Virosome: A vector in vaccine delivery

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ABSTRACT: These days vaccines are considered the best economical and effective technique for preventing and handling bacterial infections, like human papillomavirus (HPV) or meningitis, allergies, autoimmune Relevant Disorders, Microbial Infections, and many more viral diseases. Annually millions of lives are saved from death-causing infections and other relevant disorders with the help of proper immunization with the help of vaccines. But still, many diseases are not yet preventable by vaccines and there is a chance of the improvement of current vaccines with help of many approaches. One of these is a compound known as a virosome, which is produced when pure haemagglutinin and neuraminidase projections of the influenza virus surface are removed from viral envelope and deposited on the exterior of unilamellar liposomes.Studies have shown that in order to attain the desired therapeutic effect, a physical attachment between the target antigen and the virosomal carrier is necessary. A virosome can bind to and "infect" host cells and deliver the antigen directly into the processing pathway which is an Alternatively, the virosome may be phagocytosed by an APC. So Our review gives a novel idea of the vector technology for vaccine delivery, its properties, method of preparation, and importance as well as some applications of different virosomal vaccine discoveries.

Keywords: Vaccine, viral diseases, virosomes, unilamellar liposomes.

1 Introduction

These days, vaccines are considered the best economical and effective technique for preventing and handling bacterial infections, such as human papillomavirus (HPV) or meningitis, allergies, autoimmune Relevant Disorders, Microbial Infections, and many more viral diseases. Annually millions of lives are saved from death-causing infections and other relevant disorders with the help of proper immunization with the help of vaccines. But still, many diseases are not yet preventable by vaccines and there is a chance of the improvement of current vaccines[1]. Remarkably in past years Covid-19 Affected the world and included the scope of vaccination targets as they are subjected to immune therapy, which has given good therapeutic effects in certain patients, thanks to the advances made in the Virology, Biotechnology, Pharma, and all relevant fields for their diligent work of building immunity with the research in Vaccines[2].Vaccines were previously solely created for defensive or guarding against viral infections because it is so easy to make complete organisms that have been attenuated or damaged to diminish their capacity to produce disease and intrinsic pathogenicity. The capacity of today's vaccinations to stimulate the host's immune system even after injection helps the patient build a strong defence against similar microorganisms. In order to find additional methods to improve vaccine-induced protecting the host against illnesses, the discovery of novel antigens with more intense and protective immune responses is consequently a crucial research emphasis. [3].An FDA-approved carrier system with remarkable bioinspiration and biomimetic power against viral infections is called Virosome. It is a lipophilic nanomaterial.They are surface modified with critical viral fusion proteins is the main foundation of vaccine development[4].

Antigens Presented at the Surface interact with the specifically targeted receptors presented on the host cells that activate cellular, as well as humoral immune responses with the help of antibody-producing B cells and,are incorporated by endocytosis-mediated pathways[5].To date against life-threatening and widely spreading infections, several virosomal nano vaccineformulations haveexpectedto be commercialized. The development of a virosome-based vaccination platform against the novel SARS-CoV-2 virus was the focus of intense work a few months ago. Few of them concentrated on the immunity of the mucosa of the upper airways, Specifically the nasal cavity to give effect with more efficacy against the primary SARS-CoV-2 site of replication[6].The main motive of any drug is delivered to the targeted cell, tissue, and organ. Most drugs show positive results in In vitro but fail in In Vivo due to a lack of drug transported to cells, tissue, and organs.To get the better of unproductive delivery to target the cell, tissue, and organ Novel drug molecule delivery system carrier systems are developed (i.e.,Virosomes)[7].Virosomes are essentially recreated viral envelopes that function as a delivery vehicle for cells containing various macromolecules that are added to vaccinations. Virosomes are reconstructed viral envelopes that serve as both vaccinations and delivery systems for various macromolecules in cells[8]. The perspective of this virosome-based carrier system is to use this system for the field of interest of Innovations and research field as they are biodegradable, biocompatible, non-poisonous, and

nonauto-immunogenic[9]. Virosomal carrier systems have been employed as adjuvants or antibodies and drug delivery systems for organic remediation. This strategyis used to establish the bioactive material and fuse them into the virosome, they are also used to convey peptides, nucleic acids medications like anticancer agents, anti-toxins, and steroids[10].

2 History of different carrier systems

The leading type of carrier is a liposome, which is mostly utilised as a the nanocarrier for medicinal molecules. So many modifications are done in Liposomes with time to make better and upgrade the physicochemical and natural features, stimulants responsive liposomes, performing in long-circulating and ligand- targeted among others[11].In this Research on carrier systems, different Nomenclatures were reported in various articles and literature. In many cases, the new names suggested becoming known as new nanocarriers, whichcaused confusion these vesicle systems are new vesicles or consider modified liposomes[12].So above we prepared table 1which gives a general introduction to the vehicleswith the suffix "some"which can be used in the delivery of drugs as well as other therapeutically active materialswith different proportions of the lipids as well as others hydrophilic or lipophilic composites[13].

Table 1. Different vehicle systems with the suffix "somes"

Year	Name	Chief components	Therapeutic application	Size	Reference
1965	Liposome	Phospholipids (naturalor synthetic)	Mostly in drug, vaccine, and gene delivery	50–1000 nm	[14]
1979	Niosome	Non-ionic surface active agent Cholesterol	Mostly in gene and drug molecule delivery system	100 nm- > 1000 nm	[15]
1997	Ethosome	Phospholipids Propylene glycol Cholesterol Ethanol	Mostly in drug molecule delivery system	50–500 nm	[16]
1996	Enzymosome	Phospholipids ethanol (edge activator) Cholesterol Stearylamine	Mostly in enzymes delivery	100–800 nm	[17]
1995	Transferosome	Non-ionic or single chain surface active agent edge activator) Phospholipids Cholesterol	Mostly in drug molecule delivery system	60–200 nm	[18]
1989	Phytosome	Phytoconstituents Phospholipids	Mostly in phytochemicals delivery	60-nm − 20 µm	[19]
1986	Pharmacosome	Phosphatide (natural or synthetic)-drug conjugate	Mostly in pro-drugs	70 nm − 150 µm	[20]
1973	Virosome	Haemagglutinin (HA) and neuraminidase (NA) derived from virus and lipids. Phospholipids	Vaccine	>200 nm	[21]

Virosomes were first generated by[22]., when the virus that causes influenza contains haemagglutinin as well as neuraminidase projections on the surface were relocated from the envelope of the virus to the outermost layer of unilamellar liposomes & purified, the virosome structure was created. In the 1970s, virosomes were first implicated as a vaccine is proposed.Thisresulting structure was examined with the help of an electron microscope, and it looked like an original virus, so the "virosome" name is proposed for this new entity[23].

