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Virosomes as drug delivery system: An updated review

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Abstract

Amid the era of development in all sphere of Biotechnology, Biochemistry and Pharmacology, it would not be an exaggeration to say that we have a myriad of molecules available to us in labs with promising results against many diseases. The limitation lies in the fact that some molecules are toxic when they act on organs other than their targets. Yet others cannot reach their targets in the desired concentration, be it due to easy degradation in the gut or high first-pass metabolism or very short half-life, etc. Thus, to overcome this, we have an alternate drug delivery system, namely Virosomes. The purpose of this review is to understand the drug delivery aspect of the virosomes, its type, structure, method of preparation, mechanism of action, administration routes and the application in the medical field. Virosomes are the regenerated viral envelope having a central empty space that can act as the vehicles for drug delivery. The central empty space can be filled with the desired bioactive molecule. Virosomes are most commonly prepared by using the reconstituted viral envelope of the Influenza virus, but other virus such as Sendai or HVJ, Hepatitis B Virus, HIV, New castle Disease Virus etc., can also be used for the preparation of virosomes. Virosomes has a great potential as the drug delivery system for almost all types of the drugs, and this encourages the researchers and therapeutic industries to enhance their pharmacological profiles, clinical result and stability.

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INTRODUCTION

The major goal of any drug is the delivery to the targeted organ, tissue and cell. Many drugs which show promising results in vitro fail to do so in vivo because they are not effectively delivered to targeted organs, tissue and cells (Kalra *et al.*, 2013). To overcome the difficulty of ineffective delivery to the targeted organ, tissue and cell, a novel drug delivery carrier, i.e., virosomes, are developed. Virosomes are reconstituted viral envelopes, having lipid membrane and viral spike glycoprotein, but free from viral genetic material and hence making inter-

nal compartment of virosome empty while external surface contains spike protein protruding from the membrane, resembling the virus particle.

Virosomes are semi-synthetic complex obtained from a nucleic acid-free viral particle. They have regenerated empty virus envelop where viral nucleocapsid is interchanged by compound of interest (Almeida, 1975). These compounds can be drug, protein, carbohydrate, antigen, or a gene which can be incorporated either in the aqueous interior or virosomal membrane of virosomes (Felnerova *et al.*, 2004; Lund *et al.*, 2010). Since virosomes retain the property of fusogenic activity consequently deliver the incorporated compound to the targeted organ, tissue and cell. Moreover, the receptor-binding property of viral envelop glycoprotein is preserved and thus further assist in the delivery of drugs. The peculiarity of virosomes is that it protects the incorporated compound (drugs) from proteolytic degradation and low pH within the endosomes and thus permit the content to remain unharmed when they reach the cytoplasm of the targeted cell.

Virosomes are completely biocompatible, biodegradable, non-toxic and non-auto immunogenic. Many attempts were made to the use of virosomes as a drug delivery system and they are also used as a vaccine or adjuvant.

HISTORY

The term virosomes was first proposed by Kara in 1971 for oncogenic sub viral ribonucleoprotein particle of *Rous sarcoma virus* (Kára *et al.*, 1971). However, virosomes were first prepared by Almeida (1975) by placing purified spike protein into preformed liposomes, using influenza A as a parental virus. Morein *et al.* (1978) proposed the use of virosome as a vaccine and also expanded the concept of virosomes to other enveloped viruses.

Types of Virosomes

Depending on the viral envelope, there are several types of virosomes can be generated (Table 1). Some of them are as follows:

1. Influenza Virosomes (Zurbriggen, 2003)
2. Sendai Virosomes (Uchida, 1979; Kaneda, 2002)
3. HBV Virosomes
4. HIV Virosomes
5. NDV Virosomes

