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Virosome Delivery System: Concepts and Applications

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ABSTRACT

A virosome is a revolutionary hybrid drug delivery system that combines the advantages of viral and non-viral vectors. According to study, virosomes may carry a range of physiologically active molecules such as nucleic acids, peptides, proteins, and small chemical compounds. Surface modifications in virosomes might be leveraged to allow for tailored drug administration using virosome-based devices. On the market today, there are a number of virosome-based preventative and therapeutic drugs with outstanding safety profiles. Cancer treatment is a key battlefield for virosome-based medication delivery technologies. This research covers the fundamental concept, manufacturing techniques, operating mechanisms, preclinical studies, and clinical applications of virosomes.

Key Words: Virosomes, Drug Delivery, Vaccine, Mechanisms, Preparations, Applications

INTRODUCTION

Delivery systems that enable target medications to be administered to particular host tissues and cell types via regulated release and receptor-mediated uptake are included in innovative therapeutics for neurological or cancer disorders. For resolving these difficulties, virosomal technology provides a novel and demanding delivery technique. Molecules are inserted directly into cells to boost the efficacy of gene delivery. Virosomes are fusion genic viral cover proteins that incorporate antigen, medicine, and DNA, among other things. Improving virosome delivery ability in vivo is a major objective in virosome research. Despite advances in non-viral and viral vector systems, overcoming the plasma membrane's permeability barrier, followed by controlled release inside the cytoplasm, remains a substantial hurdle to delivering medications and other macromolecules into the target cell type. Virosomes are regenerated influenza viral envelopes that are empty. Infectious nucleocapside should be replaced with a chemical of your choice wherever it is found. Virosomes are pure fusion activity vesicles that carry an incorporated substance, such as medicine, antigen, or genes, into the target cell. Virosomes protect pharmaceutically active substances from proteolytic degradation and low pH inside endosomes, allowing them to reach the cytoplasm intact (Figure 1). Recently discovered drug delivery vehicles, such as proteoliposomal and liposomal carriers, were surpassed by the virosome carrier technology [1].

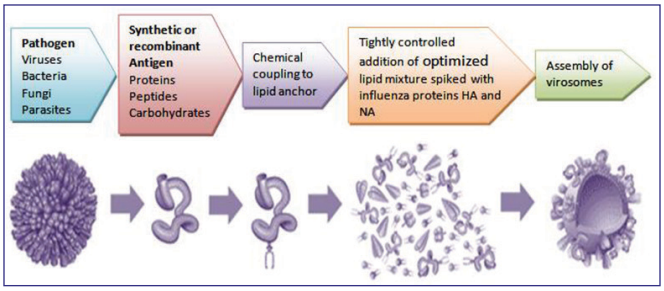

Figure 1: Assembly of virosome in a controlled manner.

STRUCTURE

Virosomes are spherical or unilamellar phospholipid bilayer vesicles with a typical diameter of 120-180 nm (Figure 2). The influenza virus is the most common virus used to synthesize virosomes and genetic material. Virosomes are incapable of replication unless they are in the presence of pure fusion active vesicles. Immune-stimulating Regenerate

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Influenza Virosomes (IRIVs) include the bulk of naturally occurring phosphatidylcholine (PC) and phospholipids (PL). Over 70% of the virosomal structure is made up of PC. The remaining 30% of membrane components include influenza virus envelope phospholipids, which aid in the delivery of hemagglutinin (HA) and neuraminidase (NA) glycoproteins. By modifying the number or kind of membrane lipids used, virosomes may be modified to improve drug inclusion or have a higher physiological effect. Depending on whether there are positively or negatively loaded phospholipids in the membrane, carriers for antisense oligonucleotides or other genetic molecules may be generated. Peptides, cytokines, and monoclonal antibodies are all ligands that may be integrated into the virosome (MAbs). It was also discovered on the surface of the virosomal nucleus. Virosomes may be coupled to tumor-specific monoclonal antibody fragments to drive the carrier to particular tumour cells (Fab) [2].

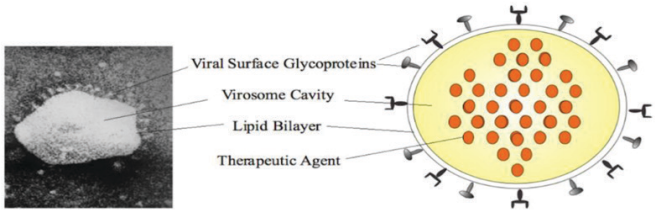

Figure 2: Basic structure of virosomes.

