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Virosome: A Virus Created Specifically To Deliver A Vaccination

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Abstract

Liposomes are particularly interesting as a novel medication delivery technology because of their potential as gene carriers and capacity to reduce drug toxicity. Optimized lipid components have been developed to prevent the uptake of reticuloendothelial system (RES). The liposome surface has been altered with antibodies or ligands that are recognized by particular cell types in order to increase tissues localization. Liposomes and fusiogenic viral envelope protein have been combined to form new virosomes, which introduce molecules directly into cells, hence improving the efficiency of gene delivery. Efforts had been made to use virosomes as adjuvants or antibodies, and also for means of drug delivery and organics, for therapeutic applications because they are biocompatible, nontoxic, non-auto-immunogenetic and, biodegradable. In contrast with conventional methods of vaccine development, a vaccine based on virosomes represents a new era in the field of immunization since it strikes a balance between acceptability and efficacy because of its immune-stimulating mechanism. The ability of virosomes to function as a therapeutic target and vaccine adjuvant, as well as their capacity to transport a different kind of substances, such as proteins, peptides, and nucleic and their ability to target specific drugs. The main topics of this article are the basics of virosomes, their formulation, composition, and advantages, development, current clinical status, interactions with the immune system, recent developments, and virosome-related research, as well as the safety, effectiveness, and tolerability of vaccines based on virosomes and their prospects for the future.

Keywords: Virosome, Nanovaccine, liposomal drug delivery system, Vaccination, Gene Delivery, Virosome based vaccine.

Introduction:

Current cancer and neurological disease treatments require delivery systems that direct medications to particular cell types and tissues that host them via receptor-mediated uptake along with regulated release. Simple inactive nanocarriers that can provide powerful protection with a single dosage may be useful as a unique method for a variety of applications.(1) Both natural and artificial sources can produce these nanomaterials. Vaccines against pathological microorganisms have also been developed using nanocarriers.(2,

3) For the production and synthesis of nanovaccines of both synthetic and natural origins, several different forms of nanomaterial have been used. Promising nanocarriers for the delivery of vaccines include bacterial spores, liposomes, nanobacteria, virus-like particles (VLP), proteosomes, bacteriophages nanoparticle-based nanobeads, exosomes, etc.(3, 4)

A new smart carrier system based on virosome technology is available that overcomes the drawbacks of traditional vaccination delivery methods. Viral envelope proteins that merge with liposomes were fundamentally used to create virosomes from liposomes.(5) Virosomes are recreated viral envelopes that contain viral spike glycoproteins and membrane lipid but lacking the genes of the viral agent. Like viruses, virosomes cannot replicate; instead, they are essentially just fusogenic shells.(6) Virosomal glycoproteins can be conserved due to receptor-binding properties and the membrane-fusion therefore virosomes are intended to be used as an active targeted transport carrier for the cellular administration of drugs .(7) The use of virosome-based delivery vehicles carrying different types of antigens, such as oligonucleotides, proteins, peptides, virions, plasmids, etc., can also be accomplished in the management and mitigation of infections and active precise immunotherapy for cancers, which frequently require the stimulation of an effective immunological responses.(8) The ability of numerous therapeutic substances to be attached in the hydrophilic interior membrane or on the of virosomes, like liposomes, during the reconstitution of virosomes is a remarkable feature of virosomal carriers. Virosomal envelopes have also been covalently attached with a number of antigens that contain small proteins. This is accomplished by altering phosphatidylethanolamine (PE), a phospholipid, with a conjugator. This allows proteins to be conjugated covalently, mostly through a disulfide link. Hydrophilic drugs can be entrapped in the core of the shell which is hydrophilic and subsequently exploited by chimeric virosomes to transport the content inside the cell. With receptor-mediated engulfment and the fusogenic qualities of virosomes, this further improves the therapeutic agent's high entrapment efficiency (8, 9).

Comparing this unique manufacturing approach to the traditional ones, the quantity of encapsulated water-soluble peptides increases by around 30 times. They can also be used as an effective vehicle for RNA and DNA delivery due to these fusogenic shells act similar as a virus. Additionally, virosomes display adjuvant properties, which are thought to be a crucial quality for improving immune response stimulation.(9-11)

Structure of Virosomes and its composition:

In terms of lipid content, virosomes are as flexible as liposomes, and they also carries membrane proteins that are either made via recombinant technology or derived from the virus itself .(12) Virosomes are reconstituted, spherical, unilamellar, genetically-free viral vesicles having a mean diameter approximately 150 nm that are made up of membrane lipids on the surface and viral spike proteins . The virosome's exterior resembles a complete virus with peplomer proteins that are projecting from the membrane.(13-15) The major phospholipid found in virosomes, phosphatidylcholine in particular, is a naturally occurring phospholipid. Only phosphatidylcholine is in charge of around 70% of the virosomes' structure. Haemagglutinin (HA) and neuraminidase (NA) glycoproteins are synthesized by virus-made envelope phospholipids, which make up the remaining 30% of the membrane's contents.(9-12, 14-16)