Influenza virosomes

They are most commonly used virosomes and are obtained from the influenza viruses, which are part of the Orthomyxoviridae family. Influenza viruses have eight segmented single-stranded RNA genomes covered with a viral envelope (Suarez and Schultz-Cherry, 2000; Noda and Kawaoka, 2010). The external surface of the viral envelope contains two types of membrane protein, hemagglutinin (HA) and neuraminidase (NA). HA is consisting of two polypeptides, HA1 and HA2. HA1 is responsible for binding with sialic acid on the surface of a host cell and thus cause attachment between the host cell and viral particle (Skehel and Wiley, 2000). HA2 polypeptide causes endosomal membrane fusion with a virosomes membrane (Fukuyama and Kawaoka, 2011). However, membrane fusion will not occur in neutral conditions and it will achieve its fusion activity through a conformational change only in acidic conditions. Fortunately, endosomes provide the required acidic condition.

Further, influenza virosomes are classified into two types based on the presence of additional lipid, namely virosome without additional lipid published by Stegmann (1987) and Immunostimulating Reconstructed Influenza Virosomes (IRIV) describe by Glück (1992). The only difference between IRIV's and Virosomes by Stegmann is that IRIV's contain additional nonviral lipids, whereas Virosomes do not have any additional lipid.

Sendai Virosomes

They are obtained from the Hemagglutinating virus of Japan (HVJ) or Sendai virus and are part of the Paramyxoviridae family. HVJ have single-stranded RNA genomes covered with envelope (Curran and Kolakofsky, 1999). The external surface consists of two membrane glycoprotein, hemagglutinin-neuraminidase (HN) and fusion protein (F) (Okada, 1993). HN glycoprotein is responsible for binding with sialic acid and thus adhere to the surface of a host cell (Takimoto, 2002) and F enables the membrane fusion between the host cell and viral particle (Asano and Asano, 1982). Interestingly, fusion protein does not require acidic condition as it works well in neutral condition for membrane fusion. Hence, unlike influenza virosomes, HVJ virosomes do not require reaching the endosome for membrane fusion.

HBV virosomes

They are obtained from the Hepatitis B virus and members of the Orthohepadnaviridae family. HBV is an enveloped virus containing a partially double-stranded DNA genome. The external surface of the

envelope contains three surface proteins, small (S), medium (M) and large (L) proteins. However, L protein can be purified by ultracentrifugation and used as a safe vehicle for drug delivery systems having high targeting specificity to human hepatocyte in vivo and in vitro (Yamada *et al.*, 2003).

HIV Virosomes

They can be obtained from the Human Immunodeficiency Virus (HIV) and members of the Retroviridae family. HIV composed of two congruent single-stranded RNA genome that is enclosed in an envelope. The external surface contains two envelop glycoproteins, gp120 and gp41. Moreover, p17 and p24 are matrix and core proteins, respectively. After the process of purification and centrifugation, virosomes obtained have enveloped glycoprotein (gp120 and gp41) and p17 matrix protein. However, the p24 core protein was not found (Cornet *et al.*, 1990).

NDV Virosomes

They are obtained from the Newcastle disease virus (NDV) and are the member of the Paramyxoviridae family. NDV contains a single-stranded RNA genome, and viral nucleocapsid is enclosed in an envelope. The external surface of the viral envelope contains two membrane proteins, hemagglutinin-neuraminidase (HN) and fusion protein (F), resembling the Sendai virus. HN protein is responsible for binding with sialic acid and thus help in attaching with a host cell. F protein is responsible for fusion with the targeted cell (Kapczynski and Tumpey, 2003).

However, the influenza virus is most commonly used for the formation of virosomes as a drug delivery system (De Jonge, 2006) and thus, in this review, we will talk about influenza virosomes.

Structure of Virosomes

Virosomes are the semi-synthetic spherical unilamellar and lipid bilayer vesicle of mean diameter ranging from 120-180 nm. They are free from the viral genome and thus create an empty aqueous interior in which bioactive drugs can be entrapped (Figure 1). Viral glycoproteins are embedded in the lipid bilayer of virosomes. Two primary components of the virosomes are phospholipid (PL) and phosphatidylcholine (PC). PC set up about 70% of the virosomal structure, and the remaining 30% of membrane component provide the envelope phospholipid, which in turn give two glycoproteins, neuraminidase (NA) and haemagglutinin (HA), embedded in the viral envelope. These two glycoproteins help in the successful uptake of virosome by the host cell (Cusi, 2006).

