BioGecko Vol 12 Issue 01 2023

ISSN NO: 2230-5807

Deciphering Virosomes: Unveiling Structure, Fusion Activity, and Cell Interaction - A Comprehensive Exploration of Types, Preparation, Mechanism, and Recent Progress

Sai Satthya Dhanasekaran[1], Jebastin Koilpillai[2], Damodharan Narayanasamy[3*]

[1]Postgraduate, Department of Pharmaceutics, SRM College of Pharmacy, SRM Institution of Science and Technology, Kattankulathur, Chengalpattu, India.
Email: saisatthya10@gmail.com
ORCID ID: 0009-0001-3841-5725
[2]Research Scholar, Department of Pharmaceutics, SRM College of Pharmacy, SRM Institution of Science and Technology, Kattankulathur, Chengalpattu, India.
E-mail: pushpakjebastin0@gmail.com
ORCID ID: 0000-0003-4430-1553
[3]Professor & Head, Department of Pharmaceutics, SRM College of Pharmacy, SRM Institution of Science and Technology, Kattankulathur, Chengalpattu, India.*

Corresponding Author: Damodharan Narayanasamy[3*]
[3]Professor & Head, Department of Pharmaceutics, SRM College of Pharmacy, SRM Institution of Science and Technology, Kattankulathur, Chengalpattu, India.*
Email: damodharan@srmist.edu.in
ORCID ID: 0000-0003-4246-5351

Abstract

Virosomes, are innovative vesicular carriers with significant implications for drug delivery and vaccine development. Virosomes are lipid-based structures derived from viral envelopes, possessing both the fusogenic properties of viruses and the biocompatibility of liposomes, thus it outperforms conventional liposomes in terms of immunogenicity, stability, and specificity. This unique combination allows virosomes to efficiently deliver therapeutic agents into target cells. The abstract explores the structural and functional attributes of virosomes, emphasizing their ability to encapsulate a diverse range of cargo, including drugs and antigens. Utilizing the receptor-binding and membrane-fusion properties of the viral envelope glycoproteins can serve as vehicles for cellular delivery of biologically active macromolecules such as drugs, nucleic acids, or genes. The review highlights the pivotal role of virosomes in vaccine formulations, showcasing their capacity to enhance antigen presentation, characterization and stimulate robust immune responses. Furthermore, the abstract discusses recent advancements in virosome technology, such as surface modifications for improved targeting and strategies to optimize payload delivery. Virosomes' inherent immunostimulatory properties contribute to their success as adjuvants, augmenting the efficacy of vaccines against various pathogens. The abstract concludes by underlining the versatility and promising applications of virosomes in pharmaceutical and biotechnological fields. Their potential to revolutionize drug delivery systems and enhance vaccine efficiency positions virosomes as a focal point for future research and development, paving the way for innovative therapeutic strategies.

Keywords: Virosome, HIV virosomes, vesicular carrier, Newcastle's disease virus, Crucell's Epaxal.

1. Introduction

Innovative therapeutics for neurological or cancer disorders include delivery systems that allow target medications to be administered to specific host tissues and cell types via regulated release and receptor-mediated uptake. Novel therapeutics for neurodegenerative or cancer disorders employ delivery systems to target specific host tissues and cell types (1). Virosomal technology provides a novel delivery system that improves gene delivery by combining antigen, drug, and DNA from viral cover proteins, thereby increasing the effectiveness of targeted drug delivery and demanding delivery technique for overcoming these challenges (2). The study's objective is to enhance the in vivo delivery capability of virosomes, which are vesicles with pure fusion activity that transport encapsulated substances such as antigens, drugs, and genes into the target cell (3). Notwithstanding advancements in non-viral and viral vector systems, traversing the permeability barrier established by the plasma membrane remains the primary obstacle in drug delivery. Pharmaceutically active substances are shielded from proteolytic degradation and low pH within endosomes by virosomes. To improve the efficacy of gene delivery, molecules are inserted directly into cells. Virosomes are viral cover proteins that combine antigen, medicine, and DNA, among other things. Improving virosome delivery in vivo is a major goal in virosome research (4–6) Despite advances in non-viral and viral vector systems, overcoming the permeability barrier of the plasma membrane, followed by controlled release inside the cytoplasm, remains a significant barrier to delivering medications and other macromolecules into the target cell type (7). Virosomes are empty regenerated influenza viral envelopes wherever infectious nucleocapsid is found, it should be replaced with a chemical of your choice(8). Virosomes are pure fusion activity vesicles that transport an incorporated substance into the target cell, such as medicine, antigen, or genes. Virosomes protect pharmaceutically active substances inside endosomes from proteolytic degradation and low pH, allowing them to reach the cytoplasm intact. The virosome carrier technology has surpassed previously discovered drug delivery vehicles such as proteoliposomal and liposomal carriers (9). Virosomes are reconstituted viral membranes containing therapeutic molecules such as proteins, nucleic acids, and drugs that are created by removing nucleocapsids from enveloped viruses. They are similar to native viruses and were first used to purify influenza spike proteins in 1975 (10). The reconstitution of viral envelopes using enveloped viruses has resulted in their remarkable efficacy as vaccine agents, owing to their capacity to transport immunogens into host cells and stimulate the cellular and humoral immune systems without inducing adverse effects (11). They can also bind to receptors and fuse with membranes, which makes them good vehicles for getting compounds into cells. The need for an optimized drug delivery system for targeted specific delivery within the human body has sparked interest in virosomal technology, which aims to improve molecule delivery directly inside the cell (12,13). This technology is now being used in a new way to deliver antigens during vaccinations and to carry biological molecules in an empty space inside the cell (14). Virosomes, which are biocompatible and biodegradable, outperform both viral and non-viral vectors in terms of safety and the ability to release substances directly inside target cells. In addition to preventing endosome-mediated proteolytic degradation of pharmacological substances, this system represents a potentially effective remedy for contemporary therapeutic approaches targeting cancer and neurodegenerative disorders (15,16). Their efficiency is related to viral capsid proteins' or membrane glycoproteins' ability to bind to cellular receptors and pass through or fuse with cellular membranes. However, the safety of the viral vector system remains a major concern, and issues such as insertion mutagenesis, as seen with retroviruses, and the induction of undesirable immune responses and inflammation remain significant challenges (17–19). Chemical approaches (e.g., cationic lipids, cationic polymers, and nanoparticles) and physical methods (e.g., gene gun, electroporation) are being used for non-viral gene delivery. Cationic liposomes have received the most attention (20–22). Overall, virosomes protect pharmaceutically active substances from proteolytic degradation and low pH within endosomes, allowing them to reach the cytoplasm intact.

This is a significant advantage of virosomal carrier systems over other drug-delivery vehicles, such as liposomal and proteoliposomal carriers (23).

