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Virosomal Drug Delivery System: A Novel Vaccination Technology.

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ABSTRACT

Virosomal Drug Delivery System is the novel drug delivery system available which has been a great revolutionary technology in drug delivery in recent years. Virosomes are the immunogenic compositions that include methods of eliciting an immune response. Virosomes are spherical, unilammellar phospholipid bilayer vesicle with a mean diameter of range 120-180nm. These represent reconstituted empty influenza virus envelopes, which contain 70% phosphatidylcholine and remaining 30% neuraminidase (NA) and haemagglutinin (HA) glycoproteins. A virosome can include at least one viral surface envelope glycoprotein expressed on the surface of the virosome. The virosome can also optionally include at least one adjuvant molecule expressed on the surface of the virosome. A virosome is a drug or vaccine delivery mechanism incorporating virus derived proteins to allow the virosome to fuse with the target cell. Virosomes cannot replicate but are pure fusion active vesicles. Virosomal drug delivery depends on the methods used to prepare the encapsulated bioactive material their incorporation into the virosomes and followed by the characterization and formulation of the finished preparations. All these features allow us to consider influenza virosomes as a promising model for antigen and molecular delivery, which could be helpful for the development of new vaccines or immunotherapeutic protocols that combine safety with immunogenicity and their applicability in different fields of medical research. This technology can potentially be used to deliver peptides, nucleic acids or genes, and drugs like antibiotics, anticancer agents, and steroids. In this paper reviewed about the challenges in drug delivery, advantages of virosomes in successful delivery of immunogens, formulation, Virosomal Technology and its various approaches.

Keywords: Virosomes, influenza virus, liposomes, hemagglutinin and Neuraminidase.

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INTRODUCTION

The transfer of genes to target cells by various delivery vehicles are mainly divided into viral and non-viral vectors systems. Viral vectors are obtained by replacement of one or more viral genes by a gene of interest and are considered to be the most efficient transducing system. Their efficiency relates to properties of the viral capsid proteins or membrane glycoproteins, such as the ability to bind to cellular receptors and to pass through or fuse with cellular membranes. But the safety of viral vector system remains a matter of major concern, and issues related to insertion mutagenesis as observed with retroviruses and the induction of undesirable immune responses and inflammation still pose major challenges.

For non-viral gene delivery, chemical approaches (e.g. cationic lipids, cationic polymers, and nanoparticles) and physical methods (e.g. gene gun, electroporation) are being employed. Cationic liposomes are the most extensively studied vehicles. In these systems, cationic lipids condense DNA through electrostatic interactions with the negatively charged phosphate groups of the nucleic acid, thereby forming so-called lipoplexes. With respect to in vivo use, liposomal delivery systems combining the characteristics of cellular interaction of viral vectors with the safety of liposomal delivery systems. Virosomes are the reconstitutes viral envelopes, having lipid membranes, viral spike glycoproteins, but absence of viral genetic material.

Externally virosomes resemble that of a virus particle, with spiky proteins protruding from their membrane, and their interior compartment is empty. Almeida et al., were the first to prepare Virosomes, who inserted purified influenza spike proteins into preformed liposomes. Virosome technology is developed in order to overcome the problem of incomplete delivery to target cells, tissues, and organs. The new generation of therapeutics against cancer or neurodegenerative disorders which require the delivery system that target drugs to specified cell types and host tissues by receptor-mediated uptake and controlled release.

Thus, the virosomal technique represents a novel sophisticated delivery system to meet all the above challenges and drawbacks. There after a wide range of viral envelopes have been reconstituted, including those of Sendai virus and sindbis virus. Because virosomes display viral envelope glycoproteins, which in their native conformation stimulate humoral responses. Moreover, since the receptor-binding and membrane-fusion properties of the viral envelope glycoprotein can be preserved, virosomes can be used as transport vehicles for cellular delivery of biologically active macromolecules. In this article, we provide a brief overview of virosomal drug delivery. Overall, virosomes protect pharmaceutically active substances from proteolytic

degradation and low pH within endosomes, allowing their contents to remain intact when they reach the cytoplasm. This is a major advantage of virosomal carrier systems over other drug-delivery vehicles, including liposomal and proteoliposomal carrier systems [1-12].

The prospect of drug delivery and targeting using virosomes is an interesting field of research and development. As virosomes are biocompatible, biodegradable, nontoxic, and non-auto immunogenic, and various attempts have been made to use them as vaccines or adjuvants as well as delivery system for drugs, nucleic acids, nucleic acids, or genes for therapeutic purposes.