NA is the tetramer having four equal subunits with central stalk hydrophobically planted in the viral envelope of Immunostimulating Reconstructed Influenza Virosomes (IRIV) (Blom, 2017). Moreover, NA present on the surface of IRIV's helps to enhance its pathogenicity by a very similar technique as used by the influenza virus; it catalyzes the cleavage of N-acetylneuraminic (salic acid) from the bound sugar residue leading to a reduction in viscosity of the host mucus, and this will allow the influenza virus an easy entry to host epithelial cell. The same mechanism results in the destruction of the HA receptor present on the host cell membrane to which virosomes or viruses will bind and thus prevent aggregation.

HA is the dimer of two polypeptides, HA1 and HA2, responsible for receptor binding and membrane fusion, respectively. HA1 polypeptide is a globular head having a receptor site and is mainly responsible for the binding with salic acid present on the surface of the host cell and thus attach with the host cell. On the other hand, HA2 polypeptide mediated the fusion of virosomes with the endosomal membrane (Cusi, 2006). Although, membrane fusion will occur only in acidic conditions present on host cell endosomes (approx.5.0) that trigger a conformational change in HA2 and expose the fusogenic peptide, which is essential for membrane fusion.

The choice of bilayer content determines the characteristic of virosomes and they can be improved for the incorporation of drug or physiological effect by altering the content or type of membrane lipid used.

Advantage of virosomes

1. FDA has approved the virosomal drug delivery technology for human use and it shows a high safety profile.
2. Virosomal technology is applied for a diverse form of the drug, like an anticancer drug, antibiotics and fungicides.
3. They are totally biocompatible, biodegradable and non-poisonous in nature (Huckriede *et al.*, 2005).
4. They are non-replicative and thus have no disease transmission risk.
5. Virosomes shows non-auto immunogenic.
6. They lack of anaphylaxis effect (Huckriede *et al.*, 2005).
7. They prevent incorporated compounds (drugs) from degradation by protease enzyme and low pH in endosomes.

Table 1: Different types of virosomes

S. No.	Virosomes	Parent virus	Family	Viral glycoprotein
1.	Influenza Virosomes	Influenza	Orthomyxoviridae	HA, NA
2.	Sendai Virosomes	Sendai or HVJ	Paramyxoviridae	HN, F
3.	HBV Virosomes	HBV	Orthohepadnaviridae	S, M, L
4.	HIV Virosomes	HIV	Retroviridae	gp120, gp41, p17
5.	NDV Virosomes	NDV	Paramyxoviridae	HN, F