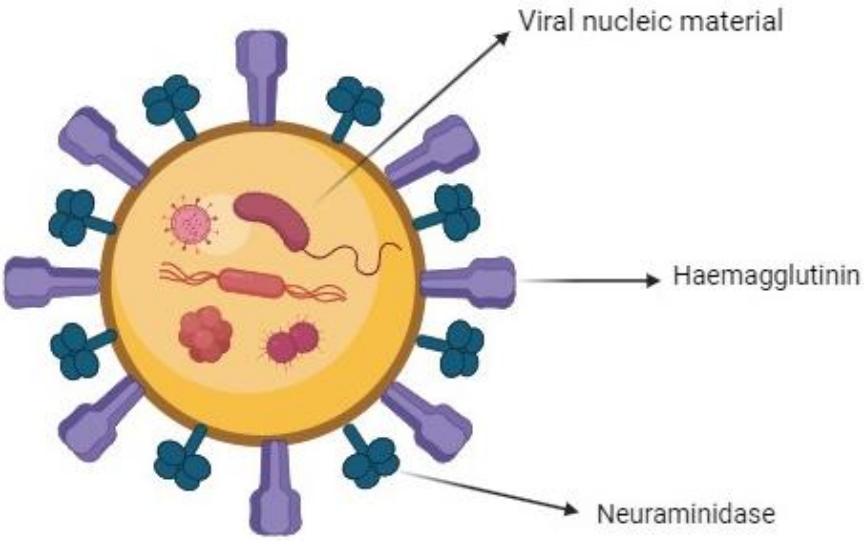

Fig. 1 Schematic structure of virus

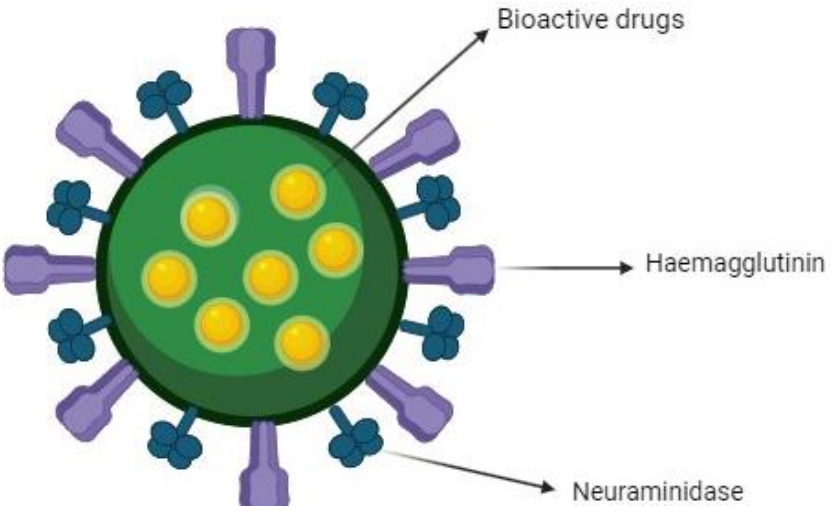

Fig. 2 Schematic structure of virosome

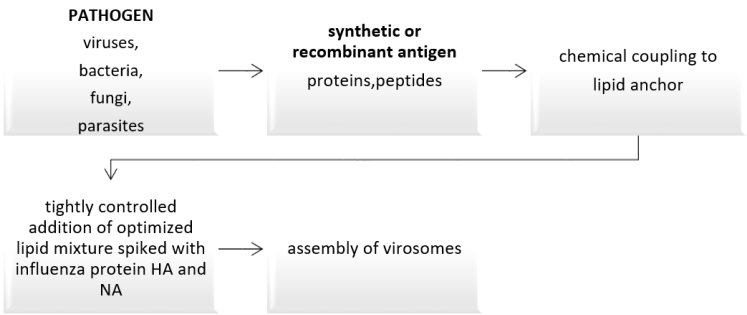

Fig. 3 Schematic illustration on assembly of virosome

Structure of virosomes

The influenza virus is frequently employed to manufacture virosomes and serves as the primary source of genetic material. Virosomes cannot multiply, unless in cases where pure fusion-active vesicles are present (24). Virosomes are composed of phospholipid bilayer vesicles, which can be either spherical or unilamellar. The surface is made up of membrane lipids and viral spike proteins. These vesicles typically have a mean diameter ranging from 120 to 180 nm. In the context of lipid composition, virosomes exhibit similar adaptability to liposomes. Furthermore, virosomes possess membrane proteins, which are either obtained from the virus itself or generated via recombinant technology (25). The virosome's outer surface exhibits a resemblance to an entire virus, featuring peplomer proteins that extend outward from the membrane (26,27). Virosomes are formed by various enveloped viruses, the most common of which is influenza. Purified influenza hemagglutinin and neuraminidase envelope proteins are incorporated into synthetic and natural viral lipids to form them. For fusion activity, the hemagglutinin (HA) membrane protein is required (Fig. 1) (Fig. 2). Virosomes made from the Hemagglutinating virus of Japan (HVJ) are made up of a phospholipid bilayer containing viral F proteins and HN protein. Respiratory Syncytial virus, Chikungunya virus, Cytomegalovirus, and Herpes Simplex virus are among the viruses used to generate virosomes (11,12,14,15,28). The Virosome mostly consists of Immune Stimulating Regenerate Influenza Virosomes (IRIVs), which are composed primarily of naturally occurring phosphatidylcholine (PC) and phospholipids (PL). Phosphatidylcholine is the primary constituent contributing to approximately 70% of the virosomes' structural composition. The remaining 30% of membrane constituents consist of envelope phospholipids that are produced by the virus and contain haemagglutinin (HA) and neuraminidase (NA) glycoproteins (29–32). Virosomes are essentially reconstructed hollow virus envelopes that lack genetic material, rendering them incapable of replicating like their original pathogenic counterparts (33). In contrast to liposomes, virosomes consist of functional glycoproteins hemagglutinin (HA) and neuraminidase (NA) that are incorporated into the viral membrane. The outstanding attributes of virosomes can be attributed, in part, to the presence of the immunologically active HA glycoprotein that is incorporated into their membrane. The haemagglutinin glycoprotein serves to maintain the uniformity and structural integrity of virosomes. Additionally, it significantly enhances the immune-stimulating capabilities of virosomal particles, distinguishing them from other proteoliposomal and liposomal delivery systems. The structure of HA primarily comprises two protein sections, HA1 and HA2, which are formed by the process of translational cleavage of HA. These two subunits are connected by a disulfide link (34). The HA2 component possesses an N-terminal fusion protein and is integrated within the virosomal membrane. At a pH of approximately 7, the HA1 subunit effectively constrains the HA2 subunit in a metastable state, even in its inactive state. This constraint is achieved through the regulation of fusion peptides by a complex network of hydrogen bonds. The conformational modification of the HA protein results in the exposure of hydrophobic areas in HA2, leading to the fusion of viral particles with the membrane of the target cell. This transformation occurs following a shift in pH from a neutral to an acidic environment. During the course of influenza virus infection, a fusion event occurs between the endosomal and viral membranes, resulting in the release of genetic material into the cytoplasm of the target cells. In an in vitro setting, when target cells are absent, hyaluronic acid (HA) often becomes deactivated in an acidic environment with a pH of approximately 5 and a temperature of 37°C. Consequently, its ability to induce fusion is diminished (35). The NA glycoprotein, which is also present on the virosomal surface, is a tetramer of an enzyme with four subunits that hydrophobically attaches to the membrane via a stem. The subunit head region contains the enzyme loci. Also, the activity of neuraminidase (NA) results in the cleavage of N-acetylneuraminic acid (NAM), commonly known as sialic acid, from its attached sugar residues(36). Virosomes have the potential to be improved for maximum medication inclusion or to provide the largest physiological effect by altering the composition or type of membrane lipids utilized. It is feasible to produce carriers for antisense

oligonucleotides or other genetic molecules by incorporating positively or negatively charged phospholipids into the membrane (Fig. 3).

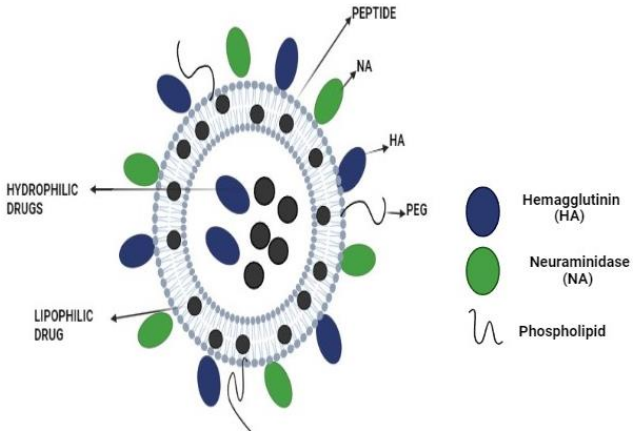

Fig. 4 Virosome-endowed adjuvant properties and functioning as a carrier

A range of ligands, such as peptides, cytokines, and monoclonal antibodies (MAbs), have the potential to be integrated into the virosome structure. Additionally, it is seen on the surface of the virosome. The conjugation of tumor-specific monoclonal antibody fragments (Fab) with virosomes can facilitate targeted delivery of the carrier to specific tumor cells.

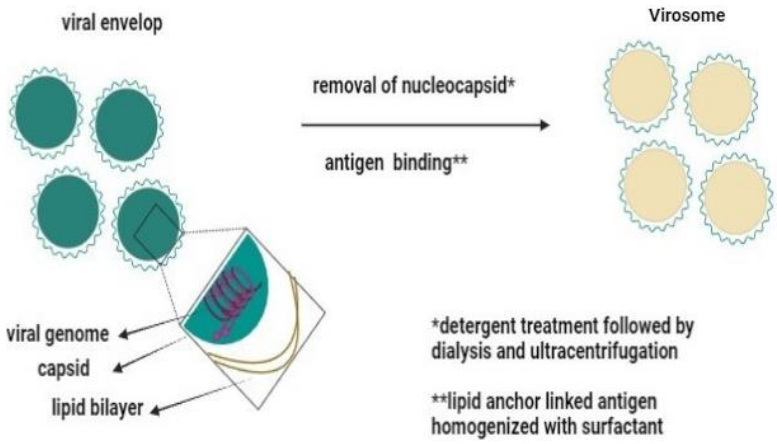

Fig. 5 Schematic illustration of virosome

Table. 1 First generation virosomes

INDICATION	PRODUCTS	VACCINE COMPOSITION	REFERENCE
Seasonal Influenza	Invivac ®	Virosomes from 3 Influenza strains A(H1N1), A(H3N2)B	(37,38)
Seasonal Influenza Intranasal application	Nasalflu®	Virosomes from 3 Influenza strains A(H1N1), A(H3N2), B & HLT Adjuvant	(39)
Hepatitis A Child	Epaxal ® Junior	A(H1N1) virosomes & inactivated Hepatitis A virus	(40)
Seasonal Influenza All age groups	Inflexal ®	Virosomes from 3 Influenza strains A(H1N1), A(H3N3), B	(41)
Hepatitis A Adult	Epaxal ®	A(H1N1) virosomes & inactivated Hepatitis A virus	(42)

Table. 2 Second-generation virosomes

DISEASE	TARGET	EFFECTOR	ADMINISTRATION ROUTE	ANTIGEN CONFIGURATION	REF
HIV	Gp41	antibody	Intramuscular prime & intranasal boost	1Peptide membrane-anchored	(43)
malaria	Plasmodium falciparum	AMA-1 & CSP antibody	Intramuscular	2Peptide membrane-anchored	(44)
Breast cancer	her-2/neu	Antibody	Intramuscular	3Peptide membrane-anchored	(45)

Fusion activity

Because influenza HA is present in the membranes of virosomes, they have unusual fusion properties. HA not only gives virosomal formulations structural stability and consistency, but it also aids virosome fusion. Virosomal HA improves receptor-mediated endocytosis by adhering to the target cell surface. The acidic environment of the endosome causes HA-mediated membrane fusion, allowing the therapeutically active chemical to escape from the endosome and enter the target cell's cytoplasm.

As a result, virosomal HA significantly improves cytosolic delivery. Endosome virosomes protect pharmaceutically active substances from proteolytic degradation and low pH before they reach the cytoplasm. This is an advantage of the virosomal carrier system over liposomal and proteoliposomal carriers, which provide less protection for therapeutic macromolecules in hostile compartmental microenvironments (46)**.**

Virosome-cell interaction

Virosome development replicates in vivo contaminated express, allowing resistance players and macromolecule organisation to specific action regions. Virosomes, such as flu virosomes, attach to the same receptors as viruses and are used to deliver nano-sized proteins, corrosive nucleic acids, or medication particles to the targeted action site. This connection has been used to treat a variety of illnesses, including parasite infections, viral infections, neurological disorders, and metabolic problems. Flu virosomes have been used to create vaccines against RSV and to establish epitopic B-cell sites, which are especially effective against intestinal disease. The use of virosome architecture also helps to keep a strategic distance between immunisation doses in a variety of disorders (47).

Comparison of virosomes with liposomes

Liposomes are well known as vehicles for targeted biomolecule delivery to living cells in vitro and in vivo, but they fail to deliver encapsulated molecules in host cell cytosol. This is due to their inability to fuse with host cells. Virosomes, on the other hand, are endowed with functional glycoproteins of viral origin that have membrane-fusion and receptor-mediated binding properties, allowing for optimal delivery of required molecules within host cells' cytosol. Nakamura et al. discovered that HVJ-virosomes delivered oligonucleotides intracellularly with up to three times the efficiency of cationic liposomes (48). Apart from providing binding and fusion properties to virosomes, the presence of influenza HA inside the influenza virosomal membrane provides structural stability and homogeneity to virosomal formulations. Low pH inside host cell endosomes accelerates HA-mediated cell fusion, where the molecule packed inside the virosome membrane is released from the endosome microenvironment into the cytosol of the target cell, enhancing cytosolic delivery. This is one of the benefits of using virosomal technology over liposomal systems, which provide little protection for therapeutic biomolecules from harsh microenvironments such as low pH and high acid content within organelles (32). Virosomes can

also stimulate the host immune system with their immunogenic properties, acting as an adjuvant as well as a carrier to deliver antigens. Furthermore, unlike liposomes, virosomes are not rapidly cleared by the body's reticuloendothelial system (49).