Challenges in drug delivery:

State-of-the-art-drugs must meet diverse medical and pharmacological demands. They have to be efficient, reliable and safe. Severe side-effects have to be avoided. Market aspects also need to be considered. Due to the high number of competing products on the market, drugs should be cost effective. Stability is an issue as well, because drugs are often subject to unfavorable delivery and storage conditions. Moreover, the market potential of a drug also rises with its compliance and convenience. Apart from these requirements, novel drug delivery technologies should provide improved bioavailability, minimizing the quantities of the drug needed to be taken by the patient. Several drugs in clinical tests are discontinued at various stages in the development process because of the lack of suitable delivery technologies. An appropriate drug delivery system may rescue some of the products by overcoming these difficulties. Furthermore, the new generation of therapeutics against cancer or neurogenerative disorders needs delivery systems which guarantee targeted drug delivery to specified host tissues, receptor-mediated uptake and controlled release into the lumen of the cell. All these challenges with the development of a highly sophisticated carrier system based on its proprietary virosomal technology should be met.

Advantages of Virosomal Drug Delivery[10, 12]:

- Virosomal technology is approved by the FDA for use in humans, and has a high safety profile.
- Virosomes are biodegradable, biocompatible, and non-toxic.
- No disease-transmission risk.
- Patent protected.
- No autoimmunogenity or anaphylaxis.
- Broadly applicable with almost all important drugs (anticancer drugs, proteins, peptides, nucleic acids, antibiotics, fungicides).
- Enables drug delivery into the cytoplasm of target cell.
- Promotes fusion activity in the endolysosomal pathway.

- Protects drugs against degradation.
- Encapsulation of drug protects patient against side effects.
- Target-specific delivery of antigens and amplification of the immune response.
- Extended uptake, distribution and elimination of the drug in the body.
- Virosomes allow patient specific modular vaccine regimen.
- Up-scaling according to standard procedure.
- The fully functional fusion-activity of virosomes enables receptor mediated uptake and natural intracellular of the antigen, which leads to stimulation of both arms of the immune system such as humoral and cellular immune responses.
- The antigen is partially protected from extracellular degradation and the resulting depot effect greatly facilitates immune potentiation.

Virosomal Ultrastructure and Modifications:

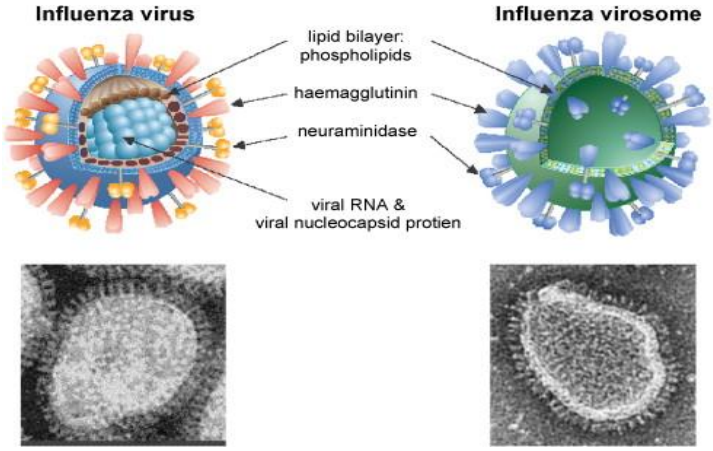

Figure 1: Virosomes are reconstituted influenza virus envelopes devoid of inner core and genetic information

Virosomes is a drug or vaccine delivery mechanism consisting of spherical unilamellarphospholipid membrane vesicles with a mean diameter of around 150 nm incorporating virus derived proteins to allow the virosomes to fuse with target cells. Virosomes cannot replicate but are pure fusion-active vesicles.

Influenza virosomes:

In contrast to liposomes, virosomes contain functional viral envelope glycoproteins: influenza virus hemagglutinin (HA) and neuraminidase (NA) are intercalated within the phospholipid bilayer membrane (Figure 1).

Essentially, virosomes represent reconstituted empty influenza virus envelopes, devoid of the nucleocapsid including the genetic material of the source virus.

The unique properties of virosomes partially relate to the presence of biologically active influenza HA in their membrane. This viral protein not only confers structural stability and homogeneity to virosome-based formulations, but it significantly contributes to the immunological properties of virosomes, which are clearly distinct from other liposomal and proteoliposomal carrier systems. It has been shown that a physical association between the virosome and the antigen of interest is necessary for the full adjuvant effect of virosomes. Such physical association can be achieved by a variety of methods, depending on the properties of the antigen. Antigens can be incorporated into virosomes, adsorbed to the virosome surface, or integrated into the lipid membrane, either via hydrophobic domains or lipid moieties cross-linked to the antigen.

Virosomes therefore represent an innovative, broadly applicable adjuvant and carrier system with prospective applications in areas beyond conventional vaccines. They are one of only three adjuvant systems widely approved by regulatory authoritiesand the only one that has carrier capabilities.

Non-influenza virosomes:

They are also being considered for HIV-1vaccine research. They were used as a drug carrier mechanism for experimental cancer therapies. Further characteristics of virosomes depend on the choice of bilayer components. Virosomes can be optimized for maximal incorporation of the drug or for the best physiological effect by modifying the content or type of membrane lipids used. It is even possible to generate carriers for antisense-oligonucleotides or other genetic molecules, depending on whether positively or negatively loaded phospholipids are incorporated into the membrane.