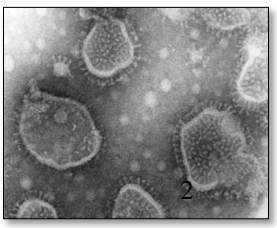

FIGURE 1. Analysis of virosomes of influenza under electron microscope negative stain electron micrograph[4].

3 Comparison of virosomes with liposomes

As there are so many carrier systems available for targeting biomolecule delivery for the delivery to living organismsboth in vivo as well as in vitro but they sometimes fail to give good results for delivering the encapsulated molecules in the cytosol of the host cell. This is because of its potential to connect with the host cell. As we know Virosome containthe active glycoproteins of the origin of viruses, they have the property of receptor-mediated binding as well as membrane fusion which gives the optimum delivery of that specific molecule inside the cell ofthe host's cytosol[6].

Some comparative studies are done on virosomes with liposomes in earlier periods one of them is that HVJ (Hemagglutinating virus of Japan) virosomes intracellularly deliveroligonucleotides and they havethree times higher potential contrasted with cationic liposomes. In virosomes, HA(one of the important components present in the influenza virosomal membrane and homogeneity, fusion, and binding properties as well as stability in the structure of the virosome.pH inside the host cell's endosome is low which boosts the cell fusion which is HA- mediatedare packed inside the membrane of virosome are released from the microenvironmentof the endosome into the host cell'sCytosol, by improving cytosolic delivery[24].

The problem associated with liposomal systems such as minimum protection for therapeutic biomolecules from external microenvironments, for instance, acidic and alkaline PH inside the organelles can be overcome by using virosomal technology.Virosomes with their immunogenic properties canstimulate theimmune system of the host which results with the advantage of as adjuvant and carrier to introduce the antigens. In addition as in liposomes, he,clearance by the body's mononuclear phagocytesystem is rapid as comparedto virosomes[25].

4 Patents regarding preparation and composition of influenza virosomes

Table2. Patents related to Influenza Virosome

Filing date	Publication number	Title	Content	Owner
08 May 1992	WO92/19267	Immunostimulant and immunopotentiations reconstituted influenza virosomes and vaccines containing them	An IMMUNOSTIMULATING RECONSTITUTED INFLUENZA VIROSOME(IRIV) comprising a mixture of Phospholipids; essentially reconstitute functional virus envelops; an influenza HA protein that is capable of inducing the fusion of said IRIV with cellular membranes and an antigen	Swiss Serum and Vaccine Institute (Crucell Switzerland AG)
11 February 2004	WO2004/071492	Virosomes-like particles	a technique for producing virus-like particles that involves dissolving encapsulated viruses in short-chain phospholipids and forming a functionally reconstituted viral envelope once the short-chain phospholipids have been removed.	By Bestewil (mymeticsInC.)
18 June 2004	WO2004/110486	Functionally reconstitute viral membranes containing adjuvant	Reconstituted viral membrane lipid bilayer vesicle that is fusion compatible and comprises an influenza virus membrane protein and an amphiphilic adjuvant	By Bestewil (mymeticsInC)
26 May 2005	WO2004/110486	A vaccine composition comprising virosomes and a saponin adjuvant	It contains IMMUNOSTIMULATING RECONSTITUTED INFLUENZA VIROSOME(IRIVand Q521 and additionally an exogenous sterol such as cholesterol)	By GlaxoSmith Kline(GSK)

20 May 2005	WO2006/045532	Virosome particles comprising antigens from the influenza virus and hepatitis B virus	Envelop proteins from the influenza virus and antigens from the hepatitis B virus make up viral chromosomes (HBV and HBC)	By Crucell Switzerland AG
21 December 2005	WO2006/069719	Lyophilization of virosomes	A composition of IMMUNOSTIMULATING RECONSTITUTED INFLUENZA VIROSOME(IRIV) with cationic cholesterol, which enables lyophilization and functional reconstitution of virosomes	By Pevion Biotech AG
15 December 2006	WO2007/068497	An adjuvant system comprising virosomes and liposomes	A mixture that contains an empty INFLUENZA VIROSOME RECONSTITUTIONAL IMMUNOSTIMULATOR and a liposome with at least one antigen trapped inside of it or attached to its membrane by a lipophilic anchor, with the pH of the mixture kept at a healthy level.	By Pevion Biotech AG
02 March 2007	WO2007/099446	Virosomes-like vesicles comprising gp41-derived antigens	The gp41 P1-peptide's C-terminus is covalently attached to a lipid molecule in virosomes for localization on their exterior surfaces.	By Pevion Biotech AG, MymeticsIn C. INSERM
21 March 2007	WO2007/107585	Intranasal influenza vaccine based on virosomes	INFLUENZA VIROSOME RECONSTITUTIONAL IMMUNOSTIMULATORwith no additional adjuvant or lipid from an external source for stimulation of systemic or local immune response against influenza virus in humans by single intranasal or inhalation administration	By Solvay Pharmaceuticals
12 July 2007	WO2008/008881	Virosomes, methods of preparation and immunogenic compositions	Virosomes with at least one adjuvant molecule and on the virosome surface, there is a single surface glycoprotein produced from a distinct encapsulated virus.	By Emory University
31 December 2007	WO2008/080628	Nonspecific immunostimulant agents	Influenza virosomes for stimulation of a nonspecific immune response against neoplastic, viral, or bacterial disease or disorder	By Pevion Biotech AG
11 June 2008	WO2008/15052	Intradermal influenza vaccine	Virosomes comprising influenza virus HA but no additional adjuvant for intradermal application as influenza vaccine in humans	By Crucell Switzerland AG
13 June 2008	WO2009/000433	Virosomes comprising HA derived from an influenza virus produced in a cell line, compositions, methods of manufacturing	Virosomes containing HA derived from the influenza virus produced in an avian cell line, featuring increased fusion activity and immunogenicity	By Pevion Biotech AG

29 Septemb er 2011	WO2012/0 41503	Generation of virosome particles	Generation of INFLUENZA VIROSOME RECONSTITUTIONAL IMMUNOSTIMULATORwith recombinant HA produced in tobacco plants, a lipid bilayer comprising a bisacyloxy propyl cysteine conjugate, e.g. MALP-2	By Franvax SRL, Itlay.

5 Composition of different reconstituted virosomes

The restructuring of virosomes from deactivated influenza virus with the help of ultracentrifugation, solubilization, clarification, and surfactant removal results in the empty unloaded influenza virosome without the genetic material as well as nucleocapsid of the source virus[26]. Thisunilamellar vesicle is spherical in shape with an average diameter of ~150nm.The main characteristic of the virosomes is the retention of the most useful viral cover of glycoproteins,i.e., Influenza virus haemagglutinin (HA) and neuraminidase (NA) then they are inserted into the Phosphatide bilayer membrane. They are represented by short surface projections with a size of 15nm[27].

