



Virosomes: A groundbreaking revolution in novel drug delivery

Sreeja C Nair*, Sreelekshmi A S, Aslam Abdul Rahiman C A, Karthik Krishnan, Gopika G, Priyanka Nair T, Unnithan Gauri Ajit

Department of Pharmaceutics, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, AIMS Health Sciences Campus, Kochi - 682041, Kerala, India

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ABSTRACT

The site-specific action of the drug has been seen from the last eras of the revolution in drug delivery technologies. Drug delivery opportunities by the use of biomimetic nanoparticles like virosomes is a stimulating area of research & development as it demonstrates targeted action by fusion with the target cell. Virosomes are vesicular particles reconstituted from viral envelopes which are non-replicating “artificial viruses” that denotes a unique system for presentation of antigen directly into the host cell. Trials have been created to use them as vaccines or adjuvants moreover as a delivery system for medicine, nucleic acids, or genes. Various attempts have been made to use them as vaccines or adjuvants as well as a delivery system for drugs, nucleic acids, or genes as they are biocompatible, biodegradable, non-toxic and non-autoimmunogenic. The production of vaccines increasingly moved away from living attenuated or inactivated whole organisms to safely killed organism. A virus that is safely killed can be a promising vector because it does not cause infection, and the viral structure allows the virosome to identify different components of its target cells. Pevion’s virus-like particle (VLP) vaccine technology, called virosomes, and its architecture is specifically designed to produce safe and efficient vaccine subunits. Virosome-based vaccination is effective in reliable regulatory and safety records as well as the feasibility of upgrading production and has been approved in more than 40 countries, including infants and older people. The prospect of drug delivery and targeting using virosomes is a vital area of research and development. This review pinpoints the various aspect of virosome and will be a milestone for the researchers in the field of drug delivery.



*Corresponding Author

Name: Sreeja C Nair

Phone: +919388600399

Email: sreejacnair@aims.amrita.edu

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INTRODUCTION

Over the past few decades, a crucial quantum leap has been undergone in the area of drug delivery system (DDS). The main drawback of the majority of the potential drugs is a failure to attain targeted action. Certain conditions such as cancer and many neurodegenerative brain disorders require targeted and controlled release actions. Most of the potential drugs are often abandoned during development due to their failure to attain the targeted effect (Nupur *et al.*, 2015). Overall, cancer treatments generally comprise chemotherapy, surgery, and radiation therapy, but limitations bound all

three of them. Both radiation therapy, as well as surgical procedures, restrict themselves to a localised tumour. Chemotherapy is used in a wide variety of cancers, including distant cancer, through systemic administration of anti-neoplastic drugs; although, it often destroys healthy cells and causes severe systemic toxicity (Saga *et al.*, 2013). In order to prevent such systemic toxicity, novel, sophisticated targeted mechanisms are required for the delivery of drugs. The distribution of biological molecules and drugs using virosomes is a modern technique that is currently available these days. Virosomal technology is being developed to overwhelm the difficulties of partial delivery towards targeted organs, tissues and cells of the body. Virosomes are semi-synthetic complexes that are produced from viral particles which are free of nucleic acid. They are unilamellar phospholipid bilayer vesicles that contain proteins derived from viruses. They exhibit desired output or action by merging with the targeted cell. These can be either used as vaccines or as a medium for the cellular delivery of macromolecules. In addition to the delivery of these substances, virosomes also helps in the enhancement of the immune response. They are not capable of replicating but are 'Fusion Active Vesicles' otherwise known as 'Virus-like Particles'. Virosomes maintain their fusogenic activity and provide the targeted action of integrated pharmaceuticals or biological products such as antigens, medications, genes, etc. Hence they can either be used as drug delivery material or as vaccines or gene carriers (Nupur *et al.*, 2015). Pathogenic antigens of interest are either trapped within the virosome lumen or are chemically bound to the surface. Because of its viral protein envelope, a virosome can bind and "infect" host cells and transmit the antigen directly to the processing pathway for MHC class 1 antigen (Germain, 1994). Virosomes are biocompatible, non-auto immunogenic, non-poisonous and biodegradable; efforts have been made to use them as adjuvants or antibodies, as well as to provide drug and organic frameworks for remedial purpose. Virosomes have also been shown to have a higher target specificity in the fight against Ebola infection. So, the successful delivery of virosomal drugs depends on the mode of preparation, the use of proper detergents and the use of short-chain phospholipids, and the function of hemagglutinins. Pharmaceutical active substance integrated into the virosome is protected from proteolytic degradation as well as low pH in endosomes, allowing it to abide intact until it reaches the cytoplasm of the cell (Zinkernagel, 2001).