Various ligands, such as cytokines, peptides, and monoclonal antibodies (MAbs) can be incorporated into the virosome and displayed on the virosomal surface. Even tumor-specific monoclonal antibody fragments (Fab) can be linked to virosomes to direct the carrier to selected tumor cells.

Virosomes: A Versatile Carrier System:-

Virosomes represent an innovative, broadly applicable carrier system with various prospective applications for the treatment and prevention of cancer, neurodegenerative disorders and infectious diseases. Various pharmaceutically active substances like antibiotics, cytostatics, nucleic acids, fungicides and antigens can be encapsulated into the virosomal carrier. Even the surface of virosomes can be readily modified.

Virosomal technology has been successfully applied for many years in vaccines. Due to the reliability and favorable side-effect profile, virosomal carriers provide high efficiency and safety.

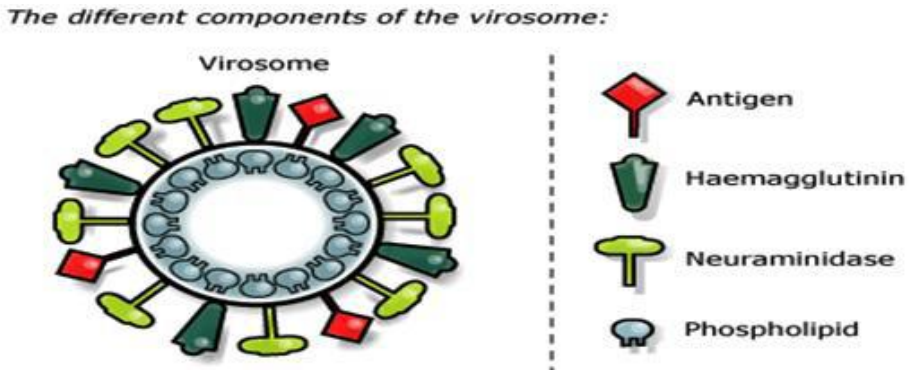

Figure. 2: Showing different components of the virosomes

Difference from liposomes[13]:

Liposomes have been considered promising vehicles for targeting and delivery of biologically active molecules to living cells both *in vitro* and *in vivo*. However, liposomes have little potential to fuse with cells and thus, generally fail to provide appreciable delivery of encapsulated molecules to the cell cytoplasm. In contrast, virosomes contain functional viral envelope glycoproteins with receptor-binding and membrane-fusion properties that enable the cellular delivery of encapsulated molecules.

Table 1: Difference between Liposomes and Virosomes

Liposomes	Virosomes
Liposomes have been considered promising vehicle for targeting and delivery of biologically active molecules to living cells both *in vitro* and *in vivo* but have little potential to fuse with cells and due to this reason fail to provide adequate delivery of encapsulated molecules to the cell cytoplasm.	Virosomes contain functional viral envelope glycoproteins with receptor-binding and membrane-fusing properties that enable the cellular delivery of encapsulated molecules 22.

Fusion activity of virosomal carriers[13, 14]:

Virosomes has the unique properties of fusion because of the existence of influenza HA in their membrane. HA is responsible for the structural stability and virosomal formulation homogeneity, also significantly contributes to the fusion activity of virosomes. Virosomal HA promotes binding at the target cell surface followed by receptor-mediated endocytosis.

The acidic environment of the endosome responsible for stimulation of HA-mediated membrane fusion, and the therapeutically active substance escapes from the endosome into the cytoplasm of thetarget cell. Thus, virosomal HA significantly enhances cytosolic delivery. Thus virosomes protect pharmaceutically active substances from proteolytic degradation and low pH within the endosomes before reaching the cytoplasm. This is a major advantage of virosomes over

liposomes and proteoliposomal carrier systems, which provide less protection for therapeutic macromolecules from different compartmental unadoptable micro-environments. (Fig-3)

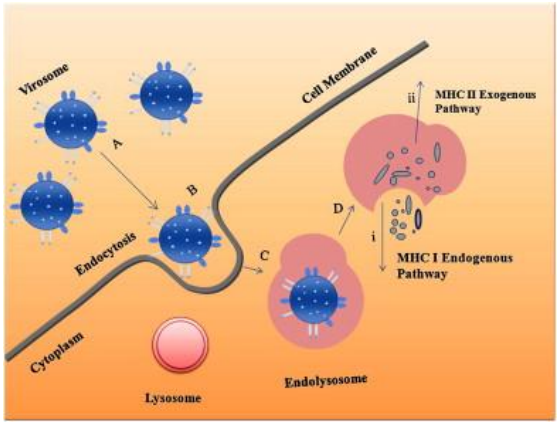

Figure 3: A) Virosomes bind with ligands and HA respectively at the selected cell. B) Receptor-mediated endocytosis enables uptake of the virosome.C) Virosomal HA has a lowering effect on the pH into the endosome. D) This acidic environment within the endosome triggers fusion of the virosomal membrane with the endosomal membrane, resulting in release of drugs into the cytosol of the cell.