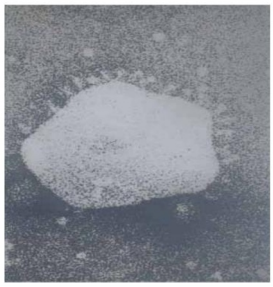

FIGURE 2. Virosome structure under electron microscope carrying two hepatitis A virion particles. The influenza glycoproteins (On the top of the image haemagglutinin (HA) and neuraminidase (NA) form thorn-likestructures that protrude from the virosome membrane[28].

Research has found that to attain the desired therapeutic effect, a physical attachment between the target antigen and the virosomal carrier is necessary. The Structure of the virosome allows various applications for antigen binding, it also depends on certain properties along with the type of immune response. Maybe the antigens are adsorbed on the surface layer of the virosomal structure, enclosed within the virosomal structure, or combined to the lipidic membrane of the virosome likewise directly through crosslinking of antigen and lipid moieties or through the hydrophobic domain[29].These are the virosomal structure of the influenza vaccine under the electron microscope that shows exact same structure of virosome with mean diameter of 150nm and a thorn-like structure of 10-15nm that replicates influenza hemagglutinin. In fact, the virosomes are Liposomes that carries two glycoproteins (HA and NA) on the surface, and it can be used for the vector for antigens or carrier. This structure also prevents internal contents from degradation[30].

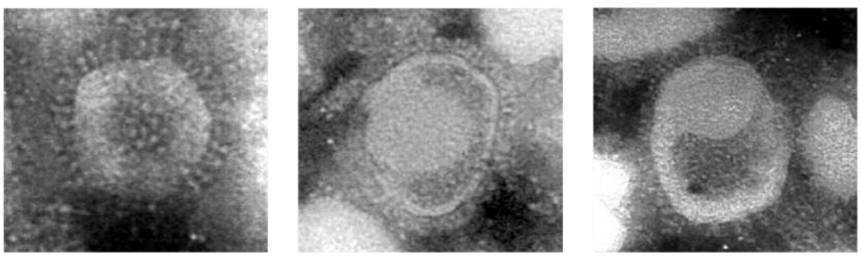

FIGURE 3. Architectural structure of the virosomal adjuvanted influenza vaccine on electronmicroscopy (Electron Microscopy Unit of the Department of Health Sciences University of Genoa)[31].

Hemagglutinin stabilizes the virosome and the recruiting memory cells against HA T cell epitopes. For intensifying tissue targeting, the liposome's exterior is modified with ligands or antibodies recognized by the specific cell types[32].

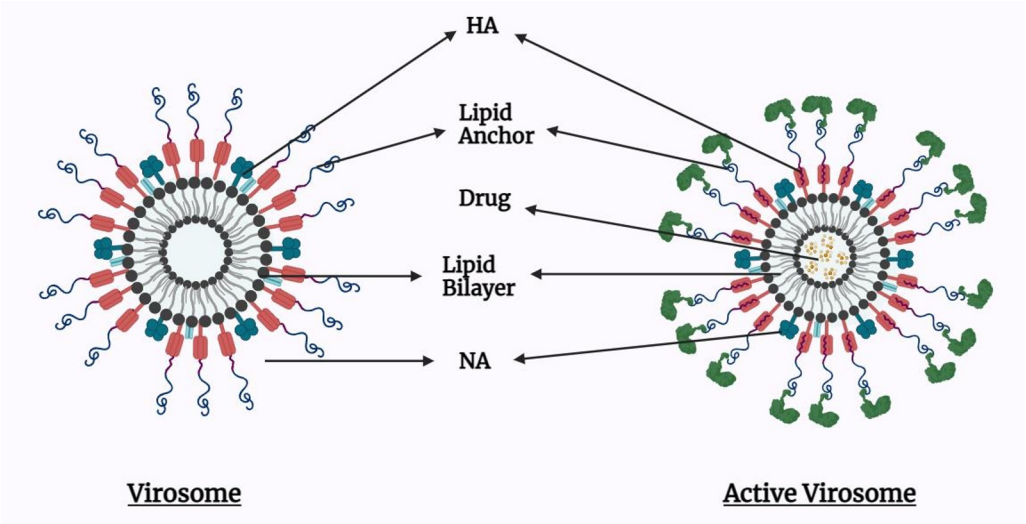

FIGURE 4. General Representation of Virosome[33].

After numerous efforts were made to develop and subsequently commercialise various vaccine adjuvants based on the virosome formulation, such as Inflexal® V for influenza, Epaxal® for hepatitis A, HIV, HPV, Cancer, and SARS-Cov-2 (some vaccine projects are ongoing), the virosome-based viral nano vaccines are now available. [34].

Virosomal vaccine against Influenza

The population is protected by conventional influenza vaccination platforms against some highly pathogenic strains, but reliable information shows that these products are insufficient against the impending epidemic. Therefore, the development of new vaccination technologies is crucial to ensuring community impunity [35].The trivalent influenza virosome vaccine known as Inflexal ® V has an expression with two inactivated strains of the virus and one strain of the B virus, both of which have the HA and NA subunits that are specific to the influenza virus[5].

The optimal quantity of cleansers has solubilized influenza contagions and removed their nucleocapsid in the manufacture of influenza virosomes, like other virosomes pharmaceutical methods. Accordingly, INFLUENZA VIROSOME RECONSTITUTIONAL IMMUNOSTIMULATORspontaneously created when viral lipids and glycoproteins are present. In reconstitutions of virosomes, phospholipids (PL), particularly phosphatidylcholines (PC), are present. To produce the NA and HA glycoproteins, envelope phospholipids from the influenza contagion were responsible for 30 of the contagious lipid content, which was assigned to PC[36].The schematic representation of an influenza virosome reconstituted is shown in the upper picture.Protection of people against somewhat highly infective strainsof influenza virus by the traditional vaccine, although appropriate report clarifies that these vaccines do not have enoughprotection against the prospective pandemic. Hence the development of novel vaccine technology has become necessary to provide public immunity.FDA-approved novel virosome-based influenza vaccines the virus-endosomal fusion and induced haverelieved the viral disease and death rate.