In the present scenario of drug discovery systems, the Vesicular drug delivery systems are promising

approaches to overcome the limitation of the drug: lower bioavailability and rapid elimination from the body. The vesicular system plays a significant role in new drug delivery (otherwise known as NDD), particularly in the sorting of a diseased cell, diagnostics, gene and genetic materials, safe, effective and targeted in vivo drug delivery. They function like a system of sustained release and minimise the elimination of rapidly metabolisable drugs. Over the last few decades, they have been extensively used as drug carriers. There are different types of vesicular drug delivery systems such as enzymosome, virosome, ufasome, cryptosome, emulsosomes, discosomes, aquasomes, genosomes, ethosomes, archaeosomes, hemosomes, vesosome, proteosome, erythroosomes, photosome, cubosome, colloidosome, layerosome, erythroosome etc. The lipid-based drug delivery systems are of four types; they are emulsion-based system, stable- particulate lipid system, vesicular system and reliable -lipid tablet system (Aiswarya *et al.*, 2019).

Liposomes, a modern vesicular drug delivery system that can overcome the snag correlated with traditional or conventional drug delivery systems and also they can minimise toxicity associated with drugs. The term liposome is derived from two Greek words: 'Lipos' meaning lipid (fat) and 'Soma' meaning body. Liposomes are small sac-like structures which contain phospholipids as their monomeric units, and they can be found in a series of sizes with either unilamellar or multilamellar construction. Its name refers to its structural building blocks – the phospholipids, not to its size. Dr Alec D Bangham, who was a haematologist at the Babraham Institute in Cambridge, was the first person to expound details regarding liposomes in the year 1964. Liposomes can be infused using drugs and can be vividly utilised for the transport and delivery of anticancer drugs as well as medications for other diseases also (Daraee *et al.*, 2016). Lipid components have been honed to abstain its consumption by the reticuloendothelial system (RES). The liposomal ability of tissue targeting can be accelerated by manipulating the liposomal surfaces using antibodies or ligands, which are acknowledged by specific cell types. Improve the proficiency of gene delivery by introducing molecules directly into cells, and virosomes have been evolved by collaborating liposomes with fusogenic viral envelope proteins. So, according to the medical definition, a virosome can be defined as "a liposome that has protein or lipid from the envelope of a virus attached to its membrane and that possesses the antigenic properties of the virus". Liposomes are used for the transport of drugs because of their unique select prop-

erties. Industrial applications comprise the use of liposomes as drug delivery vehicles in medicine, signal enhancers/carriers in analytical biochemistry and medical diagnostics, immunisation adjuvants, solubilisers for different ingredients as well as support matrices for different ingredients and cosmetic penetration enhancers (Gregoriadis *et al.*, 1987).

Difference between liposomes and virosomes

The difference between the two drug delivery system, virosomes and liposomes are shown in Table 1.

The name virosome is derived from two Greek words: 'Virus' meaning virus and 'Soma' meaning body. Virosomes are a particular class of proteoliposomes. Almeida *et al.* first prepared virosomes, where they injected distilled spike proteins of influenza into preformed liposomes (Almeida *et al.*, 1975). The most common virus of choice is the influenza virus in which the virosomes obtained are devoid of internal nucleic acid core and genetic information. After that, several viral envelopes have been reconstructed. Examples of viruses used in the preparation of virosomes and their applications (Nupur *et al.*, 2015) are depicted in Table 2.

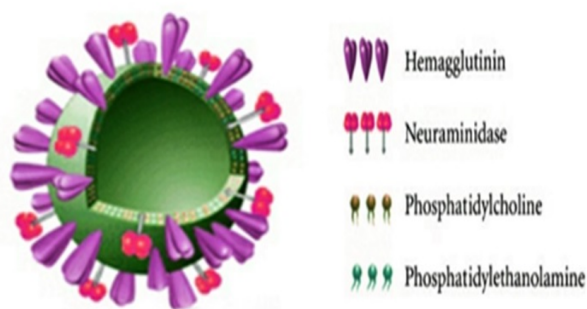


Figure 1: Structure of virosome

Necessity for the development of Virosomes

1. To attain desired targeted action - Virosomal system should be established to prevent systemic adverse effects and to achieve site-specific action.
2. To aid in the delivery of macromolecules and drugs -The most crucial factor in the therapy is the delivery of drugs and macromolecules. Since virosomes have empty inner space, they can be used for macromolecule targeting.
3. Reduce systemic side effects -Targeted action cannot be achieved with the conventional structures, which eventually results in increased toxicity. Virosome production is vital to resolve the