Rationale and optimization of virosomes

Poorly water-soluble drugs are dissolved: diazepam, vitamin A, and dexamethasone palmitate. Cytotoxic agents are delivered to specific organs. Dexamethasone palmitate, barbiturates, and physostigmine salicylate have the potential for sustained-release dosage forms (50).

To increase the duration of virosome circulation after systemic delivery, hydrophilic polymers [such as poly acryloyl morpholine, polyethylene glycol, polyvinylpyrrolidone, and poly (2- oxazoline)] may be inserted into the envelope. Furthermore, targeting molecules (such as antibodies) can be linked to these hydrophilic polymers and delivered into the virosome membrane to allow for target selection (51).

Types of virosomes

Several types of virosomes can be produced depending on the viral envelope like Influenza Virosomes, Sendai Virosomes, HBV Virosomes, HIV Virosomes, and NDV Virosomes.

Influenza virosomes

They are the most commonly used virosomes and are derived from influenza viruses, which are members of the Orthomyxoviridae family (52). Influenza viruses have eight segmented single-stranded RNA genomes encased in a viral envelope(53). The viral envelope's outer surface contains two types of membrane proteins: hemagglutinin (HA) and neuraminidase (NA). HA is made up of two polypeptides, HA1 and HA2. HA1 is in charge of binding with sialic acid on the surface of a host cell, resulting in host cell-viral particle attachment(54). The HA2 polypeptide promotes endosomal membrane fusion to the membrane of virosomes cannot take place in neutral environments and is only achieved via a conformational change in acidic conditions. Fortunately, endosomes offer the necessary acidic environment (55)(Fig. 5).

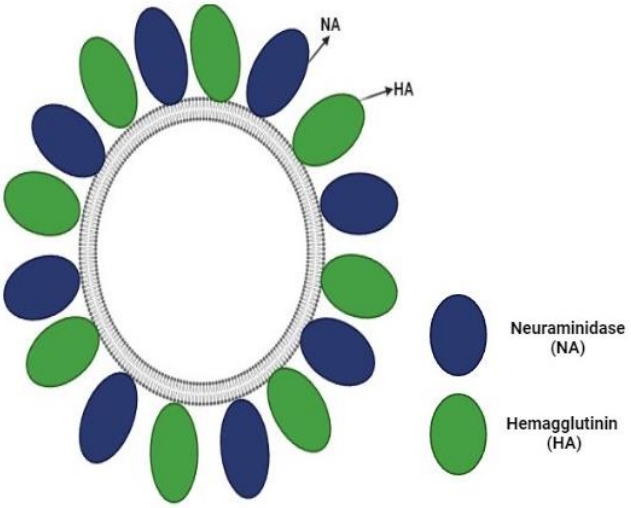

Fig. 6 Schematic influenza virosome

Sendai Virosomes

They are members of the Paramyxoviridae family and are derived from the Hemagglutinating virus of Japanese (HVJ) or Sendai virus. HVJ have single-stranded RNA genomes that are enveloped (56). The membrane glycoproteins hemagglutinin-neuraminidase (HN) with fusion protein (F) make up the external surface. HN glycoprotein is in charge of binding with sialic acid thereby

adhering to the surface of a host cell, whereas F allows for a fusion of membranes between the host cell and viral particle (57). They are derived from the Hepatitis B virus and Ortho hepadnaviridae family members. HBV is a virus with an envelope and a partially double-stranded DNA genome. The envelope's external surface contains three surface proteins: small (S), medium (M), and large (L). However, L protein can be purified by ultracentrifugation and used as a safe vehicle for drug delivery systems with high in vivo and in vitro targeting specificity to human hepatocytes (58).

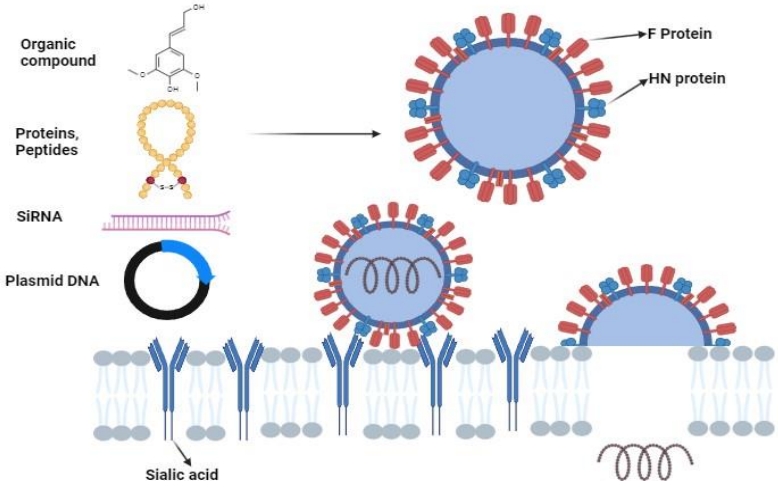

Fig. 7 The working mechanism of hvj derived virosomes

HIV Virosomes

They can be acquired from the virus that causes HIV infection as well as members of the Retroviridae family. HIV is made up of two congruent single-stranded RNA genomes that are encased in an envelope. Gp120 and gp41 are envelop glycoproteins found on the external surface. Additionally, p17 and p24 are matrix and core proteins, respectively. Following the purification and centrifugation processes, virosomes with enveloped glycoprotein (gp120 and gp41) and p17 matrix protein were obtained (12).

NDV Virosomes

They are derived from the Newcastle's disease virus (NDV) and belong to the Paramyxoviridae family. NDV has a single-stranded RNA genome and a viral nucleocapsid wrapped in an envelope. Similar to the Sendai virus, the viral envelope's external surface includes two membrane proteins, hemagglutinin-neuraminidase (HN) and a fusion protein (F). HN protein is responsible for binding with sialic acid and thus aiding in host cell attachment. F protein is in charge of fusion with the targeted cell (59).

Table. 3 Different types of virosomes

S. NO.	VIROSOMES	PATENT VIRUS	FAMILY	VIRAL GLYCOPROTEIN	REF
1	Influenza Virosomes	Influenza	Orthomyxoviridae	HA, NA	(60,61)
2	Sendai Virosomes	Sendai or HVJ	Paramyxoviridae	HN,	(62)
3	HBV Virosomes	HBV	Orthohepadnaviridae	S, M,	(63)
4	HIV Virosomes	HIV	Retroviridae	gp120, gp41, p17	(64)
5	NDV Virosomes	NDV	Paramyxoviridae	HN,F	(65)

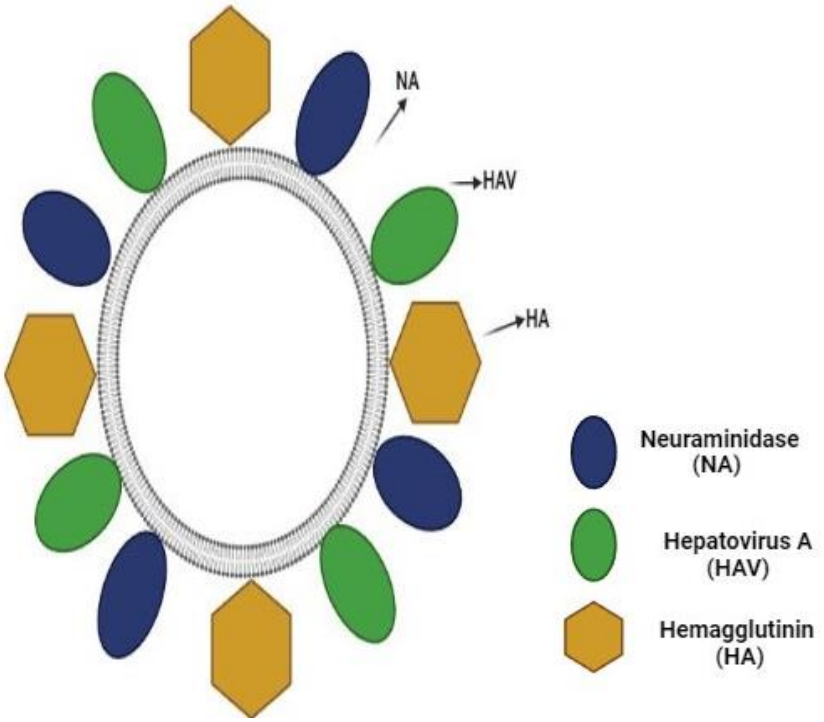

Fig. 8 Structure of hepatoviral

Preparation of virosome

selection of virus

Virosomes are viral envelopes that have been reconstituted from various viruses. The subject of discussion is the influenza virus. The envelope is primarily utilised for the production of virosomes. However, it is worth noting that virosomes can also be derived from the Sendai virus. The Sindbis virus, Epstein-Barr virus, and Friend murine leukaemia virus are three examples of viral pathogens. The topic of discussion is the virus known as herpes simplex virus (66).

Selection of antigen

The selection of antigens is based on our specific requirements. Antigens, such as bacteria, parasites, carcinogenic cells, or whole cells, are utilised in the context of immunology. Cell components such as RNA, DNA, or plasmid may also serve as antigens (67).

Reconstituted of virosome

Virosomes were dissolved in detergents such as (octaglucoside, nonidert p-40). Because of detergent solubilization, genetic material and internal viral protein will sediment after detergent is removed from supernatant using various methods such as hydrophobic resins and dialysis. The development viral matrix protein and nuclei capsid are removed using ultracentrifugation. Antigen that has been linked to a lipid anchor is combined with a surfactant or polymer solution. This solution is achieved through the process of virosome mover antigen bound virosome (68).