Method of formulating virosomes[15-17]:

For virosome preparation, viral membrane-fusion protein such as HA-generally preferred fusion protein for virosomes which is either purifies from the corresponding virus or recombinant produced using gene manipulations. For virosomes as a successful vaccine or delivery system, the major requirement is reconstituted membrane proteins that retain their immunogenic properties and those having receptor-binding and membrane-fusion activities.

a) Selection of virus:

Influenza virus envelope is most often used to produce virosomes but it can also be made from sendai-virus, Epstein-burr virus, sindbis, semliki-forest virus, friend murine lukaemia virus, herpes simplex virus, Newcastle disease virus.

b) Selection of Antigen:

Antigens such as parasite, carcinogenic cell, bacterium or whole cell can be used as antigens, which are selected to our requirement. Cell components such as DNA, RNA, and Plasmid can also be used as antigen. This antigen is coupled to lipid anchor so antigen will be ready to load on virosome.

This involves reconstruction of influenza virus membranes, based upon solubilizing viral membranes by detergents having no denaturing tendency. Influenza virus envelopes incorporated

with HA can also be solubilized with nonionic detergents of lower critical micellar concentration (CMC). Other nonionic detergents can also be used.

c) After Solubilization:

The viral nucleocapsid, containing endogenous viral genes, removed by ultra-centrifugation. The viral membrane reconstitution depends upon removal of $C_{12}E_8$ followed by its adsorption on a hydrophobic resin. In this method production of virosomes depends upon pH similar to that of native influenza virus. This method has inherent drawback particularly to maintain sterility as method involves batch processing, often in open systems. Compound encapsulated in virosomes adsorbed inactivated by the hydrophobic resin and difficulty in removal of low-CMC detergents & removal of detergent by dialysis can increase the above mentioned complications.

Dialysis requires the use of detergents with relatively high CMCs, such as N-octyl- -D-glucopyranoside (octylglucoside) that can effectively solubilize influenza virus envelopes. However, fusogenicvirosomes are not readily prepared by subsequent removal of the octylglucoside detergent.

During dialysis, the HA concentrates primarily in lipid-poor aggregates with a very limited aqueous space, while the viral lipid is recovered in protein-poor vesicles. Although these vesicles exhibit some HA-mediated membrane fusion activity, only a small fraction of the HA is recovered in these vesicles. Researchers are in pursuit of novel detergents and detergent-like compounds that can be almost completely removed by dialysis. These will be crucial for refining an effective dialysis procedure to reconstitute influenza virus membranes for industrial purposes.

Other lipids also can be added to the membranes during preparation. These lipids include cholesterol and phospholipids such as phosphatidylcholine, sphingomyelin, phosphatidylethanolamine and phosphatidylserine. Cationic lipids also are added to concentrate nucleic acids in the virosomes or to facilitate virosome-mediated cellular delivery of nucleic acids or genes. These include, DOTAP: (N-[1-(2, 3-dioleoyloxy) propyl] - N, N, N-trimethylammonium chloride), DODAC: (N, N-dioleyl-N, N, dimethyl ammonium chloride), stearylamine, etc. DODAC is the preferred cationic lipid for complexing nucleic acids to the virosome to ensure cellular delivery of nucleic acids. Concentrations of DODAC in the range of 25–45% are particularly good to ensure cellular delivery of nucleic acids.

Additional components can be added to the virosomes to target them to specific cell types. For example, virosomes can be conjugated to MAbs that bind cellular epitopes present on the surfaces of specific cell types.

Fine-Tuned Manufacturing Process[19, 25, 26]:

Biologics in general and vaccines in particular are highly complex products. The challenge is to establish a sleek, robust, cost-effective manufacturing process, which yields a precisely defined nanoparticle structure composed of multiple components for medical use, the virosome.

Modular assembly:

A virosome particle is a suitable carrier and adjuvant for a wide variety of antigens of interest, such as synthetic peptides, recombinant proteins, bacterial toxins, or carbohydrates. For optimal induction of antibodies against the antigen of interest, multiple copies of the antigen have to be displayed on the surface, tightly associated with the virosome structure. How this is achieved with highest efficiency depends on the biochemical properties of the antigen of interest.

If the antigen of interest features a lipophilic domain (e.g. transmembrane proteins), it integrates directly into the virosome membrane without further modification. In most cases, however, the antigen has to be linked to a lipid molecule in order to anchor it in the virosome membrane. Pevion has developed extensive know-how in these conjugation methods and has several approaches at its disposal. The source of the antigen (viruses, bacteria, fungi, parasites or cancer cells) is not relevant for the formulation, but synthetic or recombinant antigens are preferred for their higher purity and potential to focus the immune response on relevant epitopes.

Precise product specifications:

The controlled assembly process allows a fine tuning of the concentrations and ratios between the individual components (antigen of interest, lipids, adjuvant proteins) to achieve the desired product properties with respect to size, stability, and immunogenicity. Virosomes therefore represent a true platform technology with very broad applicability.