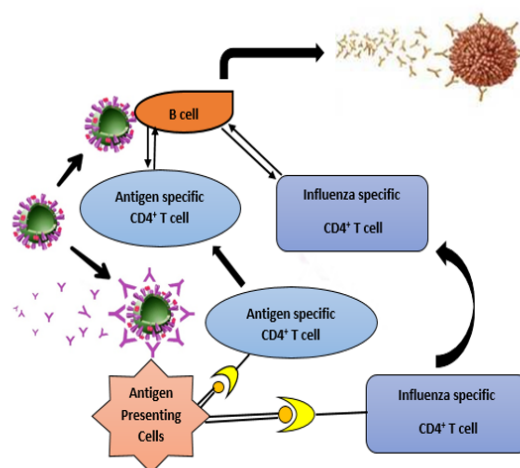


Figure 2: Mechanism of action of virosome

factor.

4. To protect the pharmaceutically active substance from proteolytic degradation - Active ingredient degradation typically occurs in the stomach after the oral intake. There is also concern about proteolytics and degradation due to low endosomal pH. Using virosomes can help prevent this problem (Nupur *et al.*, 2015)

Advantages of virosomal drug delivery system

1. Virosomal technology has a high safety profile and is approved by FDA.
2. No chance of transmission of ailments and no auto immunogenicity.
3. Anaphylaxis is absent.
4. They are biocompatible, biologically degradable, and non-toxic.
5. Serves as all vital drugs – antineoplastic agents, proteins, enzymes, peptides, nucleic acids, antibiotics, anti-infection agents, anti fungals.
6. Facilitates the mixture drive of endolysosomal trajectories.
7. Degradation of drug is prevented. Allow medication to be transmitted into target cell cytoplasm. Defends drugs from corruption.
8. Target targeted antigen distribution and immune response amplification.
9. Drugs have protracted uptake, distribution and elimination in the body.

Table 1: Difference between virosomes and liposomes

Virosomes	Liposomes
More potential to fuse with cells	little potential to fuse with the cells
Efficiently delivers the encapsulated molecules to the cytoplasm	Fails to deliver encapsulated molecules to the cytoplasm
Membrane fusion property is present	Membrane fusion property is absent
More protection for therapeutical macromolecules from harsh compartmental microenvironment.	Less protection for therapeutical macromolecules from harsh compartmental microenvironment.
Macromolecules are protected from proteolytic degradation and low pH.	Proteolytic degradation and low pH affect the macromolecules.

Table 2: Virus used in virosome production and its applications

Viruses used for the preparation of virosomes	Applications of virosome
Semliki Forest virus (SFV)	Propagation into membrane vesicles
Sendai virus	Anticancer immune activators and apoptosis inducers
vesicular stomatitis virus (VSV)	Vehicle of gene transfer to animal cell
Sindbis virus	Hybrid vehicle for efficient and safe drug delivery commonly in cancer treatment
Influenza virus	Delivery vector for TAAs and TAA-expressing plasmids

Table 3: Detection of fusion activity of molecules

Fusion Molecules	Visualization Methods
Biological or artificial target membranes	Fluorescent resonance energy transfer assay (RET).
In vitro fusion with an excimer	pyrene-labeled lipids
Indirect method	Hemolytic activity

- | | |
|--|---|
| 10. Up-scaling as per normal procedure. | 6. Short half-life - disintegration in the blood compartment is a speedy process possible virosome-related problem. |
| 11. Virosome allows standardized vaccine regimen unique to patients. | 7. Inadequate quality control assays. |
| 12. Can be administered through injection or by nasal route (Cusi et al., 2000). | 8. Data relating to chronic use of virosomes not available (Kammer et al., 2007). |

Disadvantage of Virosome

1. Manufacturing problems.
2. They can bring about humoral immune responses as they have surface viral glycoprotein.
3. Prolonged Payload.
4. Rapid disintegration.
5. Unsatisfactory quality of raw materials.

How to overcome the disadvantage of virosomes

1. Hasty disintegration can be overwhelmed by increasing virosome firmness or by allowing virosomes hit target sites within a short period of administration.
2. Quality control assays can be performed using futuristic methods, and batch variability can be tested.
3. Application of high-quality products with a protocol for better purification.

