VIROSOME – DRUG AND VACCINE DELIVERY SYSTEM

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ABSTRACT
Virosomes are reconstituted viral envelopes that can serve as vehicles for cellular delivery of macromolecules. The prospect of drug delivery and targeting using virosomes is an interesting field of research and development. Because virosomes are biocompatible, biodegradable, nontoxic and non-autoimmunogenic. Attempts have been made to use them as vaccines or adjuvants as well as delivery systems for drugs, nucleic acids or genes for therapeutic purposes. Influenza virus is the most common virus of choice. The success of virosomal drug delivery depends on method used to prepare the encapsulated bioactive materials and incorporate them into the virosomes. Virosome technology could potentially be used to deliver peptides, nucleic acids or genes and drugs like antibiotics, anticancer agents, and steroids. This review summarizes the method of preparation, administration, characterization, evaluation, and future aspects of virosomes.

Keywords- Virosomes, Influenza virus, Vaccine, Haemagglutinin (HA), Neuraminidase (NA).

INTRODUCTION
Promising drugs are often discontinued during development because they are not suitably delivered to target cells, tissues and organs. The new generation of therapeutics against some diseases or disorders requires delivery systems that target drugs to specified cell types and host tissues by receptor-mediated uptake and controlled release. Virosomal technology presents a novel sophisticated delivery system to meet these challenges. Virosomes are spherical, unilamellar phospholipid bilayer vesicles having mean diameter in range 120-180 nm.
Basically, virosomes are reconstituted empty influenza virus envelopes, where infectious neuclocapside is replaced by compound of choice. Virosomes are not replicate but they are pure fusion activity vesicles thus deliver the incorporated compound (antigen, drug, genes) inside the target cell. Virosomes protect pharmaceutically active substances from proteolytic degradation and low pH within endosomes, allowing their contents to remain intact when they reach the cytoplasm\(^1\).

**Virosome structure**

Main constitution of immunostimulation reconstitution influenza virosomes consist of phospholipids (PL) and phosphatidylycholine (PC). PC form 70% of virosomal structure and remaining 30% of membrane components are envelope phospholipids which originate from influenza virus. Influenza virus provides Neuraminidase (NA) and Haemagglutin (HA)\(^2\). Virome structure is illustrated in Fig.1 A and Fig.1 B.

![Fig.1 A) Influenza virus](image1.png) ![Fig.1 B) Influenza virome](image2.png)

Neuraminidase (NA) is tetramer and it is involved in virus budding. It is composed of 4 equal spherical subunits which are hydrophobically embedded in Immunopotentiating reconstituted influenza virosomes (IRIV) membrane by a central stalk and can readily intercalate into phospholipid membrane. NA present on IRIV’s surface shows its action by mechanism through which it enhances influenza virus pathogenicity, it also catalyses the cleavage of N-acetyl-neuraminic acid (sialic acid) from bound sugar residue resulting in decreased viscosity of host’s mucus, which allows influenza virus an easier access to epithelial cells. The same procedure leads to destruction of HA receptors within cell membrane to which viruses and IRIVs bind. This allows virus particles to avoid aggregation, as newly formed virus particles do not adhere to infected host cell membrane after budding. The HA is major influenza
antigen which is formed from 2 polypeptides HA1 and HA2, that are involved in receptor binding and membrane fusion.

HA1 - Globular head contain a receptor site that has a high affinity for sialic acid present on surface of antigen presenting cells (APCs), such as macrophages and lymphocytes therefore facilitates binding of IRIVs.

HA2 - The actual fusion of IRIVs with endosomal membrane is then mediated by HA2 polypeptide.

STEPS INVOLVED IN METHOD OF PREPARATION OF VIROSOME

A) Selection of virus
B) Selection of antigen
C) Reconstitution of virosomes

A) Selection of virus
Influenza viral envelope is most commonly used to produce virosomes. Virosomes are reconstituted viral envelopes that can be derived from different virus.
Virosome can also be made from Sendai virus, Epstein burr-virus, HIV, Sindbis, Semlikiforest, Herpes simplex virus.

B) Selection of antigen
Antigen is selected as per requirement such a parasite, bacterium or whole cell. Carcinogenic cell is used as antigen cell components. DNA, RNA or Plasmid also can also be used as antigen. This antigen is coupled to lipid anchor so that antigen will be ready to load to virosomes.

C) Reconstitution of virosomes
Virosomes solubilized with detergent for eg. Octaglucoside, Triton X-100, Nonidert p-40. Due to solubilization with detergent internal viral protein and genetic material sediment, then detergent is removed by different methods such as dialysis and hydrophobic resin from supernatant. Then using ultracentrifugation process viral matrix protein and nucleicapsid is removed. Viral phospholipid and viral protein is recovered.