FUSION ACTIVITY

Because influenza HA is present in virosome membranes, they have peculiar fusion properties (Figure 3). HA not only provides structural stability and consistency to virosomal formulations, but it also aids virosome fusion. By adhering to the target cell surface, virosomal HA improves receptor-mediated endocytosis. The acidic environment of the endosome triggers HA-mediated membrane fusion, which allows the therapeutically active chemical to escape from the endosome and into the cytoplasm of the target cell. As a consequence, virosomal HA significantly enhances cytosolic delivery. In general, virosomes in endosomes shield pharmaceutically active substances against proteolytic degradation and low pH before they reach the cytoplasm. The virosomal carrier system has this advantage over liposomal and proteoliposomal carriers, which offer less protection for therapeutic macromolecules in hostile compartmental microenvironments [3].

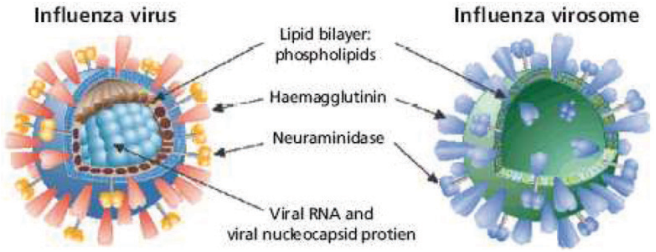

Figure 3: Virus and Virosome.

VIROSOME-CELL INTERACTION

The ability of the virosome innovation to mimic an in vivo contaminated express, which may aid in drawing resistant players and macromolecule organisation to a specific region of action, is its key benefit. Virosomes identify and attach to the same receptors that are engaged when a virus infects a person. For example, flu virosomes use sialic corrosive receptors. Once the infection detects the cell receptor, the combination of viral and endosomal layers are examined. When flu virosomes emerge, the HA viral protein, for example, uses its dipartite get together for the same goal. The NA enzyme is also included in the virosome assembly process because it enhances immunogenicity and virosome concentration in a specific tissue. The relationship between virosomes and receptors has been investigated for the treatment of a range of illnesses, including parasite infections, viral infections, neurological disorders, and a variety of other metabolic issues. The fundamental goal in each case is to deliver a nano-sized protein, corrosive nucleic acid, or medication particle to the predicted action site. The glycoproteins on the virosome's surface have been effectively linked with peptides and proteins. By integrating the monitoring proteins of the Hepatitis C infection surface proteins with the virosomal proteins positive recruitment of cytotoxic, white blood cell insusceptible response, vaccines against the Respiratory Syncytial Virus (RSV) have been developed using flu virosomes. In response, flu virosomes were used to establish epitopic B-cell sites, which are particularly powerful against intestinal disease. In addition, the use of virosome architecture has been examined in a number of disorders. In this vein, this approach benefits in maintaining a strategic distance between several immunization doses [4].

RATIONALE

- Solubilization of poorly water-soluble drugs: diazepam, vitamin A, dexamethasone palmitate.
- Site specific delivery to various organs: cytotoxic agents.
- Potential for sustained-release dosage forms: dexamethasone palmitate, barbiturates, physostigmine salicylate [5]

OPTIMIZATION OF VIROSOMES

Hydrophilic polymers [such as polyacryloylmorpholine, polyethylene glycol, polyvinylpyrrolidone, and poly(2-oxazoline)] may be inserted into the envelope of virosomes to increase their circulation duration following systemic delivery. More importantly, targeting molecules (such as antibodies) may be linked to these hydrophilic polymers and

delivered into the virosome membrane to allow for target selection [6].

ADVANTAGES

- Allow the drug to enter the target cell's cytoplasm.
- Virosomes naturally decay.
- Prevents the tainting of medications by corruption.
- Biocompatible and non-lethal
- There is no danger of disease spread.
- No signs of autoimmunity have been found.
- Anticancer drugs, proteins, peptides, nucleic acids, antibiotics, and fungicides are all common medications.
- Promotes endolysosomal pathway combination movement.
- Antigen delivery to specific targets and amplification of immune responses
- Drug absorption, distribution, and excretion in the body are all increased.
- Upscaling should be done as normal.
- Virosome enables a modular immunization plan that is customized for each patient.
- It may be controlled with a nasal spray or an injection.
- A cell's active substance is released into its cytoplasm. The antigen is protected to some degree from extracellular degradation, and the resulting depot effect improves immune potentiation greatly.
- Virosome enables tolerable tailored modular vaccination regimens with antigen delivery to particular targets and immune response amplification [7].