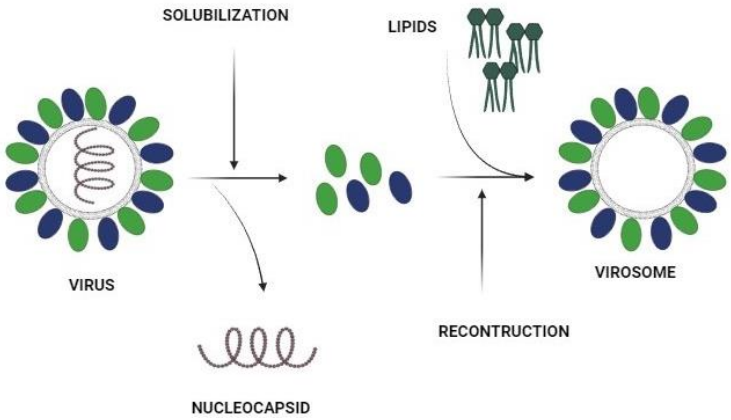

Fig. 9 Preparation method for virosomes

Challenges in the preparation of virosomes

The selection of the glycoprotein on the viral envelope, which controls the virosomes' fusion activity and receptor selectivity. Although influenza viruses are most frequently used, other viruses have also been employed, including the Sendai virus, the Semliki Forest virus, the vesicular stomatitis virus, and the Sindbis virus. The encapsulated bioactive substance and the membrane lipids should be compatible with the glycoprotein of the viral envelope(10). The process used to incorporate the bioactive material into the virosomes, which may have an impact on the material's release, stability, and effectiveness. Several techniques exist for incorporation, including reverse-phase evaporation, co-solubilization, co-lyophilization, freeze-thawing, and dehydration-rehydration. Every technique has benefits and drawbacks that vary based on the type and quantity of the bioactive substance (69). The virosomes' purification and characterisation, which might be difficult because of the complexity and heterogeneity of the virosomal preparations. The methods used for purification and characterisation should guarantee the elimination of impurities, the maintenance of the virosomes' structural and functional integrity, and the identification of their physicochemical and biological characteristics. Several techniques are employed, including dynamic light scattering, gel electrophoresis, density-gradient centrifugation, size-exclusion chromatography, electron microscopy, and fluorescence spectroscopy (70). The immune system's reaction to virosomes, which may lead to a drop in virosome levels in the bloodstream and a decrease in delivery effectiveness. Nevertheless, the induction of a preventive or therapeutic response against the virus or the bioactive substance can also benefit from the immune response. The selection of the viral envelope glycoprotein, the dosage and mode of delivery, and the inclusion of adjuvants or immunomodulators can all affect the immune response (71).

Characterization of virosomes

Protein Detection

Virosome preparation typically yields a relatively uniform protein-to-lipid ratio. SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) can be used to confirm the presence of Haemagglutinin (HA) protein in virosomes.

Structure and Size

A negative stain microscopy with electrons must be used to detect the size and ultrastructure of virosomes. To avoid acid-induced conformational changes in Haemagglutinin (HA), the staining solutions should preferably have a neutral pH (46)(72)**.**

Fusion Activity

Virosome generally exhibits the pH dependent film assembly movement of a negative influenza virus. Virosomal fusion with organic or artificial target film is frequently assessed in vitro with an

excimer measure based on pyrene-labeled lipids, where a decrease in surface thickness of the pyrene-phosphatidylcholine label on mixture with an unlabeled membrane corresponds to a decrease in excimer fluorescence of fusion activity. It can also be passively checked by measuring haemolytic activity, which is closely related to fusion activities and has the same pH dependence as fusion (73).

Modification of virosomes

The activity of virosomes can be improved by incorporating hydrophilic polymers such as polyethylene glycol, polyacrylmorpholine, polyvinylpyrroliodone, and poly(2-oxazoline) into the viral envelope, which results in a longer time of circulation after systemic administration (52). Virosomes can be customised by incorporating different ligands on their surface, such as cytokines, peptides, and monoclonal antibodies, for specific targeted delivery of the incorporated drug (1). Virosomes can clearly target cancer cells that have specific siRNA. This was accomplished by presenting HER2 (human epidermal growth factor 2, a protein that causes breast cancer cells to grow) affibody molecules on customised virosomes (74).

Virosome uptake by cells

This includes the binding of virosomes to cell receptors via HA, which are a film glycoprotein or glycolipid with a terminal sialic corrosive. If specific virosomes are found, Fab sections are linked to the virosomal surface via a cross-linker with a spacer arm. Specific virosomes will also detect antigenic structures on the target cell surface, resulting in a connection to target cells via two distinct restricting components. As a result, specific virosomes use selectivity for different cell types (75).

Penetration

Following virosome penetration, virosomes enter the cell via receptor-mediated endocytosis. The virosomes are caught in endosomes as a result of the acidic combination of the virosomal film and the endosomal layer. The viral spike glycoprotein hemagglutinin (HA) intervenes in the combination. The layer combination response in the endosome liberates virosomes from their lipid envelope and allows embodied medications to enter the cytosol (76).

Functions by the Carrier

The incorporation of the antigen into the higher structures of the virosomes molecule balances out the antigens, protects the local status of B cell epitopes, and protects the antigens from degradation. Furthermore, presenting the antigen as a monotonous surface structure improves its recognition by counteracting agent producing B cells (77).

Memory Support

The proximity of flu-inferred hemagglutinin (HA) causes a memory reaction because the majority of people have a level of common, prior susceptibility to flu. This includes both humeral and cell invulnerability: previous flu-specific antibodies proficiently tag virosomes for rapid take-up and handling by antigen introducing cells (APC). Memory T partner cells rapidly multiply and release cytokines to aid and upgrade Antigen delivery to specific targets and immune response amplification (78).

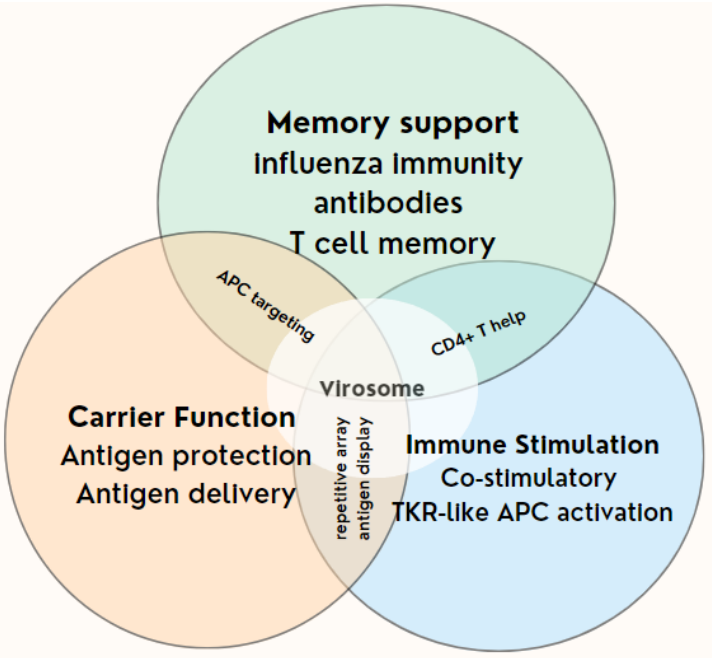

Fig. 10 Multifunctionality of virosome

Mechanism of action of virosomes

Virosomes act as carriers and adjuvants with distinct functions for inducing immune responses, with the carrier function involving the embedding of viruses. positive antigens into virosome particles. The effect of antigen integration on the stability of the antigen on the surface of virosomes replicates the original pathogen and generates the antibodies. Surface antigen induction immunises. The creation of B cells in such a complex form is the main focus for targeting immune cells, this is important for the induction of immunity (79).

Adjuvant function refers to the stimulation of immune properties of virosomes and immune system components without causing nonspecific inflammation. Adjuvant function depends on the presence of pre-existing haemagglutinin antibodies against influenza. Binding to virosomes results in rapid uptake and processing by antigen-presenting cells (APC). The size and surface of virosomes, which are crucial for initiating immune response, is one of the factors which render virosomal particle attractive target for uptake and processing by immune cell. APC activates pre-existing influenza-specific helper T cells, which proliferate and secrete cytokines to stimulate immune cells (80).

Route of administration of virosome

When the desired bioactive drug is delivered to the targeted cell via a different route, the first step is virosome binding to the host cell via the HA1 glycoprotein to the terminal salic acid cell receptor. Furthermore, virosomes surface with fragments of Fab (Fragment antigen-binding) that are cross-linked with spacer arms for efficient binding. Aside from that, some virosomes recognise antigenic receptors on the surface of targeted cells, resulting in two distinct binding processes to the targeted cell.

As a result, different virosomes have different selectivity for different types of targeted cells.

After that, penetration will take place via receptor-mediated endocytosis. After virosomes bind to the surface of the host membrane, they become entrapped inside endosomes. The viral glycoprotein HA2 aids virosome fusion with the endosomal virosomal membrane.

However, this fusion will take place in an acidic environment provided by endosomes. This fusion causes the release of bioactive drugs from the virosome's lipid bilayer, allowing the bioactive drug to enter the cytoplasm of the targeted cell (79).

Table.4 Marketed instances of various virosomal vaccines

NAME OF THE VACCINES	INDICATIONS	MANUFACTURER	TYPES OF ANITIGEN	STAGES OF DEVELOPMENT	REF
NasalFlu®	Nasal flu	Berna biologics	Flu virus antigen coadministered with E.coli heat labile enterotoxins (LT)	Marketed in Switzerland	(81,82)
Epaxal™	Hepatitis A	Berna biologics	Aluminum free Hepatitis A virus vaccine	Marketed in Switzerland, South American, Asian countries	(83)
Gardasil®	HPV Cervical cancer	Merck & Co	Self-assembled particles of human papillon virus (HPV)	Developed and marketed	(84)
Recombivax E ngerix-B	Hepatitis B	Merck & co. GlaxoSmith Kline	recombinant Hepatitis B virus (HBV)	Developed and marketed	(85)
Inflexal® V	Influenza	Berna biologics	Subunits virosomal influenza vaccine	Switzerland	(86)

Evaluation parameters

Protein detection, surface charge detection, lamelarity determination, free drug content calculation, drug release, pyrogenicity detection, surface chemical analysis, and percentage of free medication were all used in the study to detect virosomes. Sodium dodecyl sulphate polyacrylamide gel electrophoresis was used to detect protein, free gel electrophoresis was used to determine surface charge, and photo correlation spectroscopy, transmission electron microscopy, dynamic light scattering, gel permeation, and gel exclusion techniques were used to determine lipid content. Static secondary ion mass spectroscopy was used for surface chemical analysis (24).

Applications

To survive, viruses engage in intracellular parasitism, and a medicine delivery system based on the viral model of cell disease is developed. Virosomes can be used to consolidate various types of medication particles because they are biocompatible and biodegradable. Hydrophobic drugs can pass through the lipid bilayer, whereas hydrophilic drugs form focal lacunae. Virosomes can be linked to an immune response to ensure that beneficial operators reach the right place at the right time. Antibodies bind to specific cell receptors, allowing medication atoms to reach their targets. Medication, nucleic acids, and proteins have been found to be transported through viral chromosomes by hepatocytes, erythrocytes, safe cells, and glioma cells. The FDA has approved a number of virosome-based products for human use. Surface glycoproteins from infections such as influenza, hepatitis, and vesicular stomatitis have been successfully combined in various antibody and medication delivery systems. Virosome-based sedate delivery is more efficient, safer, and practical than competing technologies (6).