Fundamentally, virosomes are hollow virus envelopes that have been rebuilt but lack genetic material, making them unable to reproduce like the original harmful virus.(6) A HA glycoprotein that is immunologically active is found embedded in the membrane of virosomes, which contributes to their exceptional properties. The immune-stimulatory features of virosome, which is notably distinct from other liposomal delivery and proteo-liposomal methods, are significantly amplified by haemagglutinin glycoprotein furthermore ensures the structural stability and the homogeneity of virosomes. In its most basic form, HA is made up of two protein regions that form during translational cleavage of HA into two subunits, namely HA2 and HA1, both are connected with a disulfide link.(17, 18) The globular head of the HA1 subunit has a receptor binding region that makes it easier for virosomes to attach to different sialic acid residues on the surface of APCs (antigen presenting cells). (19, 20)

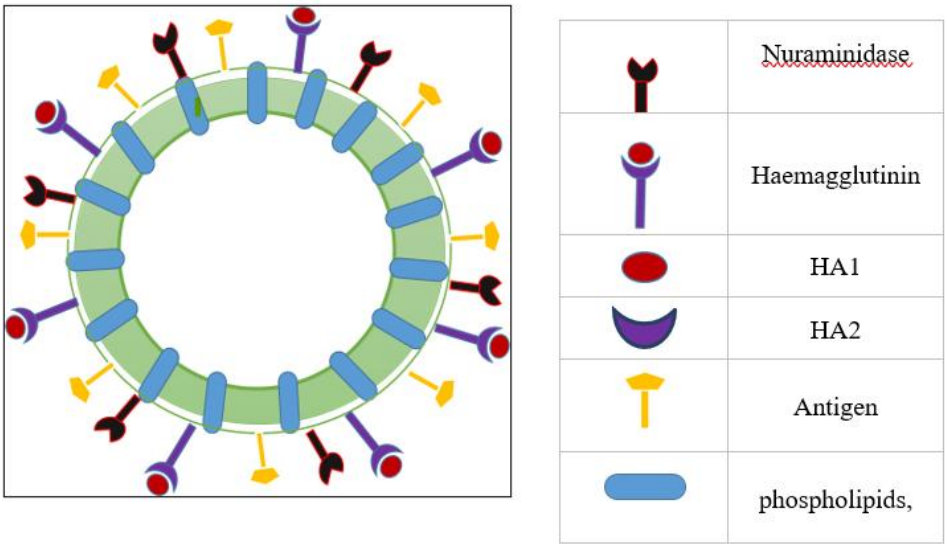

Figure no 1 : Structure of Virosomes and its composition:

The HA2 subunit, on the other hand, is encased in the virosomal membrane and it has N-terminal fusion protein. The HA1 subunit confines the HA2 subunit at a pH of approximately 7, where a number of hydrogen bonds controls the fusion peptides.(21) The HA go through a conformational shift that expose the hydrophobic portions of HA2 and causes the virosomes to fuse to the target cell membrane when the pH changes from neutral to acidic. This type of fusion occurring between the viral & endosomal membranes occurs during influenza virus infection, which ultimately results in release of genetic material into the cytoplasm of the target cells. Without presence of a target cell, HA typically deactivates in approximately pH 5 i.e. acidic environment at a temperature of 37 °C, losing its fusogenicity.(22) The NA is another glycoprotein that may be seen on the virosomal outer surface. It is a tetramer enzyme made up of four different subunits that are hydrophobically attached to the membrane by a stem. The subunit head region contains the enzymatic loci. Furthermore, sialic acid (NAM) is separated from bound sugar residues by the action of NA.(23)

Molecular communication and the virosome's mechanistic pathway:

The alleged internalization mechanism, virosome intracellular penetration, and Ag encapsulated with APC (antigen presenting cells). Similar to an alive virus, virosomes bind to sialic acid-containing receptors on APCs, including DCs (Dendritic cells).(24) Virosomes then enter the cell with the help of receptor-facilitated endocytosis. After encapsulated Ag is released into the cytosol, the endosome's acidic pH induces the union of the virosome and endosomal membrane. In order to trigger cytotoxic T lymphocytes (CTL) responses, antigen that is trapped inside of virosomes could enter the traditional major histocompatibility complex (MHC) class I conduit. The endosomal membrane is not expected to merge with all virosomal carriers, though; some virosomes must pass via the endosomal pathway. The breakdown of the peptides or proteins occurs as a result of Ag-encapsulated virosomes' continued presentation in the endosomal or lysosomal conduit. In presence of MHC class II molecules, the synthesized peptides become apparent. Similar to the complete parental virus, virosomal carriers delivers the encapsulated Ag for presentation in MHC class II and MHC class I, significantly stimulating the immune system. (25, 26) When diphtheria toxin (DTA), a membrane impermeable macromolecular endotoxin, was coupled with virosomes, the subunit A could be incorporated into the cell. DTA can't enter cells, therefore it is non-toxic ; nevertheless, when it is liberated into the cytosol with the help of virosomes, DTA is hazardous to cells because it stimulates an enzyme called elongation factor 2 that is involved in protein synthesis in the cell.(27) The confocal laser microscopic method was also used to establish the Sendai virus's virosomal cellular transport of bovine serum albumin (BSA). Liposomes and virosomes were used to contain fluorescently dyed BSA. The EL4 thymoma cells were then pulsed with these liposomes and virosomes containing fluorescently tagged BSA. Only cells treated with virosomes that included tagged BSA displayed cytosolic staining, demonstrating virosomes' enhanced ability to spit up the encapsulated contents of cells.(28) It has also been shown that ovalbumin (OVA), the reference protein Ag, can be delivered via the virosomal delivery approach very effectively for MHC class I presentation by DC derived from murine bone marrow. Furthermore, virosomes markedly upregulated the expression of co-stimulatory molecules on DC, including intercellular adhesion molecule (ICAM-1), CD40, CD80 (B7-1), MHC class II and class I, and CD86

(B7-2).(13) It has also been noted that influenza virosomes are unable to cause co-stimulatory molecules produced on plasmacytoid DC surfaces to become more active.(29) Basically, type I interferons are secreted by plasmacytoid DCs at higher concentrations than other types of DCs. Additionally, plasmacytoid DCs has a significant role in immunogenic activity against viral infections and this has been linked to the initiation and emergence of a number of inflammatory and autoimmune illnesses.(30) It is still unclear exactly how virosomes cause an increase in immunostimulatory signals. Thus, the virosomes created from the natural viruses may be able to increase the expression of co-stimulatory molecules, which are required to activate T cells. The releasing of Th1 (helper T cells Type 1) polarizing cytokines, such as interferon (IFN) or IL-12, and the synthesis of costimulatory molecules on DC are important processes associated with the activation of T cell-mediated immunity.(31, 32) Researchers have looked into the possibility of increasing the immunogenicity of an influenza virosome carrying a DNA plasmid that encode carcinoembryonic Ag (CEA) by co-encapsulating the plasmid expressing CD40L, a protein which is mainly expresses activated T cells and belongs to the TNF superfamily of molecules.(33) It is proposed that transfected DC that express CD40L will interact with DC that already express CD40, ultimately improving the ability of certain T-cells to prime. Lipopolysaccharide (LPS), a TLR4 and TLR2 ligand, was bioconjugated into the virosomes' lipid bilayer to activate murine B cells, and this process was ten times more effective than free lipopolysaccharides .(34) Although, using three immune-stimulating strains of influenza A virus, H2N2, H3N2, and H1N1, the antibody-dependent increase in the neutralizing of influenza A virus by the cells containing Fc receptor was evaluated. H1N1, H2N2, or H3N2 influenza virus strains were first infected mice. The virus from these mice was neutralized by sera obtained from them, and the virus was able to spread throughout the strain. The neutralization of the heterologous H2N2 strain and the homologous H1N1 strain was enhanced by sera from H1N1-infected animals. Remarkably, serum from mice inoculated against the H2N2 strain enhanced the ability to neutralize any strain of the virus, whether it be H1N1, H2N2, or H3N2. Additionally, sera derived from infected mice of H3N2 improved the neutralization of both homologous and heterologous H3N2 and H2N2 viruses. These findings revealed that antibodies that were strain cross-reactive improved the ability of the influenza A virus to be neutralized. NA antibodies may result in better neutralization of many strains of viruses. Monoclonal N2-specific antibodies improved the neutralization of the H2N2 and H3N2 influenza virus strains.(35) This suggests that antibodies that attach to influenza viruses enhance APCs' ability to neutralize the virus via the Fc-receptor. Additionally, Zurbriggen and Gluck have shown that pre-immunization with the influenza virus improved the start of immunogenicity against peptide antigens associated to the influenza virus in mice and rabbits.(36)

Benefits of delivering drugs via Virosomes:

Virosomes has numerous characteristics of nanoparticle, vaccination and medication delivery systems. The advantages of polymeric nanoparticle systems and liposomal delivery systems are combined in virosomes, which also overcome the time-dependent and in vivo instability issues related to both traditional and polymeric nanoparticle vaccine delivery methods. The structure, content, and formulation characteristics of virosomes, however, are more similar to those of liposomes.(8)