Crucell, Berna Biotech has created a trivalent influenza virus virosome vaccine that consists of two inactivated strains of viruses and one B virus strain containing influenza virus antigens for the HA and NA subunits.The preparation of other virosome is comparable to the preparation of influenza virosome. It includes that the influenza virus is solubilized in the optimal number of detergents, therefore its nucleocapsid was removed.In reconstitutions of virosomes, phospholipids (PL), particularly phosphatidylcholines (PC), are attained. The influenza virus's envelope phospholipids contributed 30% of the virus's 70% lipid content, which in turn produced 30% of the NA and HA glycoproteins. An illustration of an influenza virosome was provided.

NA function has a significant impact on viral pathogenicity and improves virosome functionality. N-acetylneuraminic acid (sialic acid) reduces the viscosity of the host's secretions, and also makes it simpler for developing viruses to spread their offspring. [37].

The virus-endosomal fusion required the HA1 and HA2 subunit bearing HA epitopes, which also caused cellular response. Additionally, only virosome-virosome fusion results in the inclusion of HA into the virosome membrane.From 1997, Inflexal® V has been produced by the Swiss Serum & Vaccine Institute in Berne, Switzerland. It is currently offered in more than 20 nations under a variety of trade names, such as Viroflu® in the United Kingdom and Isiflu® V in Italy. Up to this point, more than 2500 healthy volunteers have gotten involved in 18 scientific investigations that have proven the security and efficiency of Inflexal® V.More than 10 million doses of the vaccine

have been distributed., and this vaccine demonstrated excellent tolerability across all age ranges[38].Inflexal® V, whose additionally contained the least OVA level and had no thiomersal or formaldehyde in its formulations, abolished the limitations of the conventional influenza vaccine. The safety of vaccinations was much enhanced, and the price of preservation was reduced, when stabilized vaccine formulation was produced under various storage conditions. High HA content levels in Inflexal® V have been demonstrated to last for 24 months[39].

Virosomal vaccine against Hepatitis A

Hepatovirus A (HAV) is the cause of the potentially fatal condition known as hepatitis A. HAV was a protein-coated RNA particle with a 27–32 nm-diameter cubic or icosahedral capsid.The virus was unstable at low pH levels and sensitive to variations in temperature, although it was eliminated by heat above 85 °C. Universal immunization was carried out for high-risk populations against HAV vaccination. There were few vaccines available for HAV as Havrix®, Vaqta®, Avaxim®, and Epaxel® which are only aluminum-free products[40].

Epaxel® was developed by inactivated HAV to decorate the virosome, while the substrate utilized an adjuvant of aluminum hydroxide to attach HAV inactivated virus in other vaccines. There is a decrease in local inflammatory response due to aluminum-hydroxide. This vaccine was very potent and provides a more rapid response with a higher secretion rate on the 14th day, accompanied by a higher geometric mean titer (GMT). Epaxel® has high immunogenicity and very good tolerability, providing immunized individuals with protection for at least 9 to 11 years[41].When making Epaxel®, the influenza virus is first rendered inactive before being reassembled with lecithin and cephalin, phospholipids that form 150 nm bilayer structures, and viral envelope antigens like fusion proteins, HA, and NA. The final structure's formalin-inactivated HAV surface ornamentation offers natural adjuvant properties. When first exposed to inactivated HAV, B cells multiply and differentiate into cells that secrete antibodies. Epaxel® virosomes are more readily absorbed through HA and NA with the assistance of sialic acid-rich dendritic cells, enabling quick endosomal-mediated cell uptake. Ultimately, an immune response caused by Epaxel® combines humoral and cell-mediated immunity as a result of the activation of lymphocytes, MHC-I and MHC-II, including B cells, T helper cells, and CTLs. [42].

Virosomal vaccine against HIV

Human Immunodeficiency Virus type 1 (HIV-1) was a lentiviral retrovirus causing AIDS. AIDS was a life-threatening and rapidly contagious disease that causes around 35 million deaths in the world from 70 million infected people[43].The virosomal vaccine against HIV has proven to be incredibly effective. Notably, the HIV virosome vaccine administered mucosally in phase I clinical trials triggered a potent immune response. This virosomal vaccine has been injected intramuscularly, intradermally, or subcutaneously and is expected to be quickly available on the market. The robust immunological response induced by the mucosal dose form of the HIV virosome vaccine is particularly notable. The initial production of mucosal antibodies is critical in providing strong defence against HIV infections because of the predilection for the mucosal pathway [44].The sublingual dose form complies with the physical stability, antigen integrity, and robustness that HIV has evolved. [45]. The host cell infection via interaction of CD4 receptor on immune component cell-like helper T cells and macrophages was facilitated by gp41 antigen. Moreover, the P1 peptide improved the mobility and functionality of protein epitopes.The long-lasting immunity confronting the HIV-1 virus was developed by 3 m-052 adjuvants; also, incorporated via 18 carbon fatty-acid chain into a virosome-phospholipid bilayer. The rigidity of the virosome membrane was increased by 3 m-052 adjuvants[46].By interacting with CD4 receptors found on immune system cells like helper T cells and macrophages, the Gp41 antigen promotes host cell infection. The P1 peptide also helps protein epitopes move more freely and function better. Adjuvants, which are absorbed into the virosome's phospholipid bilayer via an 18-carbon fatty acid chain, aid in the development of long-lasting protection against HIV-1 viruses. A thermostable adjuvant called 3 m-052 strengthened the stiffness of the virosome membrane.

Lecithin, cephalin, as well as phospholipids which have been reconstituted with gp41 virosome type 1 and p1 virosome type 2, both of which types were engineered by the addition of 3 m-052 adjuvants, toll-like receptor (TLR) agonists, were used to create the thermostable HIV-1 virosomal vaccine in a separate investigation. TLR7/8 activation, similar to adjuvants, promotes the release of TNF, IFN, and IL12 cytokines, which in turn encourages dendritic cells to develop and produce co-stimulatory molecules like CD80 and CD86. Finally, TLR7/8 enhances T cell and B cell immunological responses. Immunisation with the stable HIV-1 candidate vaccine should produce the necessary protective antibodies even if the product has been stored. [47].

Virosomal vaccine against HPV

There are more than 170 different forms of the family virus HPV, of which 40 are sexually transmitted.Several investigations on the effectiveness of preventive HPV vaccines over time based on virosomes have been conducted[48].

The effects of checkpoints on the HPV cell cycle, notably on the HPV oncogenes E6 and E7, which support the virus' persistence within host cells, have been studied in relation to HPV. Additionally, E6 and E7 have complementing properties. Cellular reactions are brought on by their presence within antigen-presenting cells (APCs). The virosome-incorporated E6 and E7 proteins can fuse with the host cell membrane during the process of endocytosis, allowing the combined virosomes and endosomes to release their contents into the cell's cytoplasm at a pH of 5. Recombinant HPV16 E7 fusion with active influenza virosomes produces strong cytotoxic T lymphocyte (CTL) responses and inhibits the development of HPV16-transformed tumours, according to studies. Additionally, contact with E7-virosomes causes IgG to become immunogenic to the E7 viral oncogene [49].