The individual components of the vaccine are produced separately and are subsequently assembled into virosomes in a simple biochemical procedure. Synthetic or purified lipids, synthetic peptides, and recombinant proteins are sourced from GMP-certified providers. Under optimized conditions, the assembled nanoparticles are highly homogenous and can be used without further purification. The final product is fully amenable to direct and precise physical and biochemical analysis. This is in sharp contrast to vaccines with alum or oil-based adjuvants, both of which interfere with many analytical methods. As a consequence, clear and precise product specifications for virosome-based vaccines can be defined early on, which translate into a more reliable and focused process development, thus providing a convincing database to the attention of regulatory authorities.

Scalability:

The manufacturing process of virosomes is established on an industrial scale and under full GMP compliance, as confirmed by the continuous production of Epaxal® and Inflexal®V. The *in vitro* assembly of the virosomes is performed at high concentration and thus, in a small volume, thereby allowing for large-scale production of up to 500'000 doses per run even in small facilities.

Virosomes are the only VLP assembled in-vitro, not by host cell:

Pevion's virosome technology is a clinically and market-validated carrier and adjuvant system designed specifically for subunit vaccines. Virosomes belong to the established class of VLP (virus-like particles). They are spherical, unilamellar vesicles with a mean diameter of 150 nm. Essentially, virosomes resemble empty influenza virus envelopes composed of phospholipids and influenza virus envelope proteins. They do not contain internal proteins or genetic material

Virosomes are therefore unable to replicate but feature many properties of an Influenza virus with respect to the interaction with host cells. The viral proteins hemagglutinin (HA) and neuraminidase (NA), embedded in the spherical membrane, not only confer structural stability and homogeneity to the virosome particles; they also are the key to their immune-stimulating properties.

Virosomes are the only VLP that are assembled in a tightly controlled process *in vitro* and independent from any host cell giving rise to products of well-defined composition and high purity. This is a prerequisite for complex state-of-the-art biologics.

Virosomes are assembled in a controlled manner:

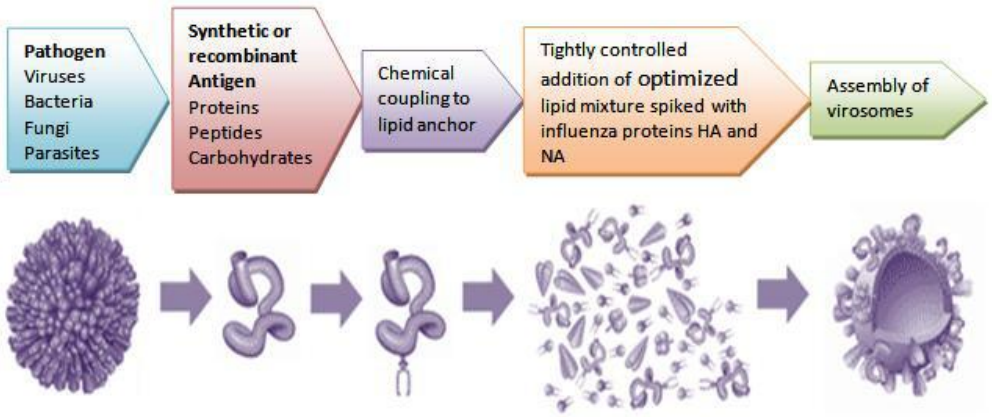

Figure 4: showing assembly of virosome in a controlled manner.

CHARACTERISATION OF VIROSOMES[17-19]:

Detection of protein:

sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) can confirm the presence of HA protein in the virosomes.

Fusion activity:

Generally virosomes exhibit pH-dependent membrane fusion activity similar to native influenza virus. Virosomal fusion with biological or artificial target membranes can be visualized with a fluorescent resonance energy transfer assay (RET). Alternatively, fusion can be assessed in vitro with an excimer assay using pyrene-labeled lipids, where the decrease of surface density of the pyrene-phosphatidylcholine-label on fusion with an unlabeled membrane corresponds to a reduction of excimer fluorescence.Fusion activity also can be indirectly monitored by determining hemolytic activity, which corresponds closely to fusion activity and exhibits a p^H dependence identical with that of fusion.

Structure and size:

Negative-stain electron microscopy can generally be used to determine the ultrastructure and size of virosomes. The staining solutions should preferably be of neutral pH, to avoid acid-induced conformational changes of HA.

Virosome technology:

Virosome technology is a tool for developing novel, predominantly synthetic vaccines against infectious and chronic diseases. A virosome is a virus-like particle that acts as a vaccine carrier and adjuvant (immunity enhancing) system. Vaccines based on virosome technology combine high efficacy with high purity, which means they are effective and safe to use even in infants and individuals with a weakened immune system.

Key Strengths:

- Virosome technology provides a versatile system for delivering virus antigens or DNA/RNA encoding specific immune-stimulatory proteins.
- Virosome technology enables target-specific delivery of antigens and amplification of the immune response.
- Virosomes stimulate both arms of the immune system, eliciting both antibody and cellular immune responses to maximize protection against the targeted disease.
- Virosomes are completely biodegradable and can exert an immune response via different routes of administration.