DISADVANTAGES

- They may provoke immunological responses due to the presence of viral glycoprotein on the surface.
- One issue with virosomes is that they break down quickly in the blood compartment and have a limited shelf life.
- Production problems.
- Low-quality raw materials
- The payload is inexcusably slow.
- There is no indication that virosomes can be used indefinitely.
- By improving virosome stability or ensuring that virosomes reach target areas immediately after injection, rapid disintegration may be prevented.
- Using high-quality products that have undergone more rigorous purifying methods.
- A quality control test may be done using modern technology, and batch to batch variability can be measured.
- Remote loading techniques are employed to alleviate payload concerns.
- To prolong shelf life, use appropriate cryoprotectants and lyoprotectants.
- Choosing the correct preparation and sterilization procedure, such as autoclaving or membrane filtering, in conjunction with an aseptic and validated pyrogen removal Lal test, may aid scale-up [8].

DIFFERENCE BETWEEN VIROSOMES AND LIPOSOMES

Viral envelope glycoproteins with receptor-official and layer combination features empower the cell. Liposomes have been proposed as suitable carriers for concentrating and transferring organically dynamic atoms to living cells in vitro and in vivo. Liposomes may interact with cells, however they often fail to provide observable transport of targeted atoms to the cytoplasm. Small particles may be transported via virosomes [9].

MECHANISM OF ACTION

Virosomes act as a drug transporter and adjuvant with a variety of roles during the recruitment of a body-safe response. The transporter research considers the advantages of implanting the antigen into a higher structure, the virosome molecule. Adjuvant capability identifies the immune-stimulating properties of virosomes and their portions on the resistant framework (Figure 4). Above all, when it comes to reviving particular invulnerability while avoiding nonspecific aggravation, virosomes come out on top.

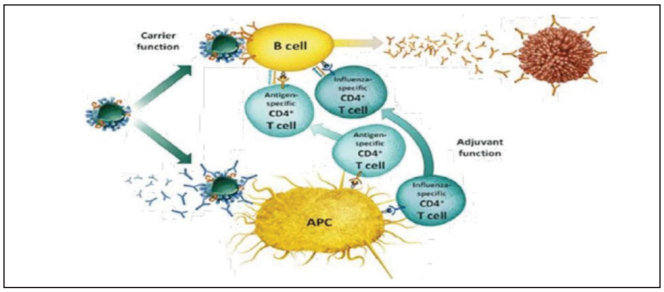

Figure 4: Mode of action of virosomes.

Carrier Function Response

The antigen is blended with the virosome's higher structure, which balances it out and protects it from debasement while jamming B cell epitopes in their local state. The antigen seems to be virosomals, which are similar to the original infection or target cell and hence encourage the development of critical antibodies for security. By displaying the antigen as a complex surface structure, the antigen's identification by immunizer-producing B-cells is enhanced. The size and surface shape of virosome particles make them an enticing target for safe cells to pick up and handle, which is a key step in the invulnerable response's early stages.

Virosomes Adjuvant Function

The closeness of flu-related envelope proteins, notably the overwhelming HA, determines the potential of virosomes to serve as adjuvants. Prior anti-flu antibodies attach to virosomes and recognise them effectively, enabling antigen-displaying cells to quickly ingest and prepare them (APC). The most common function of antibodies is to connect infection and disease. Previous flu-specific partner T cells are activated by APC that display the prepared parts of the flu proteins. Flu proteins help cells grow quickly and produce cytokines that stimulate and improve the recruitment of impact or safe cells such immune response-producing cells [10].

VIROSOME UPTAKE BY CELLS

Attachment

This requires virosomes being delivered to cell receptors by HA, which are film glycoproteins or glycolipids with a sialic corrosive at the end. If certain virosomes are found, Fab sections are cross-linked to the virosomal surface via a spacer arm. Certain virosomes will identify antigenic characteristics on the focusing cell surface, resulting in a connection to target cells through two separate restricting components. In this line, selectivity is used by certain virosomes to target particular cell types.