Table 4: Evaluation of virosomes

Parameters	Methods of Evaluation
Electrical surface potential and surface Ph	pH touchy tests and estimation of zeta potential.
Vesicle shape and surface morphology	Freeze break electron microscopy and electron microscopy transmission.
Phase conduct	Differential checking colorimetry , Freeze crack electron microscopy,
vesicle size and size dispersion	Dynamic light scrambling, photon connection spectroscopy, Transmission electron microscopy, laser light diffusion, Zeta sizer, gel avoidance and Gel saturation.
Percent of free medication	Radiolabeling, Protamine accumulation, Gel avoidance and Ion trade chromatography, Mini section centrifugation
Animal poisonous quality	Observation of survival rates, histology and pathology.
Surface charge	Free stream electrophoresis
Pyrogenicity	Rabbit fever reaction test or Limulus ambeocyte lysate (LAL) test.
Drug discharge	Diffusion cell/dialysis
Lamellarity	Small edge x-beam dissipating, Freeze break electron microscopy, 13p-NMR.
Chemical examination of surface	Static auxiliary particle mass spectrometry.

4. To overcome payload problems, we can use remote loading techniques.
5. Proper cryoprotectants & lyoprotectants can be used to increase the shelf life.
6. Scale-up problems can be compounded by choosing the right preparation and decontamination process by autoclaving or membrane filtration together with aseptic & validated pyrogen removal Lal test.
7. Narrow therapeutic index drugs can be selected ([Kapoor et al., 2013](#)).

History of Virosomes

Influenza virus belongs to the family orthomyxoviridae (family of RNA viruses). It contains a nucleocapsid with a segmented ssRNA (single-stranded RNA) genome and is protected by a viral envelope. Neuraminidase (NA) and Hemagglutinin (HA) are two types of membrane proteins that are located on the surface of the viral envelope. On the surface of the host cell, sialic acid acts as the receptor to which hemagglutinin binds, and thus virus particles adhere to the host cell. Hemagglutinin is accountable for the fusion of the viral envelope membrane with the membrane of the host cell.

Moreover, in neutral conditions, HA does not cause membrane fusion; its fusion activity only acquires

by conformational changes in the acidic environment. Influenza virosome is an artificial liposome constituting influenza membrane proteins and is produced by reconstituting the phospholipids and surface proteins of the influenza virus. On treatment with detergent, the envelope of the influenza virus is first broken down into phospholipids, and the nucleocapsid is removed from the mixture. Surface proteins and phospholipids obtained from viruses present in the influenza virosome are then restored from the combination. An influenza virosome retains its membrane fusion potential because of the presence of hemagglutinin on its surface. Therefore, by integrating them into the virosome, it serves as a diffusion vector to introduce macromolecules into the cytoplasm. Influenza virosomes have strong immunogenicity. Influenza virosomal vaccination produces beneficial levels of influenza-specific antibodies, and an influenza virosome is already approved as an influenza vaccine. Influenza virosomes often show an adjuvant effect when they are co-administered with other antigens; thus, several groups have studied the use of influenza virosomes in antitumor immunity activation ([Saga et al., 2013](#); [Almeida et al., 1975](#)).

Virosome Structure

The median diameter of virosomes is 150 nm and are unilamellar spherical vesicles. They pos-

sess a well-designed glycoprotein to envelop and the bilayer membrane- phospholipid are insinuated with neuraminidase (NA) and hemagglutinin (HA) as represented in Figure 1. Virosomes are fusion-active vesicles and cannot replicate. By amending the content or form of the lipid membrane, it can be used to achieve the best physiological effect. Depending on the phospholipid incorporated (positive or negative) into the membrane it can be used to generate antisense-oligonucleotide carrier, Tumor-specific monoclonal antibody fragments (Fab) and many peptides, cytokines, and monoclonal antibodies (MAbs) which act as ligands can even be integrated into it. Usually, the used virus is the influenza virus for the production of virosome (Jonge *et al.*, 2006).

Virosome functions and its action

Virosomes function both as a conveyor of drugs and also as adjuvant having specific capabilities in the enrollment of a healthy body response. The function of the transporter includes the beneficial influence of incorporation of the antigen into a more exceptional composition that is to the virosomal compound. The resistant mechanism of the virosomes and their segments with the immune energising properties is identified by adjuvant capacity. More than anything, virosomes dominate when it comes to improving particular invulnerability without causing an unspecific aggravation (Schumacher, 2005).

The repeatedly arranged hemagglutinins on the surface of virosome allow fast reaction of immunoglobulin receptors located at B lymphocytes. The dendritic cells (antigen containing cells) absorb the virosomes. The virosomal surface antigens and those obtained through damaged virosomes join the MHC class II pathway, triggering helper T cells. Then antigen accesses the cytosolic MHC class I pathway by fusion of a virosome, activating Cytotoxic T Lymphocytes (CTL). Ligands may be bound to the virosomal surfaces for a specific action, like medications with smaller therapeutic windows (cytotoxic medications) (Germain, 1994).