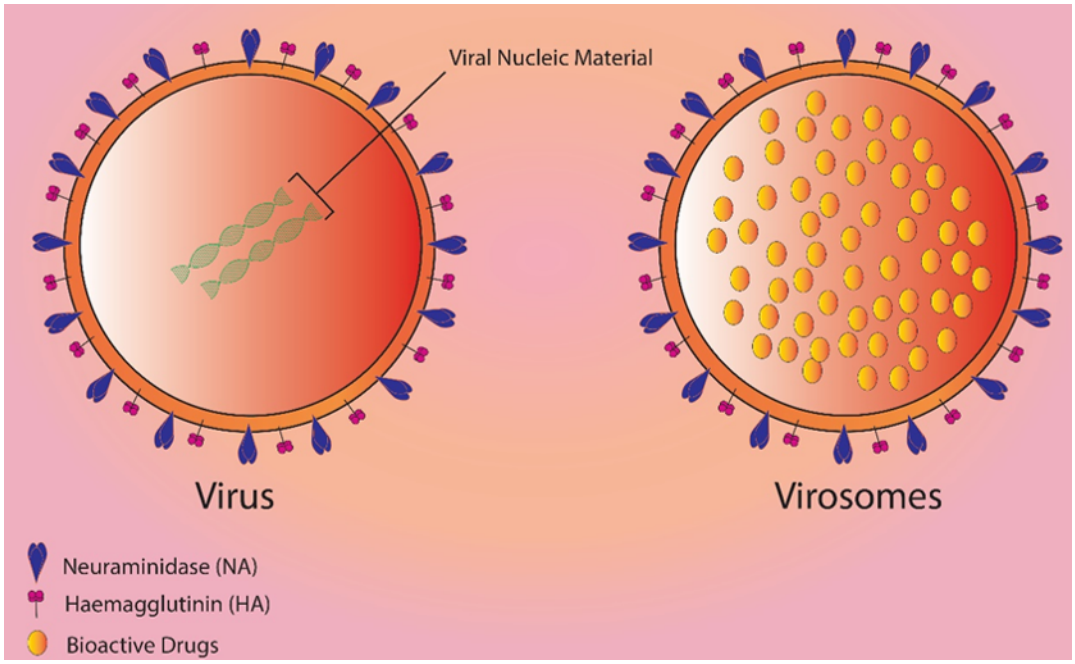

Figure 1: Virus with viral nucleic material and converted form of virus into virosomes, in which bioactive drugs are entrapped.

8. They allow the delivery of a drug into the cytoplasm of the targeted cell.

Disadvantage of Virosomes

1. There is the possibility of a negative immune response considering that some viral glycoproteins are present on the surface of virosomes.
2. Fast disintegration in blood before reaching the targeted cell will make virosomal technology ineffective and thus a short half-life.
3. Extended payload.
4. Undesirable quality of raw material.
5. Poor quality control assays.
6. Brief shelf life.
7. Some manufacturing issues.
8. Data associated with chronic use of virosomes is lacking (Kammer, 2007).

The solution to these disadvantages

1. Fast disintegration can be solved by rising virosome stability or by permitting the virosomes to hit the target in a short span of time after the administration (Liu, 2015).
2. The newly developed remote loading method can be used to resolve the payload problem.
3. Implementation of high-quality products with proper protocol for reliable purification.
4. Innovative approach and batch to batch variability test can be carried out for poor quality control assays.
5. Shelf life can be increased by using a substance like cryoprotectants and lyoprotectants.

Preparation of virosomes as a drug delivery vehicle

For the preparation of virosomes, selection of desirable virus HIV, Influenza, HSV etc.

Table 2: Therapeutics developed by virosomal technique

S. No.	Source of virosomes	Disease	Status
1.	Hepatitis A virus envelop protein (Epaxal®)	Hepatitis A	Approved
2.	Influenza virus (Inflexal®)	Influenza	Approved
3.	Diphtheria/tetanus toxoid virus envelope proteins	Diphtheria, Tetanus	Under clinical trial
4.	Peptidomimetic of the loop I from domain III of Plasmodium falciparum AMA-1	Malaria	Under clinical trial
5.	PEV6	Breast cancer	Under clinical trial
6.	Doxorubicin	Cancer, Ovarian carcinoma	Pre-clinical trial
7.	L-myc antisense ODNs	Cancer	Pre-clinical trial
8.	DNA-encoded TAA Prostate	Carcinoma	Pre-clinical trial
9.	DNA-encoded mumps antigen	Mumps	Pre-clinical trial

(Mischler and Metcalfe, 2002; Hatz, 2011; Shaikh *et al.*, 2019)

However, the Influenza virus is commonly used for the preparation of virosomes. The next step is to select the antigen according to our requirements. Then a large amount of selected virus must be cultivated in vivo or in vitro.