Penetration

Virosomes enter by receptor-mediated endocytosis after piercing. Because of an acidic interaction between the virosomal film and the endosomal layer, the virosomes get stuck in endosomes. In the combo, the viral spike glycoprotein HA plays a role. The endosome's layer combination process frees the virosomes from their lipid sheath, allowing the contained medications access to the cytosol.

Functions by the Carrier

The reconciliation of the antigen into the higher structures of the virosomes molecule balances the antigens, maintains the local state of B cell epitopes, and shields the antigens from destruction. Furthermore, displaying the antigen as a uniform surface structure improves antigen recognition by inhibiting the production of agents by B cells.

Memory Support

The presence of flu-associated HA prompts a memory response since the majority of individuals have a degree of common, past insusceptibility to flu. Previous flu-specific antibodies successfully target virosomes for fast uptake and processing by antigen-introducing cells, leading in humoral and cell immunity (APC). Memory T partner cells multiply fast and secrete cytokines to assist antigen delivery to specific sites and immune response amplification (Figure 5) [11].

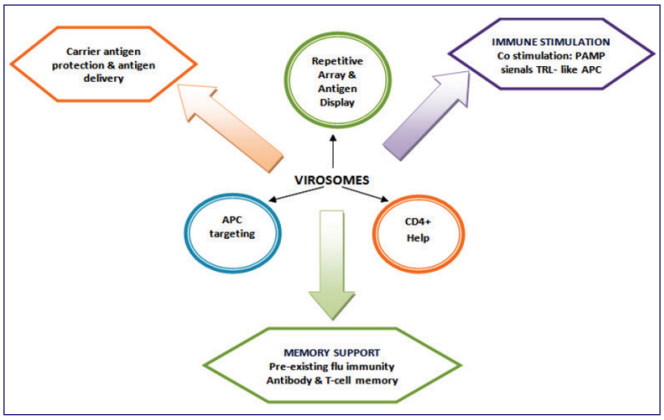

Figure 5: Activation of antibody through virosomes.

PHARMACOKINETICS

Pharmacokinetic data may be utilized to translate the pharmacological effects of liposomal-entangled and free drugs, and hence can be used for measurement planning. Pharmacokinetics regulates the retention, dissemination, and debasement of virosomal transporters in vivo. The pharmacokinetics of virosomes, with the exception of topical plans, needs a comprehension of probable accessible places after intravenous organisation, since this is the most well-known route for numerous virosomal details misused for clinical treatments. Virosomes affect a medication's tissue dispersion and absorption rate, both of which are influenced by pharmacokinetics parameters. The medicine is conveyed within the virosomal fluid stage during flow and overflows at a sufficient rate to become noticeably bioavailable when it settles in tissue or other particular sites under optimum conditions. Bioavailability is defined as the quantity of free medication that may leave the bearer's boundaries and so become easily accessible for redistribution to nearby tissue in the case of virosomal transporters [12].

PREPARATION

Selection of virus

Virosomes are viral envelops that have been reassembled from a variety of viruses. The influenza virus envelope is the most common source of virosome, although it may also come from Sendai virus, Sindbis virus, Epstein-burr virus, buddy murine leukemia virus, and herpes simplex virus.

Selection of antigen

In terms of our criteria, the antigen is the better option. Antigens include bacterial parasites, malignant cells, and whole cells. Antigens may be produced from RNA, DNA, or plasmid, among other cell components.

Reconstitution of virosome

Virosomes may be washed away using detergents such (octaglucoside, nonidert p-40). Genetic material and internal viral protein will settle after detergent solubilization, and detergent will be removed from the supernatant using different techniques such as hydrophobic resins and dialysis. Ultracentrifugation is used to extract the viral matrix protein and nuclear capsid. The antigen that has been attached to the lipid anchor is mixed with a surfactant or polymer solution. This is achieved by a process involving virosome mover antigen-bound virosomes [13]. Figure 6 shows a schematic representation of how virosomes are made.