- Virosome technology is already used in the manufacture of a number of Crucell's licensed vaccines. We can demonstrate an excellent track record for safety and manufacturing expertise in this field.

VIROSOME UPTAKE BY CELLS[19, 20]:

Entry of virosomes into target cells divided into two different steps:

Attachment:

This involves binding of the virosomes via HA to the cell receptors that are a membrane glycoprotein or glycolipid with terminal sialic acid. In case of specific virosomes, Fab' fragments are coupled by a cross-linker with a spacer arm to the virosomal surface. Specific virosomes will additionally recognize antigenic structures on the target cell surface, resulting in an attachment to target cells by two different binding mechanisms. Thus, specific virosomes exert selectivity for special cell types.

Penetration:

After attachment entry of virosomes occurs by receptor-mediated endocytosis. The virosomes are trapped in endosomes. The acidic fusion of the virosomal membrane with endosomal membrane is done. The fusion is mediated by the viral spike glycoprotein hemagglutinin (HA). The membrane-fusion reaction in the endosome librates the virosomes from its lipid envelope and provides access for the encapsulated drugs to the cytosol.

MODE OF ACTION OF VIROSOMES[21-23]:

The outstanding profile of combined efficacy and safety that virosome-based vaccines are known for has its origin in the unique mode of action of these multifunctional particles. It leads to a comprehensive induction of a complete immune response-particle structure key to multi-functionality, in contrast to single-sided triggers.

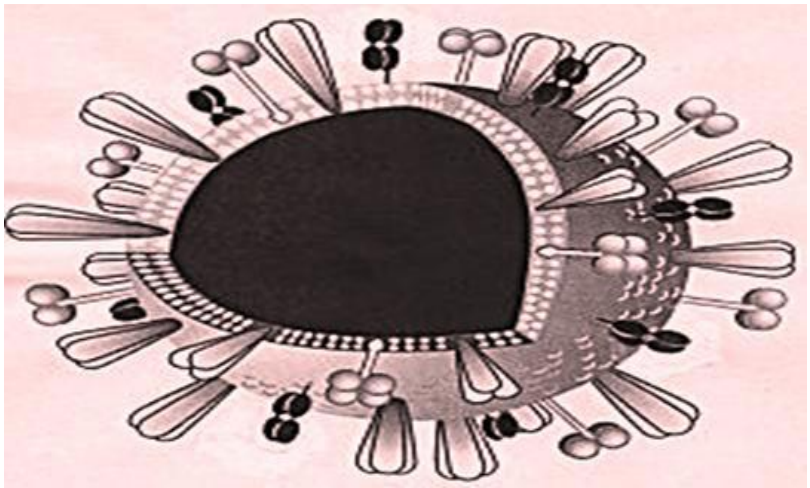

Figure. 5: Hemaglutinin (large trimers), neuraminidase (black tetramers), as well as fab' fragments with a spacerarm are anchored in the lipid bilayer of virosomes.

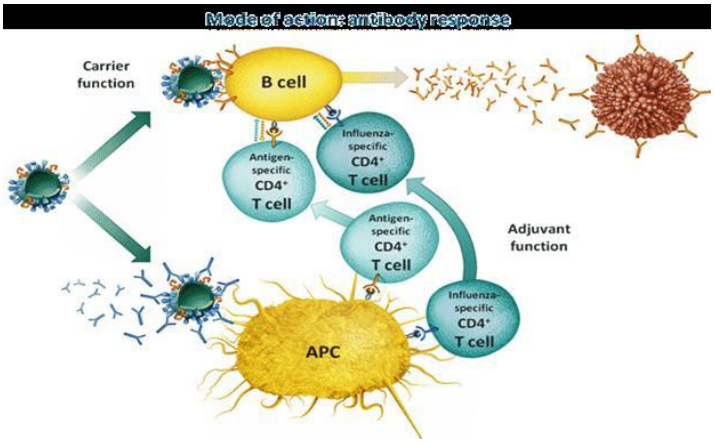

Figure. 6: mode of action of virosomes

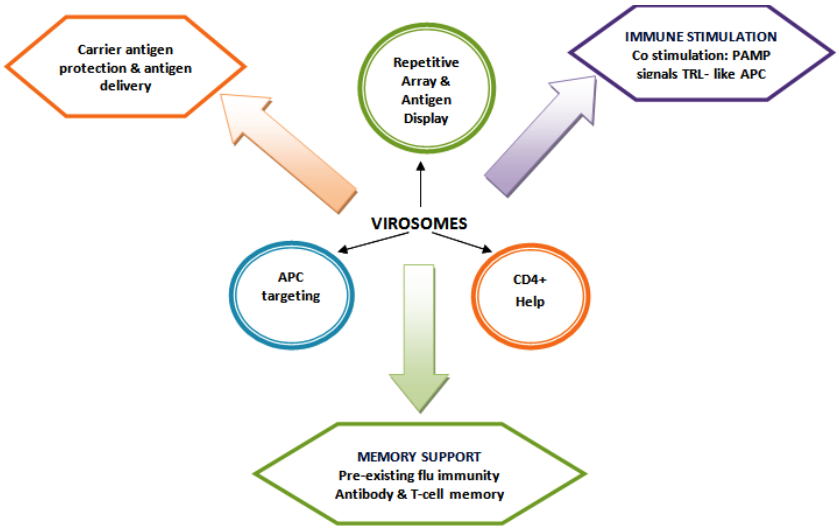

Figure 7: Showing activation of antibody through Virosome.