Immuno-potentiating Agents

Virosomes are antigen and medicine delivery vehicles that interact with cellular receptors to target specific cell types. Antigen-presenting cells that detect, uptake, and represent the antigen

incorporated into the virosome activate the immune system. Both cytotoxic and helper T-cell responses are elicited by viral genomes. Virosomes can also serve as adjuvants, directing the immune response to a specific antigen. For immunological benefits, they can attract dendritic cells and other antigen presentation cells. Virosomes deliver antigens to the immune system in a continuous and sustained manner, focusing the immune response on a single antigen. The combination of antigen and adjuvant administration can boost immune protection against a variety of diseases (87).

Drug delivery

Virosomes are utilised for transferring bioactive medicinal molecules into cells, including nucleic acids or genes. They are capable of being coated in the liquid interior or the lipid membrane, making entry into cells easier. Typically, the bioactive chemical is added to a lipid-HA-containing solution or placed in a liposome, which is then paired with a virosome having two HAs with different pH thresholds to form a virosome-liposome hybrid. Proteins can also be delivered via virosomes, as evidenced by the effective transfer of gelonin subunits A and ovalbumin from diphtheria toxin, as well as influenza nucleoprotein peptides (9).

Targeted Delivery

A system for drug delivery is critical for safely and quickly releasing healthcare professionals to the target site. Changes in medication properties can result in new synthetic components, in vivo discharge patterns, or physical structure changes. Virosomes can encapsulate various natural medications, delivering drug atoms that are hydrophilic or hydrophobic to specific tissues. They dissolve and degrade inside the cell, delivering medication particles to their intended site of action. Virosomes can also represent different types of genetic material that can be used for both preventative and therapeutic purposes. The lipid bilayer of virosomes protects them from DNA and RNA damaging proteins, and viral glycoproteins aid in the formation of films after cell type identification. Following delivery, the genetic material can be used to construct encoded features (88,89).

Cancer treatment

Virosomes have also been used in oncology to carry peptides corresponding to tumour associated antigens, such as peptides from parathyroid hormone related proteins or recombinant proteins such as her -2 neu Fab combined the anti-proliferate properties of monoclonal antibodies and the cyototoxic effect of doxorubicin in vivo (70).

Recent progress on virosome-based vaccines for sars-cov-2

The Coronavirus Disease (COVID-19) pandemic that caused the Severe Acute Respiratory Syndrome Coronavirus 2(SARS-CoV-2) outbreak originated in Wuhan, China, in December 2019, but several studies predicted the first signs of disease in February 2019 (90). More than 62 million cases have been reported in 210 countries since the last report on December 5, 2020, with 1,480,000 deaths. SARS-CoV-2 is an enveloped spherical particle with a diameter of 60-150 nm and a glycoprotein coating of club-shaped spikes. About 20 nm in length. It is a member of the Betacoronavirus genus. Coronaviridae is a virus family. SARS-CoV-2 has a large positive-strand. Ribonucleic Acid (RNA) genome with a length of 3 k nucleotides (91). The low proofreading efficiency of viral RNA polymerases results in a high mutation rate. As a result, RNA viruses are prone to developing drug resistance and evading immune surveillance. Currently, a variant strain of SARS-CoV-2 was recently identified, and recent research has revealed more than 40 kinds of them that transmit pattern and clinical symptoms. Each region's version has been localised. All of the foregoing, as well as the development of a suitable COVID 19 vaccine, remain critical challenges.

The coronaviral genome contains structural proteins crucial for infectious virions and disease severity. CoV S protein, E protein, and hem agglutinin-esterase protein (HE) are key components in receptor binding, virus-host cell fusion, and vaccine design. Virosome particles are a promising vehicle for SARS-CoV2 vaccine development, with the Transvac 2 project underway by the European MI Matrix Company (4,92). Recent research indicates that SARS-CoV E protein and hem agglutinin-esterase protein (HE) can be therapeutic targets. SARS-CoV2 surface antigens and phospholipids can be used to make virosome vaccines. Virosome particles are a promising vehicle for the development of SARS-CoV2 vaccines. The European MI Matrix Company is working on the Transvac 2 project, which is a virosomal-based vaccine. Furthermore, SARS-CoV is primarily composed of basic amino acid residues, which promote virus membrane fusion via electrostatic attraction with heparan sulphate proteoglycans found on the surface of target cells.

SARS-CoV enters the target cell via the endosome, and the low pH-dependent mechanism is required for cytoplasmic infection.

Coronavirus replication occurs in the cytoplasm, where viral RNA is synthesised in a flask-shaped compartment surrounded by a double membrane. These modifications include the formation of double-membrane vesicles, nucleocapsid inclusions, and cytoplasmic granulations (93,94).

Regulations for virosome-based nano vaccines

The development of virosome-based vaccines with long-term stability at ambient temperature is highly desirable, and this is the primary goal of the MACIVIVA project. Quality by Design (QbD) must eventually be established based on regulatory agencies such as the European Medicines Agency (EMA), the US Food and Drug Administration (FDA), the International Council for Harmonisation (ICH), and other valid regulatory organizations. In the healthcare manufacturing context, QbD refers to a high-quality vaccine formulation that includes active ingredients, excipients, and other ingredients. QbD is primarily determined by the concepts of critical quality attributes (CQAs) and quality target product profile (QTPP).

Critical quality attributes are primarily concerned with the optimization of size, polydispersity index (PDI), shape, stability, Z-potential, the surface-to-volume ratio, morphology, surface decoration, structural organization, stimuli responsiveness, and thermodynamic properties, all of which are critical to ensuring the safety and efficacy of the nanoformulation. Standard laboratory equipment, for example, must be used to assess virosome size and concentration (95).

Furthermore, virosome-based vaccine formulations must be repeatable, cost-effective, and high in output. The interaction of the CQAs parameter with the needs of the patients must then be evaluated. The QTPP was established by the vaccine's performance, indication, and administration mode. Furthermore, the international regulatory of WHO guidelines should include clinical evaluation of vaccines (95). All of the manufacturing aspects of the nanovaccine must be precisely evaluated. In the production of nasal spray vaccine dosage forms, for example, sterilization is not required, and microbial content control is sufficient. Vaccine storage conditions must be precisely controlled based on vaccine components. Freeze exposure, for example, destroys vaccines containing aluminium salt. Vaccine packages should include temperature labelling, shipping standards, and International Air Transport Association (IATA) time. Finally, nanovaccine production should be classified and packaged in accordance with World Health Organisation (WHO) Guidelines for international vaccine packaging and shipping (96).

Stimulation of cellular immunity using virosomal technique

Virosomes are an innovative vaccine technology that can be used to boost cellular immunity. They are reconstituted viral envelopes that can be used as vaccines as well as vehicles for macromolecule delivery to cells (97). Vaccination based on virosomes is currently approved in over 40 countries, including for the elderly and infants. Virosomes are biocompatible, biodegradable, nontoxic, and

non-autoimmunogenic, and various attempts have been made to use them as vaccines or adjuvants, as well as drug, nucleic acid, or gene delivery systems for therapeutic purposes. The fully functional fusion activity of virosomes containing the HA protein allows for receptor-mediated antigen uptake and natural intracellular processing, eliciting both humoral and cellular immune responses. In mice, a polyvalent virosomal influenza vaccine elicited broad cellular and humoral immune responses. Electrical stimulation can also be used to modulate the immune system, resulting in the production of immune cytokines and agents in the patient's body, or to increase the cellular uptake of these immune agents via electroporation (98,99).

In general, cytotoxic T lymphocytes (CTLs) are responsible for the neutralisation of virus-infested cells and thus for the prevention and cure of viral infection. In humans, CTLs specific to the influenza virus are primarily triggered by epitopes originating from proteins present within the virus, such as matrix proteins and nucleoproteins, whereas immunodominant epitopes are primarily manifested on the nucleoprotein in mice (100). Traditional vaccinations do not stimulate CTL action, resulting in less effective antigen carriers to the APC. Subunit vaccines lack CTL antigens such as nucleoprotein and matrix protein. Virosomal carriers can deliver Antigens directly to the cytosol, increasing CTL activity and antibody responses. Helper T cells are essential for activating B cells and changing antibody classes. They are also required for the growth of B cells and antibody class switching (49).

Furthermore, helper T cells boost cytokine production, which allows CTLs to develop. As a result, vaccines must be capable of inducing strong helper-T cell responses, which is often overlooked. Influenza infection typically induces a helper-T cell-mediated immune response, which is characterised by the secretion of Interferon (IFN) as the primary cytokine. This type of helper-T cell reaction promotes the biosynthesis of antibodies of the IgG2a subclass in mice and the IgG1 subclass in humans, as well as the development of CTLs.

Clinical status from lab to bedside

Over the last two decades, a significant number of clinical involvements with influenza vaccine in general and influenza virosomes have been gathered. Crucell's Epaxal, a hepatitis A vaccine, was the first commercially approved vaccine based on virosomes (101). In summary, Epaxal was made up of pathologically inactivated hepatitis A virus adsorbed onto the surface of a pre-made influenza virosome (102). Various clinical studies have shown that hepatitis A virus vaccines based on virosomes have nearly equal immunogenicity, significant local tolerance, and the ability to create long-term memory based on B cells when compared to alum adjuvanted products (103). Influenza vaccines are reminiscent of influenza virosomes in that the antigen is virus envelope proteins. Immunogenic effects such as increased Cluster of Differentiation 4 (CD4) and Helper-T lymphocytes are excluded from adjuvant properties. Virosomal carriers distinguish influenza vaccines from conventional subunit vaccines, but they do not contain any additional immunostimulatory constituents. Inflexal V is the only adjuvanted influenza vaccine that is appropriate for all age groups and produces a significant immune response in both immunocompromised and healthy children, adults, and the elderly (86). Nasal Flu, an influenza vaccine administered via intranasal route, contains the same virosomes as Inflexal V and is also adjuvanted with E. coli (mucosal adjuvant). It was withdrawn shortly after its release due to increased occurrences of Bell's palsy in immunised individuals (104). These adverse events were most likely caused by heat-labile toxins (HLT), as evidenced by identical findings in clinical trials with non-virosomal influenza vaccines adjuvanted with HLT and administered intranasally (105). Invivac, a virosome-based trivalent seasonal influenza vaccine for intramuscular administration introduced by Solvay Pharmaceuticals in 2004, is only available commercially for one season (106).