Viral virosome vaccines against cancer

Virosome therapeutic medicines against metastatic melanoma have been generated more extensively in multifunctional carriers. In oncologic sectors, virosomes are the preferred delivery system for immunogenic chemicals and chemotropic agents. Oncolytic viruses are made up of recombinant and naturally occurring variants that multiply in tumor cells and strengthen anti-tumoreffects. Melanoma Virulence factors (TSA) and Melanoma Antigens, among others, might be delivered by virosome (TAA). It is recommended to utilize viral vector compression since it is very safe. The HVJ and Sendai viruses can serve as multimodal cancer prevention agents[50].The potential of virosomes in the treatment of cancer has been examined in numerous research. Reconstituted influenza virus envelopes (virosomes) have been thoroughly investigated in preclinical studies and clinical trials, including with OVCAR-3 cells, in the context of treating malignancies such ovarian carcinoma. PEG-derivatized lipids were incorporated into the virosome membrane in order to control the viral HA membrane fusion activity. Then, mAb 323/A3 (anti-epithelial glycoprotein-2) Fab P fragments were joined. The HA fusion activity was decreased as a result of this tactical adjustment. The distal end of the virosomes contained the PEG-derivatized lipids. Notably, influenza virosomes showed favourable traits for transport to the cytosol. In a work by Waelti et al., it was shown that the HER-2/neu (p185her2) oncogene could be combined with virosomes that were extensively coated in HA spikes to provide a unique and selective drug molecule delivery system. This novel strategy showed promise for effective tumour growth inhibition [51].

Recent progress on virosome-based vaccines for SARS-CoV-2

The COVID-19 pandemic that resulted in the SARS-CoV-2 virus started in Wuhan, China, and spread in December 2019, despite numerous studies warning of the first illness symptom in February 2019. Since the most recent report on December 5th, 2020, there have been over 62 million incidents documented in 210 countries, and 1,480,000 of those cases have resulted in death. The glycoprotein coat of the encapsulated, spherical SARS-CoV-2 particle has 20 nm-long club-shaped spikes and a diameter of 60 to 150 nm. It belongs to the genus Beta coronavirus and the family Corona viridae. The SARS-CoV-2 positive-strand RNA genome is vast, with about 3 k nucleotides. [52].

The intrinsic restrictions in the sequencing ability of viral RNA polymerases are the cause of the mutational rate seen in RNA viruses. Because of this process, RNA viruses are more likely to develop treatment resistance and evade immune detection. Notably, a variant strain of SARS-CoV-2 has been discovered, and current research suggests that the virus exists in over 40 different variations. Geographic localisation of clinical symptoms has also been seen. These issues still exist, making the creation of a potent COVID-19 vaccine difficult and complex. [53].The coronavirus genome encodes structural proteins that are essential for both the development of contagious viral particles and the severity of the illness[54].

As an additional virulence component, the SARS-CoV's E protein has the potential to identify specific targets for therapeutic approaches, according to recent investigations. Hem agglutinin esterase protein may also be found in several coronaviruses (HE). The lectin domain of the HE protein facilitates virus-host cell adhesion. To produce virosome vaccines, particular surface antigens and phospholipids of SARS-CoV2 were isolated. Virosome particles are a potential delivery system for the SARSCoV2 vaccination. The European MI Matrix Company is working on the Transvac 2 project, a virosomal-based vaccination. By attaching to ACE2 and activating the endocytosis pathway, coronaviruses spread the infection to host cells[55]. Furthermore, the basic residue of amino acids that makes up the majority of SARS-CoV acts as an electrostatic attractor for the hepar sulphate proteoglycans on the surface of target cells, enhancing viral membrane fusion. For SARS-CoV to be infectious in the cytoplasm, a low pH-dependent route and endosomal entry into the target cell are required. A designated compartment in the form of a flask that is surrounded by a double membrane is employed to synthesise the viral RNA in the cytoplasm, where coronaviruses grow. Granulations in the cytoplasm, nucleocapsid inclusions, and the emergence of double-membrane vesicles are among these changes.[56].

6 GENERAL MECHANISM OF VIROSOMES

Virosomes retain an empty core while having their surface modified by the insertion of necessary fusion proteins. For drug delivery applications, this structure acts as a carrier system that can distribute both antigens and

medications in a targeted manner [57]. The basic mechanism underlying the operation of virosomes is their continued capacity to merge. This fusion capability makes it easier to transport crosslinked or encapsulated antigens into antigen-presenting cells via receptor-mediated endocytosis. Virosomes are also efficient in the antigen-presenting pathways of MHC class I (CD8+) and class II (CD4+), allowing for thorough immune responses[58].However, the reason behind antigen delivery vesicle cum adjuvant excellency is that it can start or initiate cytotoxic cells as well as helper T cells against as compared to vaccines [59].Hemagglutinin (HA)-mediated attachment of virosomes to cell membrane receptors made of glycoproteins or glycolipids with terminal sialic acid starts the release process. Virosomes then enter cells by receptor-mediated endocytosis.