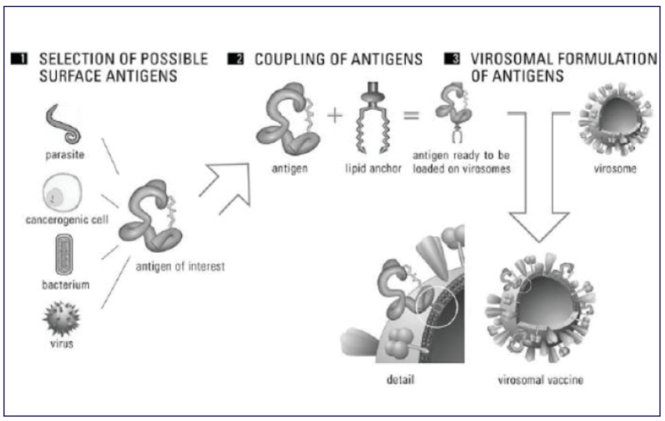

Figure 6: Method for preparation of virosomes.

PROPERTIES OF VIROSOMES

Virosomes are non-toxic, biodegradable, and compatible with living organisms. Antigens may be absorbed into virosomes, adsorbed to their surfaces, and incorporated into the lipid membrane through hydrophobic domains or cross-linked lipid moieties. They're also being studied for the creation of an HIV-I vaccine. They were used as a drug carrier mechanism for experimental cancer therapies [14].

CHARACTERIZATION

Protein detection

Protein-to-lipid ratios are usually quite consistent after virosome synthesis. The agent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is used to validate the presence of HA protein in virosomes.

Structure and size

To assess the ultrastructure and size of virosomes, negative stain electron microscopy should be performed. To prevent acid-induced conformational changes in HA, the prepared staining solutions may have a neutral pH.

Fusion activity

Similar to a local flu infection, virosomes often demonstrate pH-dependent film combination migration. Virosomal interaction with organic or counterfeit target films can be evaluated in vitro using an excimer measure based on pyrene-named lipids, where a decrease in surface thickness of the pyrene-phosphatidyl choline-name when combined with an unlabeled layer corresponds to a decrease in excimer fluorescence. Hemolytic action, which is related to combination action and has a pH dependency that is indistinguishable from combination action [15], may also be used to verify combination action in a roundabout way.

EVALUATION

- Size dispersion and vesicle size: Dynamic light scrambling, Transmission electron microscopy, Zetasizer, Photon correlation spectroscopy, Laser light diffusing, Gel saturation, and gel avoidance.
- Surface morphology and vesicle shape: Transmission electron microscopy, Freeze break electron microscopy.
- Surface pH and electrical surface potential: Zeta potential estimations and pH tests.
- Surface charge: Free stream electrophoresis.
- Phase conduct: Freeze crack electron microscopy, Differential scanning calorimetry.
- Lamellarity: Small edge X-beam dissipating, Freeze break electron microscopy, 13P-NMR.
- Drug discharge: Diffusion cell/dialysis.
- Percent of free medication: Mini section centrifugation, Gel avoidance and Ion trade chromatography, Protamine accumulation, Radiolabeling.
- Animal poisonous quality: Observing survival rates, histology and pathology.

- Pyrogenicity: Rabbit fever reaction test or Limulus ambeocyte lysate (LAL) test.
- Chemical examination of surface: Static auxiliary particle mass spectrometry [16].

ADMINISTRATION OF VIROSOMES

Several formulae have been reported. Virosomes are often suspended in buffered saline (135–150 mM NaCl), although other carriers are available as well. These compositions should be sterilised using traditional liposomal sterilisation procedures such as membrane filtration. To simulate physiological conditions, auxiliary chemicals such as buffering agents and tonicity regulating agents are frequently added in the formulation (sodium acetate, sodium lactate, sodium chloride, potassium chloride, and calcium chloride). The concentration of virosomes in the vehicle ranges from 20 to 200 mg/mL. These concentrations are changed to optimise treatment with different virosome components or for particular goals. Intravenous, intramuscular, subcutaneous, intra-arterial, and inhalable techniques are used to administer the virosomes. Topically, orally, or transdermally, virosomes may be given. For long-term distribution, the virosomes might likewise be implanted in implantable devices [17].