2. Conclusion

Virosome is an FDA-approved nanocarrier for pharmaceutical applications that provides high safety and versatility in vaccine development. It can be used in a variety of ways and in conjunction with other adjuvants. Virosome-based vaccines have been launched, and numerous products are currently in preclinical and clinical stages. Virosome induces powerful immune responses against potentially fatal viral diseases, and its adaptability allows for the development of multi-antigen epitopes versus all RNA virus strains. Rapid disintegration, on the other hand, is a significant disadvantage, and a thermostable virosome-based vaccine could revolutionize vaccination patterns in developing countries. Vaccine development is utilizing virosomes because of their capacity to deliver encapsulated antigens directly into host cells and enhance immune responses without inducing adverse effects. A wide range of bioactive molecules, including proteins, peptides, plasmids, genes, oligonucleotides, and drugs, are also transported via these nanocarriers in a targeted fashion. They can be given intranasally, intramuscularly, or intradermally and stimulate both humoral and cell-mediated immunity. Antigens can be delivered to the host body via a variety of routes, including intranasal, intradermal, and intramuscular. They can also transport cancer-fighting medications and immunomodulatory substances. Antigens and atoms such as proteins, peptides, plasmids, oligonucleotides, and medications can be transferred to cells by influenza virosomes. Their adaptability allows for a variety of vaccine delivery applications. The influenza virus-derived model is being considered for the development of a new generation of vaccines, providing pharmaceutical researchers with a new avenue to pursue. However, efforts must be made to develop long-term, cost-effective, safe, and high-clinical-output virosome modules.

Acknowledgement
The authors would like to express their administration to the College of Pharmacy, SRMIST for providing them with tremendous opportunities to accomplish this work.

Author contribution
All authors contributed to the overview. Sai Satthya: Writing- original draft preparation, conceptualization, and data curation, Jebastin Koilpillai: Editing and reviewing Damodharan Narayanasamy: Supervision

Declarations
Conflict of interest
The authors declare no competing interests.

Consent for publication
The authors give their consent for the publication.

Funding
The author(s) reported there is no funding associated with the work featured in this article.

3. Reference

1. Mastrobattista E, Schoen P, Wilschut J, Crommelin DJ., Storm G. Targeting influenza virosomes to ovarian carcinoma cells. FEBS Lett [Internet]. 2001 Nov 30;509(1):71–6. Available from: https://febs.onlinelibrary.wiley.com/doi/10.1016/S0014-5793%2801%2903112-X
2. Bovier PA. Epaxal ® : a virosomal vaccine to prevent hepatitis A infection. Expert Rev Vaccines [Internet]. 2008 Oct 1;7(8):1141–50. Available from: https://www.tandfonline.com/doi/full/10.1586/14760584.7.8.1141
3. Waelti E, Wegmann N, Schwaninger R, Wetterwald A, Wingenfeld C, Rothen-Rutishauser B, et al. Targeting her-2/neu with antirat Neu virosomes for cancer therapy. Cancer Res [Internet]. 2002 Jan 15;62(2):437–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11809693

4. Kuate S, Cinatl J, Doerr HW, Überla K. Exosomal vaccines containing the S protein of the SARS coronavirus induce high levels of neutralizing antibodies. Virology [Internet]. 2007 May;362(1):26–37. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0042682206008981
5. Genton B. Malaria vaccines: a toy for travelers or a tool for eradication? Expert Rev Vaccines [Internet]. 2008 Jul 9;7(5):597–611. Available from: http://www.tandfonline.com/doi/full/10.1586/14760584.7.5.597
6. DAEMEN T, DEMARE A, BUNGENER L, DEJONGE J, HUCKRIEDE A, WILSCHUT J. Virosomes for antigen and DNA delivery. Adv Drug Deliv Rev [Internet]. 2005 Jan 10;57(3):451–63. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169409X04002121
7. Adamina M, Weber WP, Rosenthal R, Schumacher R, Zajac P, Guller U, et al. Heterologous prime-boost immunotherapy of melanoma patients with Influenza virosomes, and recombinant Vaccinia virus encoding 5 melanoma epitopes and 3 co-stimulatory molecules. A multi-centre phase I/II open labeled clinical trial. Contemp Clin Trials [Internet]. 2008 Mar;29(2):165–81. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1551714407000894
8. Ali H, Akbar M, Iqbal B, Ali F, Sharma NK, Kumar N, et al. Virosome: An engineered virus for vaccine delivery. Saudi Pharm J [Internet]. 2023 May;31(5):752–64. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1319016423000749
9. Bron R, Ortiz A, Dijkstra J, Stegmann T, Wilschut J. [23] Preparation, properties, and applications of reconstituted influenza virus envelopes (virosomes). In 1993. p. 313–31. Available from: https://linkinghub.elsevier.com/retrieve/pii/007668799320091G
10. Almeida J, Edwards DC, Brand C, Heath T. FORMATION OF VIROSOMES FROM INFLUENZA SUBUNITS AND LIPOSOMES. Lancet [Internet]. 1975 Nov;306(7941):899–901. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673675921303
11. Tamborrini M, Mueller MS, Stoffel SA, Westerfeld N, Vogel D, Boato F, et al. Design and pre-clinical profiling of a Plasmodium falciparum MSP-3 derived component for a multi-valent virosomal malaria vaccine. Malar J [Internet]. 2009 Dec 30;8(1):314. Available from: https://malariajournal.biomedcentral.com/articles/10.1186/1475-2875-8-314
12. Cornet B, Vandenbranden M, Cogniaux J, Giurgea L, Dekegel D, Ruysschaert J-M. Virosomes reconstituted from Human Immunodeficiency Virus proteins and lipids. Biochem Biophys Res Commun [Internet]. 1990 Feb;167(1):222–31. Available from: https://linkinghub.elsevier.com/retrieve/pii/0006291X9091754G
13. Ebensen T, Debarry J, Pedersen GK, Blazejewska P, Weissmann S, Schulze K, et al. Mucosal Administration of Cycle-Di-Nucleotide-Adjuvanted Virosomes Efficiently Induces Protection against Influenza H5N1 in Mice. Front Immunol [Internet]. 2017 Sep 28;8. Available from: http://journal.frontiersin.org/article/10.3389/fimmu.2017.01223/full
14. Grimaldi S, Giuliani A, Giuliani A, Ferroni L, Lisi A, Santoro N, et al. Engineered liposomes and virosomes for delivery of macromolecules. Res Virol [Internet]. 1995 Jul;146(4):289–93. Available from: https://linkinghub.elsevier.com/retrieve/pii/0923251696805747
15. Bagai S, Sarkar DP. Reconstituted Sendai virus envelopes as biological carriers: dual role of F protein in binding and fusion with liver cells. Biochim Biophys Acta - Biomembr [Internet]. 1993 Oct;1152(1):15–25. Available from: https://linkinghub.elsevier.com/retrieve/pii/000527369390226P
16. Mayorga O, Bühler S, Jaeger VK, Bally S, Hatz C, Frösner G, et al. Single-Dose Hepatitis A Immunization: 7.5-Year Observational Pilot Study in Nicaraguan Children to Assess Protective Effectiveness and Humoral Immune Memory Response. J Infect Dis [Internet]. 2016 Nov 15;214(10):1498–506. Available from: https://academic.oup.com/jid/article-

lookup/doi/10.1093/infdis/jiw411
17. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, et al. LMO2 -Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1. Science (80-) [Internet]. 2003 Oct 17;302(5644):415–9. Available from: https://www.science.org/doi/10.1126/science.1088547
18. Taura A, Taura K, Choung YH, Masuda M, Pak K, Chavez E, et al. Histone deacetylase inhibition enhances adenoviral vector transduction in inner ear tissue. Neuroscience [Internet]. 2010 Apr;166(4):1185–93. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0306452209021526
19. Kafri T, Morgan D, Krahl T, Sarvetnick N, Sherman L, Verma I. Cellular immune response to adenoviral vector infected cells does not require de novo viral gene expression: Implications for gene therapy. Proc Natl Acad Sci [Internet]. 1998 Sep 15;95(19):11377–82. Available from: https://pnas.org/doi/full/10.1073/pnas.95.19.11377
20. Schmidt-Wolf GD, Schmidt-Wolf IG. Non-viral and hybrid vectors in human gene therapy: an update. Trends Mol Med [Internet]. 2003 Feb;9(2):67–72. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1471491403000054
21. Kumar V, Singh R, Chaudhuri A. Cationic Transfection Lipids in Gene Therapy: Successes, Set-backs, Challenges and Promises. Curr Med Chem [Internet]. 2003 Jul 1;10(14):1297–306. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=0929-8673&volume=10&issue=14&spage=1297
22. Dass C. Lipoplex-mediated delivery of nucleic acids: factors affecting in vivo transfection. J Mol Med [Internet]. 2004 Sep 23;82(9). Available from: http://link.springer.com/10.1007/s00109-004-0558-8
23. Bagai S, Puri A, Blumenthal R, Sarkar DP. Hemagglutinin-neuraminidase enhances F protein-mediated membrane fusion of reconstituted Sendai virus envelopes with cells. J Virol [Internet]. 1993 Jun;67(6):3312–8. Available from: https://journals.asm.org/doi/10.1128/jvi.67.6.3312-3318.1993
24. Singh N, Gautam SP, Kumari N, Kaur R, Kaur M. Virosomes as Novel drug delivery System : An Overview. PharmaTutor. 2017;5(9):47–55.
25. Felnerova D, Viret J-F, Glück R, Moser C. Liposomes and virosomes as delivery systems for antigens, nucleic acids and drugs. Curr Opin Biotechnol [Internet]. 2004 Dec;15(6):518–29. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0958166904001430
26. Bungener L, Huckriede A, de Mare A, de Vries-Idema J, Wilschut J, Daemen T. Virosome-mediated delivery of protein antigens in vivo: efficient induction of class I MHC-restricted cytotoxic T lymphocyte activity. Vaccine [Internet]. 2005 Jan;23(10):1232–41. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0264410X0400670X
27. Bungener L, Serre K, Bijl L, Leserman L, Wilschut J, Daemen T, et al. Virosome-mediated delivery of protein antigens to dendritic cells. Vaccine [Internet]. 2002 May;20(17–18):2287–95. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0264410X02001032
28. Perrin P, Sureau P, Thibodeau L. Structural and immunogenic characteristics of rabies immunosomes. Dev Biol Stand [Internet]. 1985;60:483–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4043539
29. Hug P, Sleight RG. Fusogenic virosomes prepared by partitioning of vesicular stomatitis virus G protein into preformed vesicles. J Biol Chem [Internet]. 1994 Feb;269(6):4050–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925817417418
30. Amacker M, Engler O, Kammer AR, Vadrucci S, Oberholzer D, Cerny A, et al. Peptide-loaded chimeric influenza virosomes for efficient in vivo induction of cytotoxic T cells. Int Immunol [Internet]. 2005 Jun 1;17(6):695–704. Available from: http://academic.oup.com/intimm/article/17/6/695/671220/Peptideloaded-chimeric-influenza-