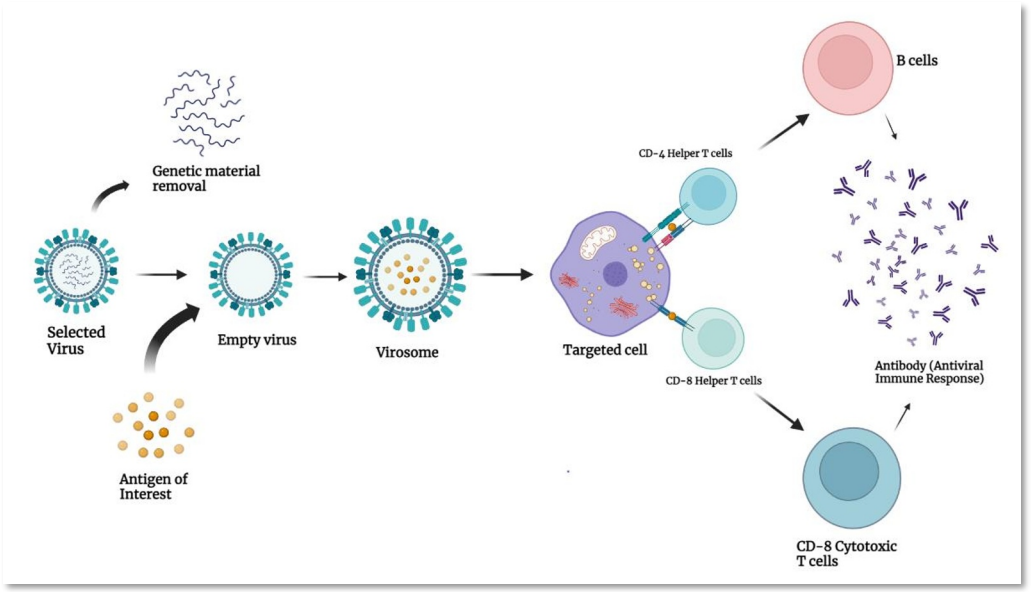

FIGURE 5. Preparation and mode of action of virosome

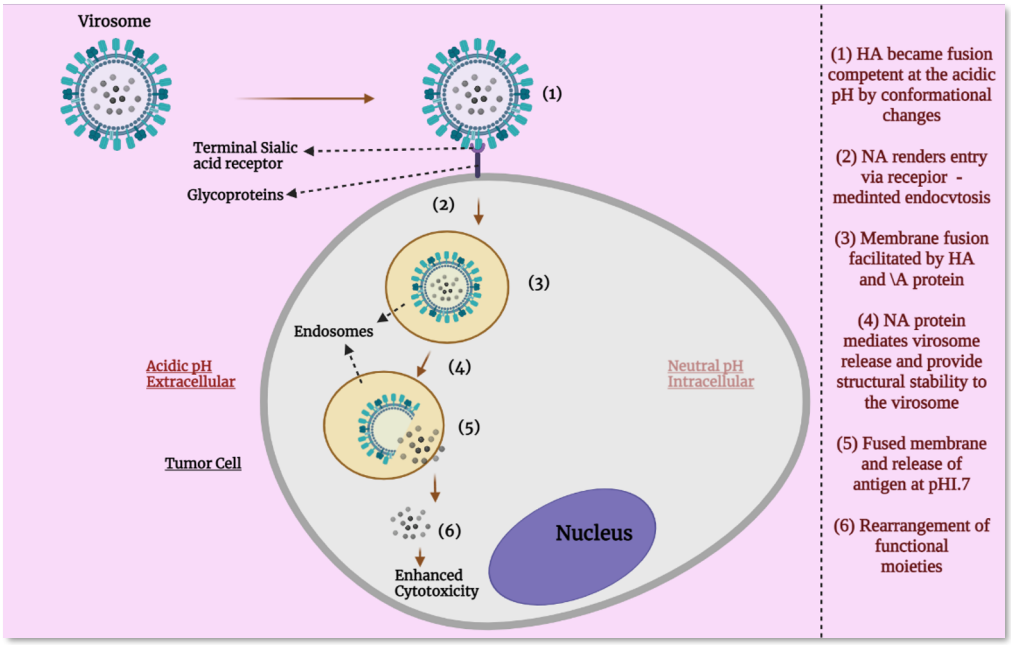

FIGURE 6. General Mechanism of action of virosome

Virosomes get stuck inside endosomes, where the internal environment causes the virosomes and the endosomal membrane to fuse together. Hemagglutinin, a viral membrane glycoprotein, promotesfusion activity (HA). The endosome's subsequent membrane-fusion action frees the virosome from its lipid sheath and provides access for the medications that are encapsulated to reach the cells' cytoplasm.

7 Method of preparation

Preparation at the laboratory level

Virosome particles may be created from viruses in vitro using a procedure called membrane solubilization and reconstitution, which involves four basic steps. First, a sufficient viral count is attained by cultivating the target virus. The virus is then purified, rendered inactive, and dispersed in detergent. Third, the nucleic acids and other viral proteins are isolated from the envelope fraction, which is composed of the membrane-associated virion proteins and lipids. Fourth, removing detergents from envelope fractions causes membrane lipids and related proteins to reassemble into vesicles[60].

Since the viral lipophilic envelop layer and viral outer membrane proteins of virus's majority of the virosomal framework, it is ideal to create a similar virosome by replacing the virus envelopes and proteins with synthetic materials. Liposomes have been utilized extensively in the past to transport a variety of medicinal compounds into cells. In the literature, methods for making liposomes with synthesized lipids have been described [29].

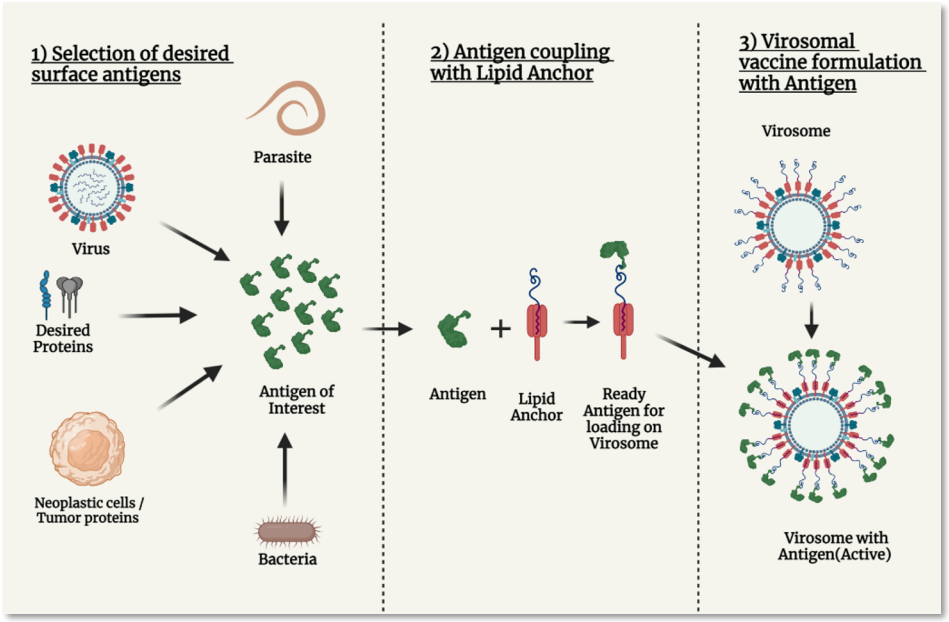

FIGURE 7. Preparation of Virosome by selecting antigen, Coupling with anchor and assembly

To prepare influenza virosomes, the influenza virus must be transformed into pellets using the ultracentrifugation process. Additionally, the particles are isolated and left overnight in 100mM C12E8. This will make it possible to completely dissolve the viral membrane. After homogenizing and ultracentrifuging prepared solutions, viral nucleocapsids are made from pellets. With the aid of detergent, BioBeads are employed for separation. Virosome suspension is purified using a discontinuous sucrose gradient to get rid of unencapsulated material. The surface of the sucrose layer is where the virosomes may be found. Additionally, the layer is eliminated using dialysis over the buffer, and the ready virosomes are filtered to sterilize them.