In comparison to conventional vaccine delivery methods, this virosomal system has various unique qualities and benefits. (12, 16, 29) (37, 38) (39)

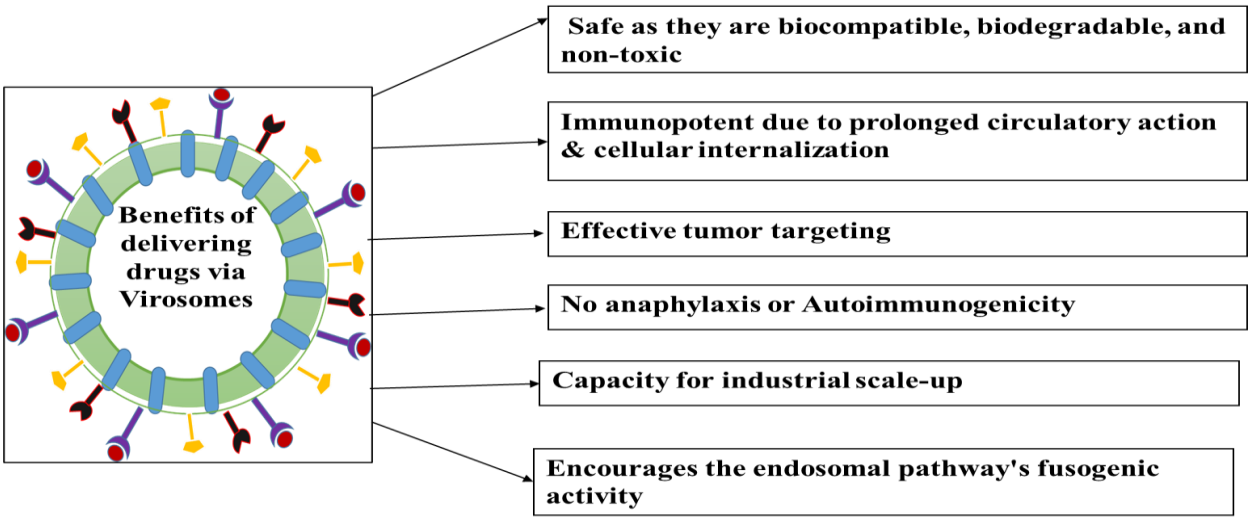

Figure no 2 : Benefits of virosomal delivery

Traditional technique for making Virosomes:

In general, biological products, and vaccines in particular, are extremely mosaic, powerful, and environment-susceptible goods. The crucial challenge is to build an efficient, reliable, cost-effective, and specific manufacturing method that can create a specially defined multicomponent nanoparticle structure for therapeutic usage of virosome. Both virosomes & liposomes share a fundamentally similar structure and makeup. The core of both virosomes and liposomes, which have a sac-like shape made of a phospholipid bilayer, can be used to deliver a nucleic acid, peptide, drug molecule, protein, etc. to the target tissues. The quick clearance of the liposomal carrier by the reticuloendothelial system (RES) is a major problem.(40)

Many changes have been suggested by the community of scientists to address these practical issues, but one crucial one is application of a PEG film to the liposomal carriers' surfaces, which can help them function as stealth carriers and avoid being cleared by RES, extending the time of circulation.(41) Since it is clear that each part of the virosomal drug delivery system is produced individually before being put together to form virosomes (12) High-quality recombinant peptides, proteins, and other ingredients are purchased for this use from vendors that have earned GMP certification. Because the neutralized virus used to make the virosomal carrier is the same substance used to commercially prepare the influenza vaccine. It can be bought commercially from companies that make influenza virus vaccines. Even today, the majority of virosomal carriers-based vaccines used on humans were created using influenza virus grown in chicken embryonated eggs. (42) However, it has been determined that viruses created in cell culture are just as useful for creating virosomal carriers.(43) Typically, solubilizing the viral envelopes with non-denaturing detergents such Triton X-100 and Octaethylene glycol mono (n-dodecyl) ether (C12E8) before removing viral nucleocapsid complexes is how virus envelopes are reconstituted.(24, 44) After the virus envelope has been dissolved, the virus's genetic material is extracted using the ultracentrifugation method. After the self-assembling phenomena of a shattered viral membrane made of phospholipids and other transmembrane glycoproteins, the virus membrane is rebuilt. As a result, discontinuous sucrose density gradient ultracentrifugation is used to separate these virosomes from unentrapped Ag.(44)