Virosomes function primarily by two methods:

By encouraging immune reaction

1. Carrier Function
2. Adjuvant Function

By transporting drugs to the site of activity

1. Drug Entrapment

2. Adsorption on Surface of Virosomes (Hukriede *et al.*, 2005)

Encouraging immune reaction

Virosomes function both as a carrier and adjuvant triggering immune reaction. They managed to activate appropriate immunity without any severe inflammation

Carrier Function

The carrier function demonstrates the decisive role of an antigen embedded.

Antigen incorporation into virosome:

1. Keeps native B cell epitope status
2. Improves antigen stability

The antigen on the virosome surface is an adaptation of the actual pathogen or target cell. Antibodies are produced against the stated condition. Once antigens are placed on the virosome surface, they strengthen the identification of B cells, creating an antibody. These complexes are tempting targets for immune cells, a significant move towards immunity initiation (Kapczynski and Tumpey, 2003).

Adjuvant Function

The function of adjuvant is reliant on hemagglutinin present. Pre-existing anti-influenza antibodies bind to and add to virosome in case of influenza derived virosomes. It promotes the Antigen Presenting Cells (APC) for taking up this complex. Now APC shows refined influenza protein fragments. The previously existing T cells particular to influenza are stimulated after the APC processing, thereby proliferating and enhancing immune cell activation (Figure 2).

By transporting drugs to the site of activity

Drug Entrapment

Medication can be lodged within the virosome. They are especially helpful in the supply of genes, nucleic acid, enzymes and peptides etc. The integration of these materials takes place at the time of preparation of the virosome. E.g., the virosomal system has effectively transmitted diphtheria toxin's gelonin subunit A and ovalbumin to the target cells.

The following steps deliver the active moiety:

Virosome binding to the target cell membrane

1. Cell membrane destruction
2. Cell membrane fusion with virosome bilayer
3. Distribution of virosomal contents inside the cell

Adsorption on the surface of virosomes

The pharmaceutically active ingredient is either adsorbed or directly attached using carrier onto the surface of the virosomes.

Methods of Preparation of Virosomes

1. Selection of virosomes
2. Selection of antigen
3. Reconstituted of virosomes

Selection of virosomes

Virosomes can be formed by reconstituting its viral envelope from different viruses. While it is possible to create virosomes from Epstein berr- virus, Sendai virus, HIV, Sindbis, herpes simplex virus, Friend murine leukaemia virus, Semliki wood. It is usually made from the influenza virus.

Antigen selection

Selection of antigen is according to our needs. This antigen is found in a fungus, carcinogenic cell, bacterium, or whole organism. Components of cells, for example, RNA, plasmid or DNA, can be the antigen. And may also be used as antigen to represent carcinogenic cells, bacterium parasites or entire cells. Once united to the lipid anchor, the antigen can be uploaded into the virosomes.

Reconstitution of virosome

Detergents (octaglucoiside, triton x-100, nonidert p-40) are used to solubilise the virosome. When the genetic material and viral internal protein solubilise with the detergent, it is extracted from the supernatant by many diverse methods such as dialysis and hydrophobic resins.

Then matrix protein of the virus and nuclei capsid are obtained using the technique of ultracentrifugation. Eighty-two per cent of viral protein and phospholipid can be recuperated.

Antigen which is by now joined with lipid anchor can be united with a polymer or surfactant solution, and this is treated with a virosome carrier to obtain antigen bound virosome (Sharma and Yasir, 2010; Singh et al., 2017).

Characterisation of Virosomes

Structure and size

Usually, electron microscopy for the Negative stain will be given to evaluate the fine-structure and size of virosomes.

Staining solution of neutral ph can avoid the acid-induced conformational changes of hemagglutinin.

Protein detection

Virosome preparation is comparatively clear protein to lipid ratios may typically be a result. The agent Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is used to confirm the presence of HA protein in the virosomes.

Fusion activity

Regularly, virosomes show combination movement of pH-dependent films such as native cold infections. Combination with a virosome and falsified target or organic films can be tracked with an excimer measure (in vitro) using pyrene-named lipids, whereby pyrene phosphatidyl choline-name surface thickness decreases with an unlabeled layer matched to a decline in excimer fluorescence. Additionally, combination action can be tested by determining hemolytic action, which shows comparable pH dependence (Kalra et al., 2013; Singh et al., 2017).

Fusion activity can also be monitored indirectly by determining hemolytic activity which closely resembles fusion activity and reveals a pH dependence identical to that of fusion.

The fusion molecules and their visualisation methods for detecting the fusion activity are portrayed in Table 3.