virosomes-for
31. Cusi MG. Applications of Influenza Virosomes as a Delivery System. Hum Vaccin [Internet]. 2006 Jan 31;2(1):1–7. Available from: http://www.tandfonline.com/doi/abs/10.4161/hv.2.1.2494
32. Huckriede A, Bungener L, Daemen T, Wilschut J. Influenza Virosomes in Vaccine Development. In 2003. p. 74–91. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0076687903730055
33. Ludwig C, Wagner R. Virus-like particles—universal molecular toolboxes. Curr Opin Biotechnol [Internet]. 2007 Dec;18(6):537–45. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0958166907001462
34. Durrer P, Galli C, Hoenke S, Corti C, Glück R, Vorherr T, et al. H+-induced Membrane Insertion of Influenza Virus Hemagglutinin Involves the HA2 Amino-terminal Fusion Peptide but Not the Coiled Coil Region. J Biol Chem [Internet]. 1996 Jun;271(23):13417–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925818413385
35. Weber T, Paesold G, Galli C, Mischler R, Semenza G, Brunner J. Evidence for H(+)-induced insertion of influenza hemagglutinin HA2 N-terminal segment into viral membrane. J Biol Chem [Internet]. 1994 Jul;269(28):18353–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925817323141
36. Kim CU, Chen X, Mendel DB. Neuraminidase Inhibitors as Anti-Influenza Virus Agents. Antivir Chem Chemother [Internet]. 1999 Aug 24;10(4):141–54. Available from: http://journals.sagepub.com/doi/10.1177/095632029901000401
37. Nalawade R, Nalawade A, Patwa H. Supplement Issue. Ann Clin Psychiatry. 2008;20 Suppl 1(2582):1–32.
38. de Bruijn IA, Nauta J, Gerez L, Palache AM. The virosomal influenza vaccine Invivac®: Immunogenicity and tolerability compared to an adjuvanted influenza vaccine (Fluad®) in elderly subjects. Vaccine [Internet]. 2006 Nov;24(44–46):6629–31. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0264410X06006207
39. Glück R. Intranasal Immunization against Influenza. J Aerosol Med [Internet]. 2002 Jun;15(2):221–8. Available from: http://www.liebertpub.com/doi/10.1089/089426802320282347
40. Van Herck K, Hens A, De Coster I, Vertruyen A, Tolboom J, Sarnecki M, et al. Long-term Antibody Persistence in Children After Vaccination With the Pediatric Formulation of an Aluminum-free Virosomal Hepatitis A Vaccine. Pediatr Infect Dis J [Internet]. 2015 Apr;34(4):e85–91. Available from: https://journals.lww.com/00006454-201504000-00017
41. Kürsteiner O, Moser C, Lazar H, Durrer P. Inflexal® V—The influenza vaccine with the lowest ovalbumin content. Vaccine [Internet]. 2006 Nov;24(44–46):6632–5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0264410X06006219
42. Lim J, Song Y-J, Park W-S, Sohn H, Lee M-S, Shin D-H, et al. The Immunogenicity of a Single Dose of Hepatitis A Virus Vaccines (Havrix® and Epaxal®) in Korean Young Adults. Yonsei Med J [Internet]. 2014;55(1):126. Available from: https://eymj.org/DOIx.php?id=10.3349/ymj.2014.55.1.126
43. Garg H, Viard M, Jacobs A, Blumenthal R. Targeting HIV-1 gp41-induced Fusion and Pathogenesis for Anti-viral Therapy. Curr Top Med Chem [Internet]. 2011 Dec 1;11(24):2947–58. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1568-0266&volume=11&issue=24&spage=2947
44. Lima LC, Marques RF, Gimenez AM, Françoso KS, Aliprandini E, Camargo TM, et al. A Multistage Formulation Based on Full-Length CSP and AMA-1 Ectodomain of Plasmodium vivax Induces High Antibody Titers and T-cells and Partially Protects Mice Challenged with a Transgenic Plasmodium berghei Parasite. Microorganisms [Internet]. 2020 Jun 17;8(6):916. Available from: https://www.mdpi.com/2076-2607/8/6/916
45. Choudhury A, Kiessling R. Her-2/Neu as a Paradigm of a Tumor-Specific Target for

Therapy. Wei W-Z, Lopez D, editors. Breast Dis [Internet]. 2004 Nov 24;20(1):25–31. Available from: https://www.medra.org/servlet/aliasResolver?alias=iospress&doi=10.3233/BD-2004-20104
46. Kalra N, Dhanya V, Saini V, Jeyabalan G. Virosomes : As a Drug Delivery Carrier. Am J Adv Drug Deliv. 2013;29–35.
47. Medarametla RT, K VG, J NSK, A KS, G M chari, A KK, et al. A comprehensive study on the review of virosomes As a novel drug delivery system. UPI J Pharm Med Heal Sci [Internet]. 2023 Oct 15;1–6. Available from: https://uniquepubinternational.com/journals/index.php/jpmhs/article/view/94
48. Nakamura N, Hart DA, Frank CB, Marchuk LL, Shrive NG, Ota N, et al. Efficient Transfer of Intact Oligonucleotides into the Nucleus of Ligament Scar Fibroblasts by HVJ-Cationic Liposomes Is Correlated with Effective Antisense Gene Inhibition. J Biochem [Internet]. 2001 May 1;129(5):755–9. Available from: https://academic.oup.com/jb/article-lookup/doi/10.1093/oxfordjournals.jbchem.a002916
49. A two-phase model of B-cell activation. Immunol Rev [Internet]. 2000 Aug 12;176(1):171–80. Available from: https://onlinelibrary.wiley.com/doi/10.1034/j.1600-065X.2000.00606.x
50. Shaikh SN, Raza S, Ansari MA, Khan GJ, Athar SHM. Overview on virosomes as a novel carrier for drug delivery. J Drug Deliv Ther [Internet]. 2019 Jan 1;8(6-s):429–34. Available from: http://jddtonline.info/index.php/jddt/article/view/2163
51. Metcalfe IC, Glück R. Virosomes for vaccine delivery. In: Immunopotentiators in Modern Vaccines [Internet]. Elsevier; 2006. p. 179–89. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780120884032500125
52. Zurbriggen R. Immunostimulating reconstituted influenza virosomes. Vaccine [Internet]. 2003 Feb;21(9–10):921–4. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0264410X02005418
53. Suarez D. Immunology of avian influenza virus: a review. Dev Comp Immunol [Internet]. 2000 Mar 1;24(2–3):269–83. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0145305X99000786
54. Skehel JJ, Wiley DC. Receptor Binding and Membrane Fusion in Virus Entry: The Influenza Hemagglutinin. Annu Rev Biochem [Internet]. 2000 Jun;69(1):531–69. Available from: https://www.annualreviews.org/doi/10.1146/annurev.biochem.69.1.531
55. Fukuyama S, Kawaoka Y. The pathogenesis of influenza virus infections: the contributions of virus and host factors. Curr Opin Immunol [Internet]. 2011 Aug;23(4):481–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0952791511000896
56. Curran J, Kolakofsky D. Replication of Paramyxoviruses. In 1999. p. 403–22. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0065352708603735
57. Okada Y. [3] Sendai virus-induced cell fusion. In 1993. p. 18–41. Available from: https://linkinghub.elsevier.com/retrieve/pii/007668799321005S
58. Yamada T, Iwasaki Y, Tada H, Iwabuki H, Chuah MK, VandenDriessche T, et al. Nanoparticles for the delivery of genes and drugs to human hepatocytes. Nat Biotechnol [Internet]. 2003 Aug 29;21(8):885–90. Available from: https://www.nature.com/articles/nbt843
59. Kapczynski DR. Development of a Virosome Vaccine Against Avian Metapneumovirus Subtype C for Protection in Turkeys. Avian Dis [Internet]. 2004 Apr;48(2):332–43. Available from: http://www.bioone.org/doi/abs/10.1637/7115
60. Sharma D, Ali AAE, Trivedi LR. An Updated Review On:Liposomes as Drug Delivery System. Pharmatutor. 2018;6(2):50.
61. Dowdle WR, Davenport FM, Fukumi H, Schild GC, Tumova B, Webster RG, et al. Orthomyxoviridae. Intervirology [Internet]. 1975;5(5):245–51. Available from: https://www.karger.com/Article/FullText/149921
62. Galinski MS. Paramyxoviridae: Transcription and Replication. In 1991. p. 129–62. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0065352708607940