Now, antigen which is already coupled to lipid anchor is mixed with polymer or surfactant solution and this solution is processed with virosoine carrier so that antigen bound virosoine is obtained.
ADMINISTRATION OF VIROSOMES

Generally, virosomes are suspended in buffered saline (135-150 mm NaCl), but other suitable vehicles also exist. These compositions should be sterilized by conventional liposomal sterilization techniques such as membrane filtration. The formulation also generally contains auxiliary substances required to stimulate physiological condition such as buffering agents and isotonicity adjusting agents eg., Sodium acetate, Sodium lactate, Sodium chloride, Potassium chloride, Calcium chloride. Concentration of virome used in the vehicle ranges from 20-200 mg/ml. These concentrations are varied to optimize treatment with different virome components or for particular purpose. The virome are administered in a variety of parental routes including intravenous, intramuscular, subcutaneous, intra-arterial and inhalable route. Virosomes can be administered topically or trasdermally as well as can be incorporated into implantable device for long term release[3].

CHARACTERISATION OF VIROSOMES

A) Protein Detection

Virome preparation should generally result in relatively uniform protein to lipid ratio. Sodium dodecyl sulfate-polyacrylamine gel electrophoresis (SDS-PAGE) can confirm the presence of HA protein in the virosomes[4].

B) Structure and size

To determine ultra-structure and size of virome, negative stain electron microscopy is used. The staining solution should preferable be of neutral pH to avoid acid induced conformational changes of HA[5].

C) Fusion activity

Generally virosomes exhibit pH dependent membrane fusion activity which can be visualized with a fluorescent resonance energy transfer assay (RET). Alternatively, fusion can be assessed in vitro with an excimer assay using pyrene labeled lipids where, the decrease of surface density of the pyrene-Phosotidylcholine label on fusion with unlabeled membrane corresponds to reduction of excimer fluorescence.

Fusion activity also can be indirectly monitored by determining hemolytic activity which corresponds closer to fusion activity and exhibit a pH dependent identical with that of fusion.
POINTS FOR EVALUATION OF VIROSOMES
A) Surface morphology and vesicle shape: - Transmission electron microscopy, Freeze fracture electron microscopy.
C) Surface charge: - Free flow electrophoresis.
D) Surface pH and electrical surface potential: - Zeta potential measurements and pH sensitive probes.
E) Lamellarity: - Small angle x-ray scattering, Freeze fracture electron microscopy, 13p-NMR.
F) Phase behavior: Freeze fracture electron microscopy, Differential scanning colorimetry.
G) Percent of free drug: Mini column centrifugation, Gel exclusion and Ion exchange chromatography, Protamine aggregation, Radiolabelling.
H) Drug release: Diffusion cell/ dialysis.
I) Pyrogenicity: Rabbit fever response test or Limulus amebocyte lysate (LAL) test.
K) Chemical analysis of surface: Static secondary ion mass spectrometry[6,7].

ADVANTAGES OF VIROSOMES
A) Virosomal technology is approved by the FDA for use in humans, and has a high safety profile.
B) Virosomes are biodegradable, biocompatible, and non-toxic[8]
C) No disease-transmission risk
D) No autoimmunogenity or anaphylaxis[8,9]
E) Broadly applicable with almost all important drugs (anticancer drugs, proteins, peptides, nucleic acids, antibiotics, fungicides)
F) Enables drug delivery into the cytoplasm of target cell
G) Promotes fusion activity in the endolysosomal pathway
H) Protects drugs against degradation

KEY PROBLEMS ASSOCIATED WITH VIROSOMES PREPARATION[10-11]
A) Shelf-life is too short
B) Scale up related problems
C) Poor quality of raw material
D) Pay-load is too slow
E) Absence on any data on safety of these carrier systems on chronic use.

But in recent years several solutions have been worked upon to overcome above mentioned problems
A) High quality products with improved purification protocols and validated analytical techniques are available
B) Quality control assay can be performed using sophisticated instruments and batch to batch variability can be checked
C) Payload problems can be sorted out using either lipophilic drug/lipophilic prodrug of hydrophilic drugs or using active (remote loading) techniques
D) Shelf life can be improved using appropriate cryoprotectant and lyoprotectant and product can be successfully freeze dried
E) Scaling up can be improved by carefully selecting method of preparation, sterilization by autoclaving or membrane filtration (0.2 micrometer) coupled with aseptic and pyrogen removal using properly validated LAL test
F) By choosing candidate potent drugs with narrow therapeutic window (eg. Cytotoxic drugs, and fungicides) the drug related safety problems can be alleviated

APPLICATIONS OF VIROSUME

A) Cancer treatment
Virosomes are used in oncology field to carry peptide corresponding to tumour associated with an antigen as in case of peptide from parathyroid hormone related protein or from recombinant proteins such as her-2 neu Fab combined the anti Fab-doxovirosome along with anti-proliferate properties of the monoclonal antibodies and cytotoxic effect of doxorubicin in vivo\textsuperscript{[12]}. 