Evaluation of Virosomes

Evaluation parameters and the methods are illustrated in Table 4 (Shaikh et al., 2018).

Uptake of Virosomes by Cells

The approach of virosomes into the target cells can be divided into four distinct steps:

1. Attachment of virosomes
2. Penetration
3. Functions by the carrier
4. Memory support

Attachment of virosomes

This includes binding of virosomes via HA to cell receptors that are glycoprotein or glycolipid membranes with terminal sialic acid. In the case of certain virosomes, the fragments of Fab are integrated into the virosomal surface by a cross-linker with a spacer arm.

Besides, certain virosomes will acknowledge antigenic structures on the surface of the target cell, resulting in the attachment of two different binding mechanisms to target cells. Different virosomes thus exert selectivity on different types of cells.

Penetration

Receptor-mediated endocytosis occurs after attachment of virosomes. The virosomes are caught in the endosome. A viral spike glycoprotein, Hemagglutinin (HA) mediates the acidic fusion of the membrane of virosomes with the membrane of endosomes. This fusion between the membranes of virosomes and endosomes results in the virosomal discharge from their lipid envelope, which in turn provides admittance of the encapsulated drugs to the cytosol.

Functions by the carrier

The antigen is balanced by a reconciliation of the antigen to higher structures of molecules of the virosomes, and this safeguards the regional status of the B cell antigenic determinant and protects the antigens from degradation. In expansion, the introduction of the antigen as a monotonous surface structure increases its recognition by counteracting agent producing B cells.

Memory support

On interaction with flu-inferred hemagglutinin (HA), a memory reaction is triggered in most of the individuals who may be due to their previous exposure to influenza. This includes both humoral as well as cellular invulnerability. The existing flu-specific antibodies can tag virosomes which are capable of managing the rapid cell-introducing antigen (APC). Also, the memory T cells rapidly multiply to aid in the target-specific delivery of these antigens along with the discharge of cytokines for amplifying the intended immune response (Singh *et al.*, 2017).

Cell Interaction of Virosome

The virosome innovation's key favoured point of view is its capacity to imitate and express the in-vivo contamination. This will be beneficial in drawing resistant groups and to organise macromolecules on a separate operation site. The virosomes sense and bind with the very same receptors which are used whenever a common viral disease may occur. One such example is flu-virosomes use corrosive sialic receptors. When the infection has accepted the cell receptor, the mixture of the viral and endosomal layer is observed. When flu virosomes, for example, arise, the viral protein hemagglutinin (HA) utilises its twin to unite for a similar cause.

Additionally, neuraminidase (NA) is incorporated in the virosomal mixture because this can improve the virosome's immune responses and sensitivity on a particular tissue. The link of virosome receptors has been investigated for the therapy of numerous diseases like parasitic and viral diseases, neurolog-

ical disorders and many other dysmetabolic problems (Liu *et al.*, 2014). The principal point in each of the cases is the marshalling of drug particles, nucleic corrosive or a nano-sized protein to the intended activity site. At the virosomal surface, the proteins and peptides were efficiently mixed with glycoproteins.

Virosomal Drug Delivery Approach

In the aqueous centre or the lipid bilayer of the virosomes, the biologically active drug compounds may be embedded to promote the incorporation of compounds to the cells. In particular, the virosomes are valuable for the supply of genes or nucleic acids. After virosomal fusion with the endosomes or plasma membrane, such compounds are delivered to the cytoplasm. In the host cells genes or nucleic acids that encode a naturally found protein is added and expressed, ensuring that the sequence of expression contains the relevant regulatory factors for cis action. During the production of virosomes, nucleic acids or drugs are inserted to the virosomes. After elimination of nucleocapsid, the biologically active compound will be added to the solution containing lipid-HA.

Additionally, the biologically active compound is first introduced into the liposomes then it is combined with two hemagglutinins embodied virosome having specific pH levels to create a hybrid of virosome and liposome. Proteins are also transmitted via virosome into cells. Example, virosomes has successfully delivered the diphtheria toxin's gelonin subunit A and ovalbumin into the targeted cells. The access gained by the encapsulated peptides and proteins to the cytoplasm is indicated by the virosomes bearing influenza nucleoprotein derived peptides or intact ovalbumin mediated robust cytotoxic T lymphocyte responses (Bungener *et al.*, 2005).