63. Liu W-X, Zhao J-W, Zuo A-X, Yang Z, Gao L, Zhou M, et al. Two novel terpenoids from the cultured Perovskia atriplicifolia. Fitoterapia [Internet]. 2018 Oct;130:152–5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0367326X18310347
64. Coffin J, Blomberg J, Fan H, Gifford R, Hatziioannou T, Lindemann D, et al. ICTV Virus Taxonomy Profile: Retroviridae 2021. J Gen Virol [Internet]. 2021 Dec 23;102(12). Available from: https://www.microbiologyresearch.org/content/journal/jgv/10.1099/jgv.0.001712
65. Alexander DJ. Avian Paramyxoviridae - recent developments. Vet Microbiol [Internet]. 1990 Jun;23(1–4):103–14. Available from: https://linkinghub.elsevier.com/retrieve/pii/037811359090140Q
66. Stegmann T, Morselt HW, Booy FP, van Breemen JF, Scherphof G, Wilschut J. Functional reconstitution of influenza virus envelopes. EMBO J [Internet]. 1987 Sep;6(9):2651–9. Available from: https://onlinelibrary.wiley.com/doi/10.1002/j.1460-2075.1987.tb02556.x
67. Bron R, Ortiz A, Wilschut J. Cellular Cytoplasmic Delivery of a Polypeptide Toxin by Reconstituted Influenza Virus Envelopes (Virosomes). Biochemistry [Internet]. 1994 Aug 1;33(31):9110–7. Available from: https://pubs.acs.org/doi/abs/10.1021/bi00197a013
68. Schoen P, Bron R, Wilschut J. Delivery of Foreign Substances to Cells Mediated by Fusion-Active Reconstituted Influenza Virus Envelopes (Virosomes). J Liposome Res [Internet]. 1993 Jan 28;3(3):767–92. Available from: http://www.tandfonline.com/doi/full/10.3109/08982109309150758
69. Metsikkö K, van Meer G, Simons K. Reconstitution of the fusogenic activity of vesicular stomatitis virus. EMBO J [Internet]. 1986 Dec;5(13):3429–35. Available from: https://onlinelibrary.wiley.com/doi/10.1002/j.1460-2075.1986.tb04665.x
70. Schoen P, Chonn A, Cullis PR, Wilschut J, Scherrer P. Gene transfer mediated by fusion protein hemagglutinin reconstituted in cationic lipid vesicles. Gene Ther [Internet]. 1999 May 1;6(5):823–32. Available from: https://www.nature.com/articles/3300919
71. Bomsel M, Tudor D, Drillet A-S, Alfsen A, Ganor Y, Roger M-G, et al. Immunization with HIV-1 gp41 Subunit Virosomes Induces Mucosal Antibodies Protecting Nonhuman Primates against Vaginal SHIV Challenges. Immunity [Internet]. 2011 Feb;34(2):269–80. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1074761311000367
72. Sreeja C Nair, Sreelekshmi A S, Aslam Abdul Rahiman C A, Karthik Krishnan, Gopika G, Priyanka Nair T, et al. Virosomes: A groundbreaking revolution in novel drug delivery. Int J Res Pharm Sci [Internet]. 2020 Sep 29;11(4):5809–20. Available from: https://pharmascope.org/ijrps/article/view/3230
73. J. Josephine Leno Jenita, Madhusudhan N T, B.WilsoN, Manjula. D SBK. World Journal of Pharmaceutical research FORMULATION DEVELOPMENT AND EVALUATION OF. World J Pharm Res. 2012;1(2):207–15.
74. Nishimura Y, Mieda H, Ishii J, Ogino C, Fujiwara T, Kondo A. Targeting cancer cell-specific RNA interference by siRNA delivery using a complex carrier of affibody-displaying bio-nanocapsules and liposomes. J Nanobiotechnology [Internet]. 2013 Dec 24;11(1):19. Available from: https://jnanobiotechnology.biomedcentral.com/articles/10.1186/1477-3155-11-19
75. Wilschut J. Influenza vaccines: The virosome concept. Immunol Lett [Internet]. 2009 Feb;122(2):118–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165247808002551
76. de Jonge J, Holtrop M, Wilschut J, Huckriede A. Reconstituted influenza virus envelopes as an efficient carrier system for cellular delivery of small-interfering RNAs. Gene Ther [Internet]. 2006 Mar 1;13(5):400–11. Available from: https://www.nature.com/articles/3302673
77. Liu J, Wu J, Wang B, Zeng S, Qi F, Lu C, et al. Oral vaccination with a liposome-encapsulated influenza DNA vaccine protects mice against respiratory challenge infection. J Med Virol [Internet]. 2014 May 30;86(5):886–94. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jmv.23768

78. Pastori C, Wahlestedt C. Involvement of long noncoding RNAs in diseases affecting the central nervous system. RNA Biol [Internet]. 2012 Jun 27;9(6):860–70. Available from: http://www.tandfonline.com/doi/abs/10.4161/rna.20482
79. Mohd Muaz Mughees, Mughees Ahmad Ansari, Afifa Mughees, Faisal Farooque, Mohd Wasi. Virosomes as drug delivery system: An updated review. Int J Res Pharm Sci [Internet]. 2021 Sep 15;12(3):2239–47. Available from: https://pharmascope.org/index.php/ijrps/article/view/4850
80. Rathore et al, 2008.pdf. 2012;3(04):1184–7.
81. Beg S, Samad A, Nazish I, Sultana R, Rahman M, Zaki Ahmad M, et al. Colloidal Drug Delivery Systems in Vaccine Delivery. Curr Drug Targets [Internet]. 2013 Jan 1;14(1):123–37. Available from: http://openurl.ingenta.com/content/xref?genre=article&issn=1389-4501&volume=14&issue=1&spage=123
82. Glueck R. Pre-clinical and clinical investigation of the safety of a novel adjuvant for intranasal immunization. Vaccine [Internet]. 2001 Oct;20:S42–4. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0264410X01002924
83. Jeong S-H, Lee H-S. Hepatitis A: Clinical Manifestations and Management. Intervirology [Internet]. 2010;53(1):15–9. Available from: https://www.karger.com/Article/FullText/252779
84. Yuan Y, Cai X, Shen F, Ma F. HPV post-infection microenvironment and cervical cancer. Cancer Lett [Internet]. 2021 Jan;497:243–54. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304383520305681
85. Trépo C, Chan HLY, Lok A. Hepatitis B virus infection. Lancet [Internet]. 2014 Dec;384(9959):2053–63. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673614602208
86. Herzog C, Hartmann K, Künzi V, Kürsteiner O, Mischler R, Lazar H, et al. Eleven years of Inflexal® V—a virosomal adjuvanted influenza vaccine. Vaccine [Internet]. 2009 Jul;27(33):4381–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0264410X09007063
87. Hodgson CP, Solaiman F. Virosomes: Cationic Liposomes Enhance Retroviral Transduction. Nat Biotechnol [Internet]. 1996 Mar;14(3):339–42. Available from: https://www.nature.com/articles/nbt0396-339
88. Kaneda Y. Virosome: A novel vector to enable multi-modal strategies for cancer therapy. Adv Drug Deliv Rev [Internet]. 2012 Jun;64(8):730–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169409X1100055X
89. Amacker M, Moese S, Kammer AR, Helenius A, Zurbriggen R. Influenza Virosomes as Delivery Systems for Antigens. In: Delivery Technologies for Biopharmaceuticals [Internet]. Wiley; 2009. p. 377–93. Available from: https://onlinelibrary.wiley.com/doi/10.1002/9780470688397.ch19
90. Wegmann N, Schwaninger R, Wetterwald A, Wingenfeld C, Gimmi CD, Waelti E, et al. Targeting HER-2/neu with antirat neu virosomes for cancer therapy. Cancer Res. 2002;62(2):437–44.
91. Hashemi SA, Golab Behbahan NG, Bahrani S, Mousavi SM, Gholami A, Ramakrishna S, et al. Ultra-sensitive viral glycoprotein detection NanoSystem toward accurate tracing SARS-CoV-2 in biological/non-biological media. Biosens Bioelectron [Internet]. 2021 Jan;171:112731. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0956566320307193
92. Coleman CM, Liu Y V., Mu H, Taylor JK, Massare M, Flyer DC, et al. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine [Internet]. 2014 May;32(26):3169–74. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0264410X14005180
93. White JM, Whittaker GR. Fusion of Enveloped Viruses in Endosomes. Traffic [Internet]. 2016 Jun 7;17(6):593–614. Available from: https://onlinelibrary.wiley.com/doi/10.1111/tra.12389
94. Gupta T, Gupta SK. Potential adjuvants for the development of a SARS-CoV-2 vaccine

based on experimental results from similar coronaviruses. Int Immunopharmacol [Internet]. 2020 Sep;86:106717. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1567576920313862
95. Asadi K, Gholami A. Virosome-based nanovaccines; a promising bioinspiration and biomimetic approach for preventing viral diseases: A review. Int J Biol Macromol [Internet]. 2021;182:648–58. Available from: https://doi.org/10.1016/j.ijbiomac.2021.04.005
96. Guidelines on the international packaging and shipping of vaccines.
97. Fonseca FN, Haach V, Bellaver FV, Bombassaro G, Gava D, da Silva LP, et al. Immunological profile of mice immunized with a polyvalent virosome-based influenza vaccine. Virol J [Internet]. 2023 Aug 21;20(1):187. Available from: https://virologyj.biomedcentral.com/articles/10.1186/s12985-023-02158-0
98. Haach V, Bastos APA, Gava D, da Fonseca FN, Morés MAZ, Coldebella A, et al. A polyvalent virosomal influenza vaccine induces broad cellular and humoral immunity in pigs. Virol J [Internet]. 2023 Aug 16;20(1):181. Available from: https://virologyj.biomedcentral.com/articles/10.1186/s12985-023-02153-5
99. Das R, Langou S, Le TT, Prasad P, Lin F, Nguyen TD. Electrical Stimulation for Immune Modulation in Cancer Treatments. Front Bioeng Biotechnol [Internet]. 2022 Jan 11;9. Available from: https://www.frontiersin.org/articles/10.3389/fbioe.2021.795300/full
100. Parker*† CE, Gould* KG. Influenza A virus—a model for viral antigen presentation to cytotoxic T lymphocytes. Semin Virol [Internet]. 1996 Feb;7(1):61–73. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1044577396900086
101. AMBROSCH F. Immunogenicity and protectivity of a new liposomal hepatitis A vaccine. Vaccine [Internet]. 1997 Aug;15(11):1209–13. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0264410X97000157
102. Glück R, Wälti E. Biophysical validation of Epaxal Berna, a hepatitis A vaccine adjuvanted with immunopotentiating reconstituted influenza virosomes (IRIV). Dev Biol (Basel) [Internet]. 2000;103:189–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11214236
103. Bovier PA. Recent advances with a virosomal hepatitis A vaccine. Expert Opin Biol Ther [Internet]. 2008 Aug 9;8(8):1177–85. Available from: http://www.tandfonline.com/doi/full/10.1517/14712598.8.8.1177
104. Mutsch M, Zhou W, Rhodes P, Bopp M, Chen RT, Linder T, et al. Use of the Inactivated Intranasal Influenza Vaccine and the Risk of Bell's Palsy in Switzerland. N Engl J Med [Internet]. 2004 Feb 26;350(9):896–903. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa030595
105. Smith DJ. Prospects in Caries Vaccine Development. J Dent Res [Internet]. 2012 Mar 25;91(3):225–6. Available from: http://journals.sagepub.com/doi/10.1177/0022034511425928
106. de Bruijn I, Meyer I, Gerez L, Nauta J, Giezeman K, Palache B. Antibody induction by virosomal, MF59-adjuvanted, or conventional influenza vaccines in the elderly. Vaccine [Internet]. 2007 Dec;26(1):119–27. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0264410X07012248