Virosomes are also produced by "Immunopotentiating reconstituted influenza virosomes (IRIV)".(12) The above-mentioned detergent solubilization method is used to create IRIV, but it also includes the inclusion of external phospholipids, which are then reconstituted once C12E8 is either separated via dialysis or adsorption onto a hydrophobic resin.(45) The target antigen, which could be plasmid or DNA, RNA is joined to a lipid anchor. Phospholipids, such as phosphatidylcholine, sphingomyelin, dioleoyl-3-trimethylammonium-propane (DOTAP) phosphatidylethanolamine, phosphatidylserine, dioleyldimethylammonium chloride (DODAC), and others, are included in the category of lipids. (46) Cholesterol is also a type of lipid. Certain antibodies, such as mAbs, that bind to the epitopes i.e. surface proteins of a particular type of cell can also be anchored to virosomes that have antigen associated with lipid load on them. Enhanced Ag encapsulation and uniform virosome particles can be produced once exogenous phospholipids are added.(47) Another method for creating a virosomal vaccine delivery system involves combining straightforward lipid vesicles (liposomes) that carry antigen with Sendai virus particles that have been UV-inactivated. (45) These virosomal carriers, which are created using the procedure described above, contain the viral RNA in contrast to influenza virosomes. Antigens such as recombinant proteins, proteins originating from pathogens, synthetic peptides, or carbohydrates can all be produced using general production techniques. The manufacture of immune goods involves a batch processing stage. The viral antigens are combined with detergent and phospholipid after extraction. After that, several sterilization and decontamination processes were permitted to run through the resultant product. To produce a final product that is stable and secure, the virosomes are often disseminated in buffered normal saline solution.(48, 49) The virosome is made stable, safe, and useful for use by these purification and formulation processes. The continuous manufacturing of and Inflexal® V and Epaxal® has demonstrated that the virosomal carrier production process is developed at an industrial scale and according to GMP criteria. The virosomal carrier self-assembles in vitro at a high concentration and, hence, in a small volume, enabling large-scale manufacturing of up to 500,000 doses per run in modest facilities.(39) Simple qualitative tests that are specifically designed for viral proteins are used to characterize the virosomal formulation. The precise shape, size, structure, and texture of the virosomes can be assessed using electron microscopy-based analytical techniques, most commonly scanning electron microscopy (SEM). The property of the material to be studied affects how virosomes' characteristics are evaluated. The very flexible and uncomplicated sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method is used to assess viral proteins. The fluorescence resonance energy transfer (FRET) method can be used to calculate fusogenic activity of virosomes.(13, 50, 51)

A novel method for creating virosomes utilizes cell-free protein synthesis:
The endocytotic pathway employed by the influenza virus to enter cells is typically coordinated by the interaction of HA1and sialic acid, which causes HA2 to fuse with the cell. Conventional techniques for producing virosomes are frequently based on influenza viruses, although they frequently do not fully mimic the membrane fusion characteristics of these viruses. The creation of proteins for virosomes can be done using the innovative protein expression method known as cell-free protein synthesis (CFPS). Since this method often does not involve surfactants, it virtually compromises the vital structure of the proteins. This method also incorporates simple production techniques. It's interesting that both membrane integration and protein synthesis occur simultaneously in this one-step process.(52)