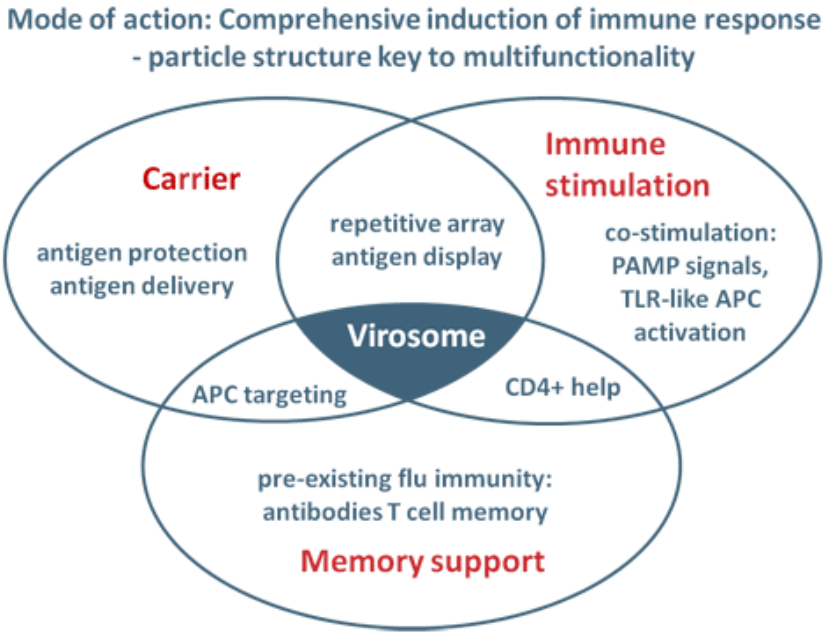

Figure 8: mode of action of virosome.

Functions by the Carrier:

The integration of the antigen into the higher structures of the virosomes particle stabilize the antigens, preserves the native status of B cell epitopes, and protects the antigens from degradation. Moreover, the presentation of the antigen as a repetitive surface structure enhances its recognition by antibody-producing B cells.

Memory Support:

The presence of influenza-derived hemagglutinin (HA) provokes a memory response, as a vast majority of people have a degree of natural, pre-existing immunity against influenza. This comprises both humoral and cellular immunity: pre-existing influenza-specific antibodies tag virosomes efficiently for rapid uptake and processing by antigen presenting cells (APC). Memory T helper cells rapidly proliferate and secrete cytokines to support and enhances the induction of effector immune cells.

Immune Stimulation:

In addition to the influenza-specific antigens, virosomes provide pathogens-associated molecular patterns (PAMP) that deliver co-stimulatory signals to APC, which leads to a TLR-like activation.

APPROACHES TO DRUG DELIVERY THROUGH VIROSOMES:

1. Bioactive drug compounds can be entrapped in the aqueous interior of the virosomes or in the lipid membrane of the virosomes for facilitated entry of the compounds into the cells.
2. Virosomes are particularly useful for delivering nucleic acids or genes. These compounds are delivered into the host cell cytoplasm on fusion of the virosomes with the endosomes or plasma membrane[23].
3. Nucleic acids or genes encoding a naturally occurring protein can be introduced into host cells and can be expressed, provided that the expression cassette contains the proper cis-acting regulatory elements[27].
4. Drugs or nucleic acids can be incorporated into the virosomes at the time of virosomes preparation. The bioactive compound is typically added to the lipid-HA-containing solution following removal of the nucleocapsid. Alternatively, the bioactive compound is initially incorporated into liposomes, which is then fused with a virosomes containing two hemagglutinins with different pH threshold to form a virosome-liposome hybrid [24].
5. Proteins also can be delivered to cells via virosome. For example, the gelonin subunit A of diphtheria toxin and ovalbumin has also been successfully delivered by virosomes to

target cells[24].