B) Gene delivery
Heam-agglutinin, the membrane fusion protein of influenza virus is known to mediate a low pH dependent fusion reaction between the viral envelope and the limiting membrane of endosomal cell compartment following cellular uptake of virus particle by receptor mediated endocytosis\textsuperscript{[13,14]}.
C) RNA/DNA: Small interfering RNA encapsulated in virosomes, are able to put adequate synthesis of newly induced and constitutively expressed protein, overcoming the lack of suitable delivery methods for these molecules. Intraperitoneal injection of SiRNA loaded virosome resulted in delivery of nucleotide to cell in peritoneal. [15]

A VEHCILE FOR VACCINE

A vaccine is a biological preparation that improves immunity to a particular infectious microorganism (microbe). A vaccine typically contains a small amount of an agent (immunogen) that resembles a microorganism. Upon administration, the immunogen stimulates the body's immune system to recognize it as foreign body which is further destroyed and remembered. Consequently, the immune system can more easily recognize and destroy or neutralize the correspondent microorganism that it later encounters, thereby avoiding severe infection and subsequently, pathologies. The immunogen is made up of some parts of the microbe, as proteins, which are seen by the immune system as the microbe itself. This immunogen usually needs a vehicle to be delivered efficiently in the body.

Virosome based vaccines

Virosomes provide a technology platform to many vaccines. Lipids, antigens, adjuvants or other materials can be added to the dissolved viral membrane or can be include in the virosome during reconstitution. The safety and tolerability of influenza virosomes have already been proven. Example: A) Influenza

![Microscopy structure](image.png)

Influenza, commonly referred as flu, is a contagious respiratory illness caused by influenza viruses. Influenza virus is spread from person to person by virus-contained aerosols (respiratory droplets of coughs and sneezes).
**Influenza vaccine approach:** A number of influenza vaccines are in the market and these vaccines are need to be injected. Usually people do not like to be injected - a large group at risk is not vaccinated. The only vaccine in the market which eliminates the need of injection is not totally secured because it is based on a “live virus”. Consequently, it can potentially infect the recipient and causes pathologies.

Another formulation developed i.e. an intra-nasal influenza vaccine which is based on virosomes and which does not contain a “live virus”. The vaccine, being administered in the nose, can therefore fight with the virus at the mucosal level by developed mucosal antibodies and then by blood antibodies. Consequently, the infection is stopped at the point of entry and prevents even the earliest onset of disease.

**B) Malaria**

Malaria is caused by a parasite called *Plasmodium falciparum*, which is transmitted via the bites of infected mosquitoes. In the human body, the parasites multiply in the liver, and then infect red blood cells[16].

**Malaria vaccine:** Malaria Vaccine develops antibodies against the *Plasmodium falciparum*, the most aggressive type of parasite that causes malaria. The parasite has two main stages of development that take place into two different organs. These are as follows.

Step 1-First, migration of the parasite transmitted by the mosquito bite (sporozoite) to the liver.

Step 2-Second, the sporozoite get transformed inside the liver cell for generating hundreds of merozoites, which then infect red blood cells.

Therefore, proteins at the surface of the parasite (sporozoites or merozoites) are constantly changing and the ideal vaccine should be targeted through different proteins from the various stages to improve the chance of obtaining protection or reducing the symptoms related mostly to the red blood cell infection.

Vaccine is one of few designed for targeting the two forms of the parasite: sporozoites (infecting liver cells) and merozoites (infecting red blood cells). The vaccine uses virosomes as a delivery platform.
C) RSV (Respiratory Syncytial Virus): Respiratory Syncytial Virus (RSV) has a high prevalence in developed countries, causes respiratory tract infections in patients of all ages, especially in patients with weak immune system. It is a major cause of lower respiratory tract infection during infancy and the main cause of pneumonia and bronchiolitis in children under the age of 1 year, responsible for up to 10% of their intensive care hospitalizations. Infections have also increasingly been found among elderly patients, causing life-threatening pulmonary disease, killing its half or as many elderly and high-risk of adults as much as influenza does. RSV is highly contagious and can be spread through droplets containing the virus when someone coughs or sneezes. It also can live on surfaces (such as countertops or doorknobs) and on hands and clothing, so this can be easily spread when a person touches something contaminated.

RSV vaccine approach: Probably, as such there are no vaccines for RSV. In the past, attenuated infectious virus vaccine candidates were found to be either over or under-attenuated and the immune response induced by inactivated virus or subunit vaccines were associated with serious safety risks, sometimes leading to more serious disease in people that were vaccinated and later infected by the virus, than non-vaccinated people (enhanced disease). A developed RSV vaccine for the elderly population, representing a target population of about 150 million in the world. It is a virosome based RSV vaccine which has gone through extensive pre-clinical testing.
FUTURE PROSPECTS

Until other carrier systems of age, virosomal technology will remain a hotspot active area for future research[17]. Virosomes represent an innovative drug-delivery system for various biologically active molecules, but especially nucleic acids or genes for numerous indications. The surface of virosomes can be suitably modified to facilitate targeted drug delivery. However, their comprehensive pharmacokinetic profile, bioavailability, clinical effects and stability should be studied thoroughly to ascertain their long-term reliability as a safe, effective and affordable means for drug delivery and targeting. In near future virosomes based delivery system with their ability to provide controlled and site specific drug delivery revolutionize disease management[18].

REFERENCES