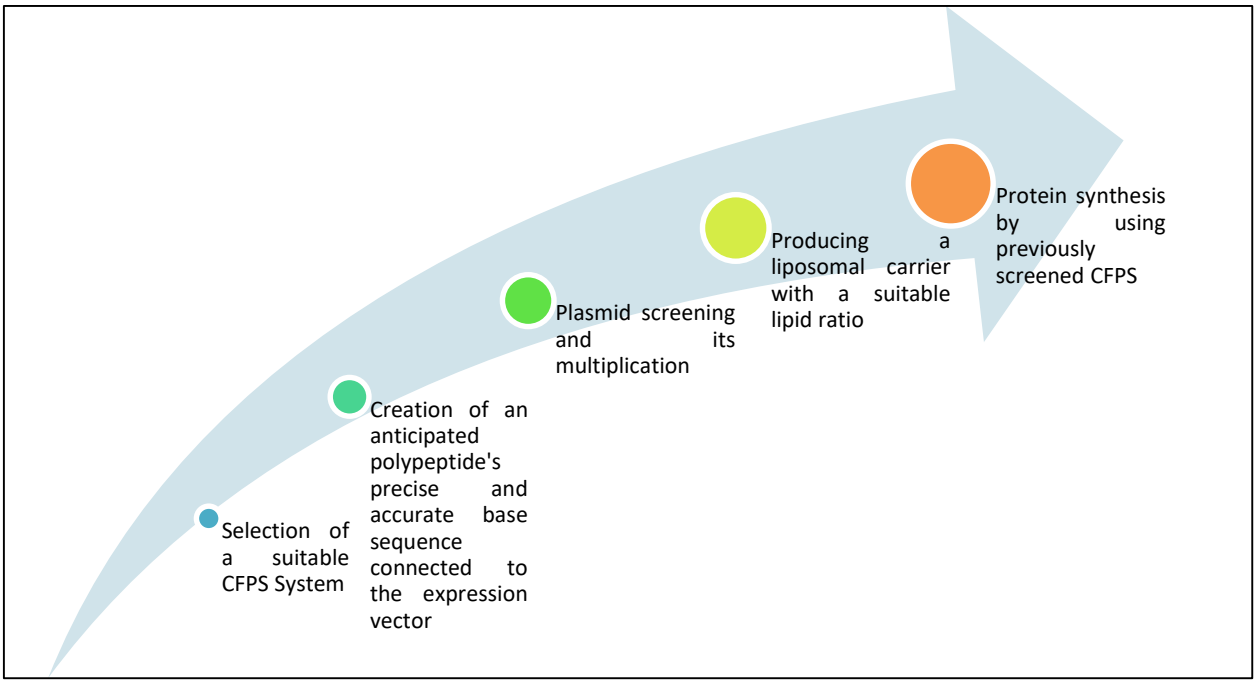

Figure 2: A flow chart showing how the cell-free protein synthesis (CFPS) method is used to synthesize virosomes

Using the CFPS process of virosomal particles manufacturing, was successfully synthesized a peptide containing a linking agent, an α-helical secondary structure, cytoplasmic tail (HA-TMR-CT) and transmembrane region with increased yield.(53, 54) HA2 virosomes were generated using a rabbit reticulocyte lysate technique. The research demonstrated the virosomes' remarkable capacity to carry siRNA and their pH dependent fusogenic activity. Successful synthesis of the MS2 bacteriophage coat protein using E-coli cell-free protein was achieved.(55) The MS2 bacteriophage protein aids in the foreign RNA's encapsulation after recognition.(56) Additionally, research has been done on using the MS2 bacteriophage protein to create virus-like particles (VLPs) that serve as carriers for exogenous RNA.(57) (58) In the CFPS, virosomes are synthesized using the MS2 bacteriophage protein, which serves as a carrier of siRNA. Gp64 is a viral glycoprotein that can transfer siRNA and CFPS. It has an expression on the Baculovirus envelope. Research has shown that the Gp64 displays a pH-dependent membrane fusion characteristic that is similar to HA2. Membrane fusion caused by Gp64 was seen at acidic pH levels, usually less than 5.5.(59) A glycoprotein called F that has been linked to the Sendai virus also shows evidence of virosome formation through cell-free protein synthesis. The Hemagglutinin-Neuramindase (HN) and glycoproteins Fusion (F) protein are present on the viral envelope of the Sendai virus.(60) It's interesting to note that glycoprotein F participates in the Sendai virus's fusion with the membrane of target cells. However, following careful isolation of the F and HN glycoproteins and examining the function of the F glycoprotein in the target cell's membrane fusion, scientists were able to demonstrate that the entire F-glycoprotein is necessary for this process. (61) Additionally, research revealed that the virosomal carriers created by combining F-glycoprotein with phospholipids exhibited the ability to initiate fusogenic properties as well as the potential for gene delivery.(62)
Consequently, in contrast to the traditional method of virosome synthesis, the CFPS method may ensure the functional and structural integrity of virosomes along with accurate folding and exact positioning of the peptides linked in virosome synthesis. These points should be highlighted for future research. Additionally, due to its significant advantages over the conventional virosome manufacturing process, CFPS, as a novel

virosome manufacturing technology, will undoubtedly spur further advancements in virosomal technology, which will ultimately function as a state-of-the-art nano-carrier technology to overcome the current obstacles in gene delivery and immunotherapy.

Virosomal method for cellular immunity stimulation:

In general, Cytotoxic T lymphocytes (CTLs) are in charge of neutralizing virus-infected cells, which means they are also in charge of reducing the severity of viral infections and curing them.(63) Human CTLs peculiar to the influenza virus are primarily triggered by epitopes generated from proteins present inside the virus, which include nucleoproteins, and matrix proteins whereas immunodominant epitopes typically show up on the nucleoprotein in mice. (64) But , other viral antigens, such as haemagglutinin, can also produce physiologically related CTL activity. Conventional vaccinations are often not a superior option as a transporter of antigens to the APC since they does not produce remarkable CTL activity. Subunit vaccines typically lack the vital CTL antigens, such as nucleoprotein and matrix protein. In order to put antigens on the MHC class I system and present them to the cytosol, virosomal carriers provide a means of doing so. This could potentially boost CTL activity by increasing antibody responses. Helper-T cells play a crucial role, particularly in stimulating B cell activity. Additionally crucial for the maturation of B cells & the alteration of antibody classes are helper-T cells.(65)

