspective

There is a wide range of diseases that have the potential to be treated using liposomes in the future (Aid Dementia Complex, tuberculosis, oncological diseases, or neurodegenerative disorders such as Parkinson's and Alzheimer's disease).

Links

Related articles

- Liposomes
- Ultrasound

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