Antisense-L-myc-virosomes

Inside the virosome, the antisense-L-myc-phosphorothioate oligodeoxyribonucleotides were enclosed. In the Small Cell Lung Cancer (SCLC) cell lines like H82, H209 AND H510 the antiproliferative effects of virosomal-embodied L-myc antisense DNA were assessed. Antisense-L-myc virosomes were applied to human SCLC cell lines which expressed, resulting in robust concentration-dependent inhibition of thymidine incorporation. Virosomes entrapped random-order OPT, and L-myc OPT only has minor consequences on the thymidine absorption (Waelti and Glück, 1998).

Administration of Virosomes

Virosomes can be delivered via parenterally, orally, topically and transdermally, the main parenteral

routes include intravenous, subcutaneous, intramuscular, inhalable and intrathecal delivery (Dahmen *et al.*, 2005). These are usually maintained in buffered saline that is 135-150mM NaCl, but there are also other appropriate solvents available. Such formulations should be purified by traditional techniques of liposomal sterilisation, including membrane filtration. They should contain auxiliary substances like isotonicity controlling agents and buffering agents such as calcium chloride, potassium chloride, sodium chloride, and sodium lactate and sodium acetate to imitate physiological status. Virosomal concentrations of 20 to 200mg/ml is used in the solvent, and this can be tailored for the function per standard. The virosomes can be integrated into implantables for the initiation of long term treatment (Rathore and Swami, 2012).

Looking Ahead

Virosomes are a new revolutionary advanced drug transport system for bioactive substances, but the genes and nucleic acids, in particular. The virosomal surface can be adjusted appropriately to allow the proper transport of drugs to the targeted site. Yet detailed pharmacokinetic profile, bioavailability, clinical impacts and protection are required and the stability studies to be extensively covered to establish long-term efficiency as a reliable, secure and inexpensive method of targeting and delivering drugs. In the cells of insects, Ebola virosomes were developed with a recombinant baculovirus expression vector system, and its effectiveness against Ebola Virus Disease was examined in mice. Virosome immunisation of mice provided full immunity against Ebola infection for vaccinated mice (Sun *et al.*, 2010). The virosomes are, therefore, an essential instrument in the production and manufacture of vaccines. Further evaluation can also help to establish virosomes for coronavirus in future.

Applications of Virosomes

Viruses conduct intracellular parasites, although for their survival they are necessarily subordinate to different host cells. This guidance brings in the creation of a system for the conveyance of medication that copies the cell disease viral example. Virosomes area unit is created from a lipid bilayer with the infective agent surface glycoproteins enlarging from the surface of those vesicles. This vesicular layer synthesis makes the virosomes biologically compatible and biologically degradable. These are ingested productively and are sorted to the external site devoid of changing the biological procedures.

Further, the virosome plan and arrangement are in a way that medicinal particles of different type can be used in it. The lipid bilayer will assimilate

the hydrophobic medicines into it effortlessly. Conversely, hydrophilic medications transform into a fragment of the focal lacunae.

Virosomes is also paired with an immune reaction to assure the tissue-specificity-focused conveyance of a useful operator. These antibodies bind to the different cell receptors that enable the transfer of drug atoms to these targets. Notably, this property can be used to convey the drug particles with minimal safety profiles. For example, anticancer drugs will be transmitted particularly to tumours by marking the virosomes with antibodies. The United States Food and Drug Administration (FDA) has approved numerous virosome-based products for human use. Virosomes have shown that macromolecules, including drugs, nucleic acids and proteins, have been transferred viable to different types of cells counting hepatocytes, healthy cells, erythrocytes and glioma cells. Virosomes that include malignant neoplastic drugs, malaria drugs, bacterial, and fungal operators have shown to be efficient in vitro and in vivo discharge profiles (Sharma and Yasir, 2010). The surface glycoproteins of contagion infection, liver disease infections and stomatitis infection are integrated with success in numerous protein and drug transport systems.

Bacterial apparitions were produced in light of the same norm. Such vesicles embody the outer shell or the supermolecule of various gram-negative microscopic organisms. Such bacterial appearances mimic a proportional pattern as we can witness in case of some distinctive illness. In any case, the virosome-based sedate conveyance is fast, sheltered and viable, rather than any other related device.

As immunopotentiating mediators

Virosomes are the molecules that can serve the purpose of providing antigens and drugs to particular types of cells. The principal property exploited by virosome architecture is the cooperation with the virus substance proteins and therefore the cellular receptors. Besides, the recognition, uptake and illustration of the antigen introduced by the specific antigen-presenting cells into the virosome help to activate the immune system. As a consequence, effective immune responses to the regulatory and the effector are produced. Both cell-mediated and immune system humoral alarms and cell-mediated alarms are activated.