APPLICATIONS

Viruses engage in intracellular parasitism because their survival is reliant on certain host cells. The creation of a medicine delivery system that resembles the viral model of cell disease is aided by this guideline. The viral surface glycoproteins protrude from the phospholipid bilayer that covers the surface of virosomes. Because of the vesicular layer's development, the virosomes are biocompatible and biodegradable. They are properly digested and delivered to the desired site without being harmed by physiological processes in the body. Furthermore, the architecture and structure of virosomes allow for the consolidation of many kinds of medication particles inside them. The hydrophobic medications may readily pass through the lipid bilayer. Drugs that are hydrophilic, on the other hand, become a part of the focal lacunae. Virosomes might be coupled to an immune response to guarantee that a beneficial operator is delivered to the right place at the right time to increase tissue specificity. Antibodies attach to certain cell receptors, enabling medication atoms to reach their intended destinations. This capability can be handy for transferring low-security medication particles. Disease chemotherapeutic operators, for example, might be delivered selectively to tumors by marking virosomes with antibodies. Hepatocytes, erythrocytes, safe cells, and glioma cells have all been found to transport medications, nucleic acids, and proteins through viral chromosomes. A variety of virosome-based products have been licensed for human use by the US Food and Drug Administration (FDA). Surface glycoproteins from influenza, hepatitis, and vesicular stomatitis infections have been effectively consolidated in various antibody and medication delivery systems. Virosomes containing cancer chemotherapeutic specialists, anti-malarial, anti-bacterial, and anti-fungal operators seemed to have effective discharge profiles both in vitro and in vivo. Bacterial apparitions have also been created using the same criterion. These vesicles contain the external shell or envelop protein of many Gram-negative microscopic organisms. These bacterial symptoms are similar to a comparative design, as shown in the instance of a particular sickness. Virosome-based sedate delivery is, in any case, quicker, safer, and more practicable than other related technologies. Table 1 lists the commercially available preparations.

Table 1. Marketed Products of Virosomal drug delivery.

Preparations	Brands	Applications
Hepatitis A virus envelope protein	Epaxal®	Hepatitis A
Influenza virus	Inflexal V®	Influenza

Immunopotentiating Agents

Antigen and medicine delivery vehicles called virosomes may be utilized to target certain cell types. The key element of the virosome design is the interaction of the virus's antigenic proteins with cellular receptors. The immune system is also triggered by the proper antigen-presenting cells detecting, uptaking, and representing the antigen incorporated into the virosome. Effective regulatory and effector immune responses emerge as a result. They triggered immune system alarms in both the cell-mediated and humoral systems. Both cytotoxic and helper T-cell responses are triggered by viral genomes. In addition to conveying the immunogen to the body, virosomes may act as adjuvants to guide the immune response to a particular antigen. Because of their particle form, they may easily attract dendritic cells and other antigen presentation cells for immunological benefits. Because of the virosome's nature, the antigen is continually and sustainably delivered to the immune system, whether intercalated into the lipid bilayer, linked to surface proteins, or present in the central cavity. This antigen release delay might be utilized to focus the immune response on a single antigen, creating a depot-like effect. Furthermore, combining antigen and adjuvant administration may help achieve improved immune protection against a number of disorders. In contrast to the delivery of nascent antigen, recent studies on murine models have revealed that virosome-based products produce a four-fold increased humoral response.

Drug delivery

Bioactive medicinal molecules may be encased in the virosome's aqueous interior or lipid membrane to make it simpler for them to enter cells. Virosomes are very efficient in transporting nucleic acids or genes. When the virosome joins the endosome or plasma membrane, these chemicals are transported into the host cell's cytoplasm. If the expression cassette has the appropriate cis-acting regulatory elements, nucleic acids or genes encoding naturally occurring proteins may be inserted into host cells and expressed. During the production process, drugs or nucleic acids might be introduced into the virosome. The bioactive chemical is normally added to the lipid-HA-containing solution after the nucleocapsid has been removed. The bioactive substance might alternatively be placed in a liposome, which would then be combined with a virosome containing two HAs with different pH thresholds to form a virosome-liposome hybrid. Proteins may also be delivered to cells through virosomes. The gelonin subunits A and ovalbumin of diphtheria toxin have been successfully delivered to target cells through virosome. Virosomes carrying influenza nucleoprotein peptides or entire ovalbumin evoked strong cytotoxic T-lymphocyte responses, showing that the encapsulated peptides and proteins were able to enter the cytoplasm.