Production of the Virosomal Influenza vaccineat Industrial level

Schematic representation of manufacturing of influenza virosomes in a commercial setting. Consists of four phases,

The first of which is the separation of the components of the influenza envelope with solubilization the inactivated influenza virus (step 1), adding the necessary excipients such as carbohydrates and lipids made in a lab (step 2).The virosome components are put together by controlled elimination of the detergent to produce the intermediate product (step 3). The atypical product incorporates the antigen of interest (API) in two different ways. The followingantigen is attached to the virosomal membrane by lipid in the first-generation B-cell vaccines. before the assembly of the particles, the anchor is added (A). The heterologous HAV antigen is adsorbed to the surface of the intermediate product in the first-generation HAV vaccine Epaxal® (B). The intermediate product is then sterile filtered and diluted to the final antigen dosage (step 4). The finished product might either be liquid or stable freeze-dried (only with second-generation influenza virosomes). On the intermediate and finished products, chemical, biological, and biophysical analysis (QC) is carried out[61].

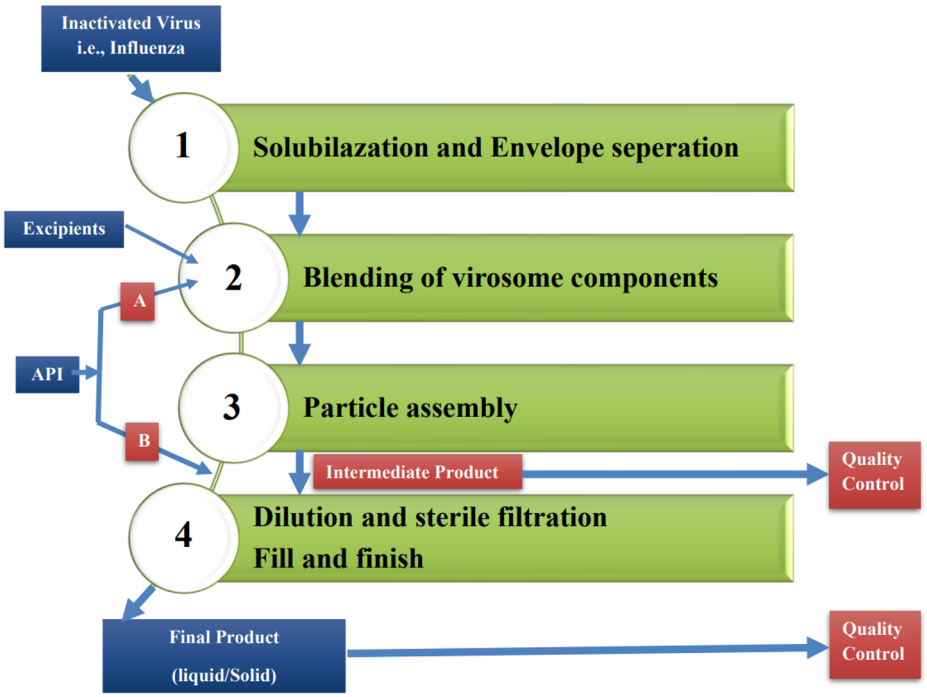

FIGURE 8. Production for the influenza virus on commercial level

8 Characterization of virosomes

Three features are often assessed to characterize virosomes:

a. Protein Detection

b. Size and Organization

c. The Fusion Process

No matter how the virosome was created or what type it was, the result should have a constant protein-to-lipid ratio. Utilizing electrophoretic methods, the HA protein in the virosomal complex can be evaluated quantitatively (SDS-PAGE being the most used). Negative stain electron microscopy can be used to see the ultrastructure and size of virosome particles.The staining solutions should be neutral in pH to prevent acid from changing the conformational configurations of HA. Virosomes also display pH-dependent film combination mobility, which is like the natural influenza virus's local flu infection features. The excimer assay (made by combining virosome with organic or fake target film) is used to visualize virosomal fusion with biological and artificial target membranes in vitro. Lipids are used in this experiment. Here, the drop in PPC label surface density following membrane fusion corresponds to a decrease in excimer fluorescence. Analysinghaemolytic activity, which has a pH dependence similar to that of the fusion process, is another way to assess fusion activity[62].
The numerous methods used to characterize virosomes are listed in below:

Bradford's assay

By incorporating Coomassie dye into the sample when it is acidic, the Bradford Protein Assay calculates the protein content. The sample turns blue when proteins bind to the Coomassie dye, turning it from brown to blue. A spectrophotometer may then be used to quantify the amount of blue to calculate the amount of protein in the sample [63].

Dynamic light scattering (DLS)

It is a monitoring method for the quick variations in laser light intensity caused by dispersed molecules or particles in solution, one may calculate size and size distribution. For many biologics, including peptide, proteins, viruses, and VLPs, DLS provides a fast and also not involving damage or destruction technique to determine size [60].

Electron scanning microscopy (SEM)

Using a beam of electrons travelling at energy level to concentrate and examine samples, the scanning electron microscope (SEM) is a type of electron microscope that scans the surface areas of microbes[64].

Transmission electron microscopy (TEM)

Visualizing virus particles, their sizes, morphologies, and locations are possible with transmission electron microscopy (TEM). The detection of virus-like particles (VLPs) in bulk harvest is feasible using TEM[65].

9 Regulations for virosome-based nano vaccines

The main objective of the Production Procedure for Cold-chain Independent Virosome-based Vaccines (MACIVIVA) project is to develop virosome-based vaccines that are sustainable for an extended period of time at room temperature as well. One of the feature of virosome-based vaccines is Quality by Design (QbD), which requires being established based on regulatory organisations like EMA, the US -FDA, and ICH, and a different legitimate regulatory organisation gradually. A high-quality vaccine composition that comprises active components, excipients, and other constituents is referred to as QbD when discussing pharmaceutical manufacturing. QbD is essentially determined by the concept of CQAs and the quality goal product profile. [66].

For instance, virosome size and concentration must be assessed using common laboratory tools. Additionally, virosome-based vaccine formulation needs to be high-yield, cost-efficient, and repeatable. The interplay between the CQAs parameter and the needs of the patients must then be assessed. The QTPP was impacted by the vaccination's efficacy, indication, and manner of administration. Additionally, the international regulations of WHO recommendations should adopt clinical evaluation of vaccinations[67].

The exact manufacturing processes for the nano vaccine must be assessed. For instance, the fabrication of nasal spray vaccine dosage forms does not require sterilization, and the management of microbiological material is adequate. Vaccine components should be used to precisely adjust vaccine storage conditions. For instance, freezing renders aluminum salt in vaccinations useless. Packages for vaccines should have IATA time, shipping regulations, and temperature labelling. The final classification and packaging of nano vaccine manufacture should be done by (WHO) guidelines for the worldwide shipping and packaging of vaccines[68].