Besides, both cytotoxic and helper-T-cell responses are caused by virosomes. Virosomes might not solely function a way of sending the immunising agent to the body, however, may additionally act as adjuvants to direct the immunologic response to the substance in question. They can quickly attract

the nerve fibre cells and different matter presenting cells to achieve medical speciality edges, being of a particulate kind. The structure of the virosome ensures that the antigen is continually transmitted to the immune system, whether it is inter-related to the lipid bilayer, conjugated to the surface proteins or exist in the central cavity. The delay in the release of the matter can function a way to concentrate the response to the precise matter for a depot-like impact to be achieved. Excessive immune defence against varied diseases can be achieved by combining the delivery of both antigen and the adjuvant. Recent studies of murine models showed up to fourfold higher body substance response within the case of a virosome-based product compared to that determined within the case of the delivery of the aborning substance. Additionally, the combined delivery of antigen and adjuvant can assist in achieving an excessive immune defence against various diseases (Radha *et al.*, 2013; Singh *et al.*, 2017). While comparing the delivery of nascent antigen and the product based on virosome, the murine models have shown fourfold humoral responses in modern studies.

As targeted drug delivering negotiators

Drugs should be altered or wrapped in a way so that restoratively real amount of drug atoms enter the specific site where the action is needed. Change may comprise modification of the medication's physical as well as synthetic parameters resulting in the creation of new elements, and other product components can be mixed to change their in vivo profiles or, sometimes can modifying the physical structures of the drug particles. It can package naturally occurring medicines into themselves. In the focal compartment, the water-seeking medications are prepared amid the virosome generation. Then again the fat-soluble drugs cannot be epitomised in this way, and are built in the lipid bilayer along these lines.

As a method of transporting these medical particles to the probable spot of operation, virosomal disintegration may take place within the cell. In various tests, the exemplification of diverse types of genetic material in the virosome to be used for prophylactic or beneficial purposes was achieved.

The virosome's lipide bilayer makes a distinction in reassuring these remedial operators of different nucleic corrosive corrupting proteins like DNAases and RNAases (Soussan *et al.*, 2011).

After perceiving the specific cell types, the viral glycoproteins aid in the film mix. When expressed, the inherited content can then be used by the cell hardware to establish the encoded qualities.

In gene therapy

A low PH-dependent fusion reaction between the limiting membrane of the endosomal cell compartment and the viral envelope is controlled by hemagglutinin which is followed by cell-by-cell receptor endocytosis intake of the virus particles (Nupur *et al.*, 2015).

In RNA and DNA

The synthesis of newly induced and constitutively conveyed protein and recently induced protein can be successfully reduced by encapsulating small interfering RNA in virosomes, and this can overcome the shortage of suitable methods of delivery. Virosome triggered with SiRNA via Intraperitoneal route can lead to the delivery of nucleotides to peritoneal cells (Muhammad *et al.*, 2012).

In malarial therapy

Virosomes represents a revolutionary method for delivery of drugs for various bioactive molecules, particularly for genes, nucleic acid and for other numerous indications. Virosomal surfaces can be adequately revised to allow targeted drug delivery. Antimalarial peptide formulated virosomes demonstrate strong permissibility and great adaptive immune response in humans. NPNA region and the AMA-1 merozoite are the two peptide region identified which can serve as an antigen for the malarial vaccine (Miyano-hara, 2012).

In cancer treatment

Virosomes have also been used in oncology to bring peptides referring to tumours injected with antigens, such as peptides from parathyroid hormone-related proteins or recombinant proteins such as her -2 neu Fab combined anti-Fab - doxovirosomes combined the anti-proliferate properties of monoclonal antibodies with the cytotoxic activity of doxorubicin in vivo (Bhattacharya and Mazumder, 2011).

CONCLUSION

The virosomal drug delivery system is an intelligent approach for targeted delivery of drugs and several other biologically active molecules, particularly nucleic acids or genes. The main motive behind the development of virosomal technique is to overcome the limitation of inadequate drug delivery towards targeted cells, tissues and organs of the body. Virosomes are biodegradable, non-poisonous, biocompatible and non-auto immunogenic unilamellar phospholipid bilayer vesicles that comprise proteins derived from viruses. Antigens can be delivered to the host body using various

routes of administration like intradermal, intramuscular and intranasal route, depending upon the aim of immunisation. Virosomes are considered as an effective prophylactic and therapeutic agent due to their added advantage of tissue targeting immune activation & potentiation over other targeted drug delivery systems. Hence, they can be widely used in the field of cancer therapy as a carrier for immunomodulating molecules as well as for targeted drugs. Application of Virosome in the field of targeted drug delivery system will bring a new opportunity & also open a new era in the modern pharmaceutical sector & human life too.

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Conflict of Interest

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