Targeted Delivery

The capacity to transport a medical expert to the target site in a safe and timely way is one of the most crucial components of a drug delivery system. Medicines should be changed or packaged in such a way that therapeutically appropriate amounts of medication atoms reach the site of activity, with the goal of supporting the focused on sedate conveyance. Changes to the medication's physical and synthetic properties may result in the formation of new synthetic components, the mixing of other substance elements to affect in vivo discharge patterns, or changes to the medication's physical structures. Virosomes have the ability to encapsulate a variety of natural medications. They might be useful for delivering hydrophilic and hydrophobic drug atoms to certain tissues. Water-loving or hydrophilic drugs are shown in the focus compartment during the virosome synthesis technique. Lipophilic drugs, on the other hand, cannot be encapsulated in this way and must instead be installed in a similar way in the lipid bilayer. The virosomes' dissolution and slow breakdown inside the cell may function as a mechanism of transferring these medication particles to the expected site of action. Many studies have revealed that the virosome may exemplify numerous types of genetic material, which can be employed for both preventative and helpful purposes. The virosome's lipid bilayer protects these restorative operators from nucleic acid corrosive corrupting proteins like DNAases and RNAases. Viral glycoproteins assist in the merging of the films after the identification of certain cell types. The cell hardware may use the genetic material after it has been delivered to construct the encoded features [18-19]. Table 2 lists the preparations that have been tested in preclinical studies.

Table 2. Pre-clinical preparations of Virosomal drug delivery.

Preparations	Applications
Diptheria / Tetanus Toxoid Virus Envelope Proteins	Tetanus, Diptheria
Peptidomimetic of loop-I from domain-III of Plasmodium falciparum AMA-1	Malaria

COMMONLY USED VIROSOMES

Virosomes reconstituted from influenza viruses

Reconstituted influenza virus virosomes are the most often used virosomes. The virosome's envelope is a phospholipid bilayer, and viral glycoproteins like HA and NA are often detected on the virosome's surface. HA is a membrane fusion protein that binds to influenza receptors and may form homotrimers and change conformations to become fusion-competent at acidic pH. The other glycoprotein NA increases viral entry into cells while also promoting the release of progeny viruses during the budding stage by removing terminal sialic acid from saccharide chains on the host cell surface.

Virosomes reconstituted from hemagglutinating virus of Japan

Because of their high efficiency in transporting nucleic acids, proteins, and physiologically active macromolecules, HVJ-derived virosomes are especially noteworthy (Figure 7). Hemagglutinating (HN) and fusion (F) proteins are used by these virosomes for cell fusion. To produce virosome and host cell membrane fusion, the HN protein binds to acetyl-type sialic acid on the host cell surface, whereas the F protein attaches to lipids like cholesterol [20].

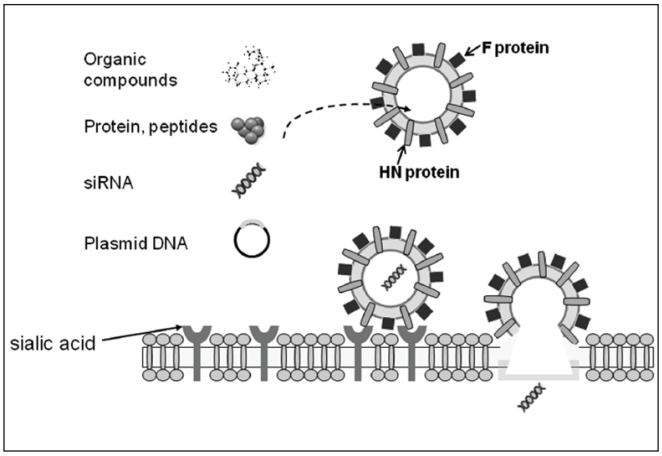

Figure 7: The working mechanism of HVJ-derived virosomes.

CONCLUSION

Depending on the objective of side-effect-free vaccination, virosomes may deliver an antigen to the host body through a range of pathways, including intranasal, intradermal, and intramuscular. Virosomes might be used to transport particular medications and immunomodulatory substances that could be used to treat cancer. One of their most enticing aspects is the ability to drive virosomes to certain cells utilizing Fab sections of monoclonal antibodies specialized for the binding. Influenza virosomes may be used to transfer antigens and atoms of many sorts to cells, such as proteins, peptides, plasmids, oligonucleotides, and even medications, and they can be exploited in a number of ways due to their adaptability.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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AUTHORS' CONTRIBUTION

All authors have equally contributed to this manuscript.

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