10 Commercial virosome-based vaccines

Four virosome-based vaccines have received human use authorization and are now on the market.Epaxal® (Crucell), which received market use approval in 1994, was the first vaccine based on influenza virosomes. The virosomes used in this vaccine were produced from the influenza strain A/Singapore/6/86 (H1N1), and following virosome assembly, they were covered with a weakened or killed form of the hepatitis A virus.The second virosomal vaccine introduced to the market in 1997 was Inflexal® V (Crucell). This vaccine is a concoction of influenza A(H1N1), A(H3N2), and B/Yamanashi virosomes. All age groups, including the elderly, adults, children, and even patients with weakened immune systems, have demonstrated an acceptable immunological response to the vaccination[69].The third virosomal vaccine, nasal flu (Berna Biotech), was introduced in 2000. This vaccine was made up of virosomes from three influenza strains: B/Beijing/184/93-like, A/Beijing/262/95-like, H1N1, and Sydney/5/97-like, H3N2. In fact, it also included a mucosal adjuvant that was a heat-labile toxin from enterotoxigenic E-coli. This vaccine was only on the market for a single year until it was discontinued in 2001 due to certain unfavourable side effects[70].

The fourth virosomal vaccine, Invivac®, was introduced by Solway in 2004. This vaccination included virosomes from the A(H1N1), A(H3N2), and B influenza viruses. However, only the 2004–2005 season saw the commercial release of this vaccination. There are further disease-prevention vaccines using virosomal technology, including those for AIDS, hepatitis C, malaria, and candida[71].

Table3. Some Virosomal vaccines with composition, Status for marketing, and Dosage form

Target vaccine	Composition	Status	Dosage form	Reference
Respiratory syncytial virus (RSV) virosome	HA, NA, RSV fusion proteins	Under GMP8	IM	[72]
HIV-1 Virosome	HA, NA, P1,gp41,TLR/81	New GMP	Mucosal	[73]
Hepatitis A virosome	HA,NA, HAV2	Marketed	IV	[41]
Influenza virosome	HA, NA	Marketed	Iv	[74]

Plasmodium falciparum virosome	AMA- 13, CSP4, pfCyRPA5	Phase 1and 2	IV	[30]
Melanoma virosome	Recombinant vaccinia virus encoding five melanoma epitopes, octo-mel- reVV6	Phase 1 and 2	Intradermal	[12]
SARS- CoV2 virosome	HA, NA, Protein 8	Phase 1 and 2	IV	[75]

For instance, Epaxal and Inflexal V are the respective virosomal vaccines for hepatitis A and influenza. Invivac and NasalFlu are two other flu vaccines that make use of virosomes[76].

10 1Epaxal®

Virosomes are essential adjuvants in the creation of the Epaxal hepatitis A vaccine. In 1996, this vaccine's licencing was approved. Epaxal is made up of isolated and formalin-inactivated RG-SB strain hepatitis A viruses that were grown in MRC-5 human diploid cell culture. These viruses have attached themselves to influenza virosome surfaces. Lecithin and cephalin are two of the lipids that make up the membranes of Epaxal. Patrick A. Bovier's thorough analysis of the study on Epaxal provides a complex and in-depth list of findings. [6].

Table4. Few virosomal vaccines with a type of antigen

Sr.No.	Name of the vaccines	Type of Antigens	Reference
1.	Invivac®	Influenza virus surface antigens (haemagglutinin and neuraminidase	[77]
2.	Inflexal® V	Subunit virosomal influenza vaccine	[2]
3.	NasalFlu®	Flu virus antigen	[78]
4.	Recombivax engerix-B	Recombinant hepatitis B virus (HBV)	[79]
5.	Gardasil®	Self-assembled particles of human papillomavirus (HPV)	[80]
6.	Epaxal™	Hepatitis A virus vaccine	[81]

10 2 Inflexal®V

Berna Inflexal® V consists of a combination of three monovalent virosome pools, every created with the unique HA and NA glycoproteins of a different influenza strain. The influenza strains are chosen in accordance with the WHO and European Medicines Agency's yearly guidelines.[82] All age ranges can get the influenza vaccination Inflexal® V without a problem.[83,84] InfectoVac® Flu, Isiflu® V, and Viroflu® are additional trade names for Inflexal®V that are used in Germany, Italy, as well as the United Kingdom, respectively[85-87].

11 Conclusion

•Because of their adaptability, influenza virosomes can be a fantastic instrument for transporting antigens and biomolecules of many kinds, such as proteins, peptides, plasmids, oligonucleotides, and even medications, to cells.

• The delivery system is a crucial component in the protection of illnesses. It has been demonstrated that APCs, particularly DCs, can process influenza virosome-delivered antigens. DCs guarantee that the antigen is presented via MHC class I or II, which triggers a humoral and cell-mediated immune system response.

• Depending on the goal of the immunization, virosomes can be used to deliver an antigen in the host by various routes, including intranasal, transdermal, or Intramuscular injections, without causing any adverse effects, as has already been demonstrated in clinical testing with available commercially virosomal-based vaccines.

• Virosomes have an adjuvant effect that can be used to boost the immune response in people without risk.

• The ability to direct virosomes to specific cells by employing Fab' segments of monoclonal antibodies designed for binding is a desirable property of virosomes. Virosomes that have been antibody-redirected maintain their capacity for fusogenesis while attaching to particular cell types only. Virosomes are more effective than liposomes, which are frequently used to transport therapeutic chemicals to target cells. This is because when therapeutic drugs are internalised by cells in liposome-encapsulated forms, they are unable to fully escape from endosomal/lysosomal confinement. The utilization of virosomes, a viable research tool in immunotherapy as well as vaccination, for both active and preventive treatment, can solve this issue.

• Targeted virosomes might be used following surgical removal of tumor, in conjunction with anticancer therapy, or in situations where persistent viral infections necessitate an active cytotoxic immune system response.

• There has also been a greater interest in the formulation procedures, such as emulsification, DNA complexation, and entrapment, for vaccine delivery systems. Studies are being conducted to better understand the immunological processes of virosome activity.

• Virosomes might be used as delivery systems for immunomodulating particles and targeted medicines, both of which are finding new uses every day, notably in the treatment of cancer. Due to all of these characteristics, influenza virosomes are thought to be a promising prototype for the delivery of antigens and/or unrelated molecules, which may be useful for the creation of novel immune therapeutics or vaccines that incorporate immunogenicity with safety as well as for their use in a wide range of medical research areas.

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