6. Virosomes carrying peptides derived from the influenza nucleoprotein or intact ovalbumin induced strong cytotoxic T- lymphocyte responses, which suggests that the encapsulated peptides and proteins gained access to the cytoplasm.
7. Antisense-L-myc-virosomes:antisense-L-myc-phosphorothioateoligodeoxyribo nucleotides were encapsulated into the virosomes. The antiproliferative effects of virosomal-encapsulated L-mycantisence DNA in the SCLC cell lines H209, H510, and H82 were evaluated. Antisense-L-myc-virosomes were added to the cells of human small cell lung cancer cell lines that expressed led to strong inhibition of thymidine incorporation in a concentration-dependent manner. Virosomes-entrapped sense L-myc OPT and random-order OPT had only minimal effects on the thymidine uptake[30].
8. Virosomes in Cancer Treatment: Virosomes have been also used in the oncologic field to carry peptides corresponding to tumor associated antigens (TAA). The application is forwarded to particularly prostate, breast tumors and bone metastase[28].
9. Virosomes in Malaria Therapy: Major advances in genomics have identified numerous protein vaccine candidates, among which only primary sequence information is available. The production of stable preparation of properly refolded recombinant proteins has turned out to be a major challenge. So synthetic antigens consisting of partial sequences are more stable which can focus immune response[28].
10. Virosomes can be used as adjuvants in vaccines, as some of the vaccines contain inactivated adjuvant which potentiates immune response to the antigen. So virosomes can be used as the alternative adjuvant.
11. These can be used for activation of Murine Lymphocyte, by incorporating LPS into the phospholipid vesicles[27].
12. These can be used in RNA/DNA Delivery and even Gene Delivery.

Targeted drug delivery:

Effective use of active substances is often hampered by their failure to act in selected tissues. For the targeted delivery of the encapsulated drugs, virosomes selectively bind through binding molecules (e.g. Fab fragments and ligands) to the target cell. The patient's non-diseased tissues are not affected. Virosomal HA triggers receptor-mediated uptake of the virosome into an endosome in the target cell. The endolysosomal pathway protects the drug from degradation since the drugs are transported directly into the cytosol of the cell. This characteristic trait of virosomes is especially desirable for cancer therapies, which often have severe side effects

because of the toxicity of the agents.

Ideally one would like to be able to target drug delivery to selected tissues. One can tailor virosomes to targets by incorporating specific molecules (e.g., Fab fragments and ligands) into the virosome's composition. The feasibility of targeted delivery of anticancer OR cytotoxic drugs by means of virosomal carrier has been demonstrated recently by two independent approaches. In one, a MAb cross-linked to the surface of virosomes mediated specific targeting of the virosomal carrier containing an anticancer drug (e.g., doxorubicin) to human cancer cells. MAbs can bind specifically to cancer-related antigens, providing a means to target systemically administered virosomes to cancerous tissues. Alternatively, ligands that bind surface receptors on the target cells also can be bound to the virosomes to achieve targeted drug delivery. Tumors of mice treated with targeted drug-loaded virosomes failed to grow, and mortality of these animals was significantly reduced. These positive results will definitely open a new field of applications for virosomal technology.[18, 19]

Administration of Virosomes[17-22]:

- Several formulations have been reported. Generally, virosomes are suspended in buffered saline (135–150 mMNaCl), but other suitable vehicles also exist. These compositions should be sterilized by conventional liposomal sterilization techniques, such as membrane filtration.
- The formulation also generally contains auxiliary substances as required to simulate physiological conditions, such as buffering agents and isotonicity adjusting agents (sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride).
- The concentration of virosomes used in the vehicle ranges from 20–200 mg/mL. These concentrations are varied to optimize treatment with different virosome components or for particular purposes.
- The virosomes are administered in a variety of parenteral routes, including intravenous, intramuscular, subcutaneous, intra-arterial, and inhalable delivery.
- In addition, virosomes can be administered topically, orally, or transdermally.
- The virosomes also can be incorporated into implantable devices for long-term release.

Future prospective:

Virosomes represent a new innovative advanced drug-delivery system for the biologically active molecules, but especially nucleic acids or genes. The surface of virosomes can be suitably modified to facilitate targeted drug delivery.

But there is need for comprehensive pharmacokinetic profile, bioavailability and clinical effects and safety and not the least stability studies to be covered thoroughly in order to ascertain long-term reliability as a safe, effective, and affordable means for targeting and delivery of drug. Virosomes are particularly well-suited to address new vaccine indications, since they are a highly versatile toolbox type system allowing the use of difficult antigens and/or targets (e.g. peptides).

Two virosome-based vaccines, Epaxal® and Inflexal® V (both marketed by Crucell Switzerland AG, Johnson & Johnson subsidiary) are licensed in over 40 countries and more than 70 million doses of these vaccines have been commercially distributed, thereby providing a solid safety and efficacy track record for the virosome technology platform.

Virosomes represent a unique combination of technical versatility and clinically proven safety and immunogenicity. In contrast to other carriers or adjuvant technologies currently in development, virosomes have a well-tuned proven equilibrium of efficacy and tolerability, a prerequisite for the development of efficient and safe vaccines.

CONCLUSION:

Vaccine development has continuously shifted away from live attenuated or inactivated whole organisms. Although this approach having good efficacy but for improvement on comparison of risk/benefit ratio which needs improvement because of highly complex compositions for safety concerns. The next generation vaccines represented as subunit vaccines, whereby the only pathogens fragments used which are relevant in inducing protective immunity. For the successful vaccination two major key requirements are safe carrier and adjuvant system, since the small, isolated pathogen fragments themselves are generally weak immunogens. It leads to a comprehensive induction of a complete immune response, in contrast to single-sided triggers.

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