Additionally, helper-T cells promote the development of CTLs by increasing the production of cytokines. Consequently, and this is a commonly overlooked point, vaccinations need to be able to elicit significant helper-T cell responses. The primary cytokine secreted by helper-T cells during an influenza infection is IFN-γ, which is a feature that triggers the immune response. The formation of CTLs is stimulated by this kind of helper-T cell response, which also promotes the manufacture of IgG1 antibodies in humans and IgG2a antibodies in mice. (63) It is discovered that the virosomal carriers having a peptide attached resemble to a well-recognized nucleoprotein epitope specific to CTLs, offering evidence of concept that can strengthen the CTL response that is nucleoprotein-specific. Increased CTL responses against the peptide were seen in mice given the peptides-anchored virosomal carriers twice (at two-week intervals) by intraperitoneal injection. A mere 0.5 μg of virosome entrapped peptide was enough to elicit a potent immunological response akin to that of an entire infectious influenza virus. CTL activation was not observed in vaccinations containing even freer peptide up to 100 μg. Since virosomal carriers lacking fusion were unable to generate a significant CTL response, the fusion process of the carrier was essential for initiating the immunological response.(66) In vitro research has shown that virosomal carriers are adept at delivering the ovalbumin (OVA) prototype antigen into DCs, stimulating ovalbumin to present as its MHC class I antigen. Additionally, research has shown that virosomal carriers are effective at inducing OVA-induced CTL responses in vivo.(67)

Vaccines undergoing clinical trials:

Virosomal vaccines can function as immunization products by inducing an immune response on their own. They might therefore make suitable adjuvant candidates. Due to their capacity to transport macromolecules such as proteins and nucleic acids, virosomal carriers are ideal for serving as medication delivery vehicles. Virosomal drug carrier systems demonstrate favorable pharmacokinetic characteristics, ensuring a safe and efficient approach to investigating a medication's therapeutic potential. The quantity of viral surface proteins affects the virosomal carriers' ability to fuse. Moreover, virosomes have certain restrictions related to batch processing and sophisticated assay procedures. Consequently, efforts must be taken in this context to create simple assay procedures. More biopharmaceuticals based on virosomes will be approved and made available if these issues are resolved and troubleshooted. (68-70)

A malaria vaccine based on a synthetic peptide (derived from Plasmodium falciparum) was clinically evaluated. A Phase I clinical trial involving healthy adult and pediatric participants showed good safety, excellent tolerability, and a high level of immunogenicity. However, a delayed and unusual parasite growth was seen in the Phase II of clinical trial. (71)

The hepatitis C vaccine is composed of three peptides: the 3rd peptide functions as a CD4 epitope and is connected to the virosome's surface, while the other two peptides represent CTL epitopes and are enclosed in the virosome. The purpose of its formulation was to treat chronic hepatitis C virus infections by use of a therapeutically active T-cell vaccination. However, the vaccination did not produce the anticipated T-cell response in the clinical trial's healthy participants.(71)

vaccination A synthetic form of secreted aspartic peptidases (rtSap2) is being researched to treat recurrent vulvovaginal candidiasis. It arises as membrane-jointed rtSap2, a recombinant, enzymatically inactive, and shortened version of the secreted protease Sap2. In essence, Sap2 is an acidic hydrolase enzyme that gives Candida its virulence factor. This enzyme promotes the generation of inflammatory cytokines by host and helps

fungus get amino acids. It can also break down certain host proteins that are involved in the immune response. Both application techniques were examined separately in a Phase I trial including healthy participants. Applications administered intramuscularly and intra-vaginally have been reported to be safe, nontoxic, and hence well tolerated.(72) For the purpose of treating breast cancer, a trivalent vaccination targeting the Her2/neu receptor was developed as a target that had been verified (by Trastuzumab). Three peptides that were linked to the influenza virosomal surface and generated from Her2/neu are included in this vaccine.(73)

Table no 1: Virosomal vaccines, type of study and phases of clinical trials (Completed)

Vaccines	Study Design	Clinical trial Phase	National clinical trial Number	Reference
Hepatitis-A	Randomized, Open study, Controlled Study	Phase- 3	NCT01307436	(74)
Malaria	Double-blind randomized placebo controlled	Phase- 1	NCT00513679	(74)
Influenza	Non-randomized Trial, Open	Phase- 3	NCT01631210	(74)
HIV	Randomized Double Blind Study	Phase- 1	NCT01084243	(74)
Influenza	Randomized Double Blind Study	Phase- 1	NCT00714229	(74)
Hepatitis-A	Randomized, Open, Controlled Study	Phase- 2	NCT01405777	(74)
Influenza	Open Label, Non-randomized	Phase- 4	NCT01457027	(74)
Vulvovaginal Candidiasis	Randomized Placebo Controlled	Phase- 1	NCT01057131	(74)
Hepatitis-A	Randomized, Open, Controlled	Phase- 4	NCT01349929	(74)
Hepatitis-C	Single-blind randomized placebo, Controlled	Phase- 1	NCT00446419	(74)
Influenza	Non-randomized Trial, Open	Phase- 3	NCT01348829	(74)
Breast-Cancer	Open, Non-randomized	Phase- 1		(73)

The concept of employing virosome delivery to deliver siRNA has been investigated in a pre-clinical context because to the differing merits and demerits of virus-based and non-virus-based carriers. The technique for delivering siRNA via the virosomal carrier encompasses many virus protein types that are used in the synthesis of virosomes. The administration of siRNA using virosomal drug carriers may be facilitated by the cell-free protein synthesis technique.(75) There have been many developments in the field of vaccinations and immunizations, but there is still a technology gap and difficulties in developing a new, efficient therapy for diseases like tuberculosis malaria, and AIDS,. Furthermore, a few of the vaccination products that are already on the market have drawbacks such as being unable to fully acquire an immune response, problems with in vivo intactness, frequent dosage requirements, systemic toxicity, and difficulties with in preservation and vitro stability. In order to address the problems mentioned above, nanotechnology has become a crucial instrument. Generally speaking, a nanovaccine system is a revolutionary kind of vaccination that uses nanoparticles (NPs) as both an adjuvant and a carrier system.(76)

Pay-Load	Marketed By	Brand Name	Year of approval	References
Adenovirus serotypes 26 and 5	Gamaleya Research Institute of Epidemiology and Microbiology, Russia	Sputnik V	2021	(77)
Recombinant incompetent Ad26 vector	Johnson & Johnson	Janssen COVID-19 Vaccine	2020	(77)
Nucleoside-modified mRNA	Pfizer-BioNTech	Spikevax	2022	(78)
Recombinant human adenovirus 26 serotype	Gamaleya Research Institute of Epidemiology and Microbiology, Russia	Sputnik Light	2021	(79)
BNT162b2 [mRNA]	BioNTech-Pfzer	Comirnaty	2021	(80)

Table no: 2: Newly approved COVID-19 nano-vaccines that have undergone clinical testing for human use.

Future Prospects:

To yet, the virosomal carrier system hasn't been thoroughly investigated. The current genetic delivery vehicles have been thoroughly examined; nevertheless, new reports from a number of clinical trials indicate that promising advancements for viral delivery methods have been made. It has become clear in recent years that the claims made about the development of a adaptable gene delivery carrier may not be accurate. Since the diseases which are related to gene therapy can cure are quite distinct, individualized treatment plans are required. Some diseases can solely be treated by regulating the expression, or generally stopping the expression of a specific gene for a longer period of time. Other medical conditions, on the other hand, may only be managed by abundant but temporary protein expression. In some circumstances, delivering siRNA via the cytoplasm is appropriate; in others, presenting one or more genes into the target cell's nucleus is necessary. In addition, the location of the diseased cells in the body and their characteristics, such as their mitotic and endocytic environments, influence how the illness is treated. These many qualities make it appealing to build nucleic acid delivery systems that are tailored for particular uses. Therefore, it is crucial that additional types of delivery vehicles—whether of viral, hybrid or non-viral origin—be made available in the future. The technology platform that can be readily modified to meet the requirements of treating a specific disease is embodied by the virosomal drug carrier platform, which will play a vital role in the future delivery of monoclonal antibodies & nucleic acids. Complications related to DNA-virosomal carriers can be resolved. One of the best methods for treating and preventing infectious diseases is vaccination. Many virosomal-based nanovaccine formulations against common and deadly illnesses have recently gone on sale. The effectiveness of SARS-CoV-2 vaccinations has not decreased over time, however sustained protection is susceptible to the emergence of novel viral variants. A lot of work is currently being done to create a vaccine delivery system based on virosomes to combat the new SARS-CoV-2 virus. Even so, more perseverance will be needed to develop a stable, reliable, efficacious, and repeatable virosome-based SARS-CoV-2 vaccine for use in clinical settings. Although virosomes provide a unique drug carrier system for the administration of a variety of therapeutically active compounds, there is still much to learn about the stability, optimization, and tolerability, IVIVC, scale-up of virosomal drug delivery systems.

Conclusion:

In summary, a virosome contains the remnants of the original virus's envelope but not its nucleic acid. Virus phospholipid membrane structure, related spike proteins, and peptides intercalated in the envelope are all present in virosomes, which are more functionally active than liposomes. The success and improvement of virosome-based vaccines with novel and diverse antigens have been supported by the clinically recognized characteristics of virosome as adjuvant and antigen carrier. Although the product's activity depends on the antigen and production costs, its straightforward structure and easy fabrication techniques may hinder its wider application for less expensive, preventative immunization products like diphtheria, hepatitis B, tetanus, etc. because of this.

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