The viral membrane is solubilized in detergents. The most commonly used detergents are octaglucoside, triton x-100, nonidert p-40 and octaethylene glycol mono ether (C12E8). Consequently, viral protein and genetic material solubilize and can be removed from the supernatant by dialysis and hydrophobic resin methods. Following this, viral matrix protein and nucleocapsid are obtained by the ultracentrifugation method further viral nucleocapsid is removed. When the detergent is removed gradually, lipid bilayer, along with viral glycoprotein, get self-assembled to generate virosome. In due process, around 82% of phospholipid is recovered and antigen which is so far incorporated to lipid anchor is mixed with a polymer or surfactant solution further processed with a virosomes carrier to procure antigen bound virosome (Cusi, 2006; Sharma and Yasir, 2010; Liu, 2015). Additionally, cholesterol and other phospholipids like sphingomyelin, phosphatidylethanolamine, and phosphatidylserine can be integrated into the membrane.

During the course of this process, the bioactive drug of both hydrophobic and hydrophilic nature can be integrated into the aqueous interior or in the lipid bilayer of the virosomes (Cusi, 2006; Chang and Yeh, 2012). In an aqueous interior usually, hydrophilic drugs are placed, whereas hydrophobic drugs are incorporated with phospholipid mixture to provide solubility of the drug.

In contrast, the bioactive drug can be first incorporated in liposomes and then combine with virosome carrying two hemagglutinin glycoproteins with varying pH threshold to produce a virosome-liposome hybrid.

Modification of virosomes

The activity of virosome can be optimized by adding hydrophilic polymers such as polyethylene glycol, polyacrylmorpholine, polyvinylpyrroliodone and poly(2-oxazoline) into the viral envelope and this result in extending their time of circulation after systemic delivery (Moghimi and Szebeni, 2003). Virosomes can be customized by incorporating different ligands such as cytokines, peptides, and monoclonal antibodies on their surface for specific targeted delivery of the incorporated drug (Mastrobattista, 2001; Wegmann, 2002). Evidently, cancer cells for which specific siRNA can be targeted by virosomes. This has been done by customized virosomes on which HER2 (human epidermal growth factor 2, a protein that causes the breast cancer cell to grow) affibody molecules were presented (Nishimura *et al.*, 2013).

Route of administration of virosome

The transport of drugs to different parts of the body is the main objective for any scientist. Till now, cancer cells, erythrocytes, hepatocytes, cells of the respiratory and gastrointestinal systems have been fortunately targeted by virosomes. To emulate normal physiological conditions, virosomes are frequently put up in saline buffer, which is 135-150 mmol L^{-1} NaCl but some other appropriate solvents are also available. Additionally, they commonly contain

some auxiliary elements such as tonicity controlling agents and buffering agents like calcium chloride, potassium chloride, sodium acetate and sodium lactate (Huckriede *et al.*, 2005). Usually, virosome concentration as a vehicle is in the range of 20 and 200 mg mL^{-1}, although they can be enhanced depending on the characteristics of the virosome component and for a specific purpose (Cusi, 2006). Importantly, this formulation requires purification or sterilization before administration, through the classical process of liposomal sterilization, namely membrane filtration. Virosomes can be administered through a wide range of routes such as intravenous (IV), subcutaneous (SC), intramuscular (IM), intra-atrial, oral, topical or respiratory route and transdermally (Harandi and Medaglini, 2010; Jabbal-Gill, 2010; Beg, 2013). Interestingly, virosomes can be integrated in implantable devices for long-term therapy.

Mechanism of virosome as drug delivery

When the desired bioactive drug reached the targeted cell through a different route of administration, the first step is binding of virosomes with the host cell through HA1 glycoprotein to the terminal salic acid cell receptor. Additionally, virosomes surface sometimes combined with the fragments of Fab (Fragment antigen-binding) which are cross-linked with spacer arm for efficient binding. Apart from this, some virosomes recognize antigenic receptor on the surface of targeted cells and thus resulting in two different binding processes to the targeted cell.

Therefore, different virosomes exhibit different selectivity toward different types of targeted cells.

Following this, penetration will occur via receptor-mediated endocytosis. After binding of virosome with a surface of the host membrane, virosomes are entrapped inside the endosomes. HA2 viral glycoprotein help in the fusion of virosomes with the endosomal virosomal membrane.

Although, this fusion will occur in an acidic condition which is provided by endosomes. This fusion results in a release of bioactive drugs from the lipid bilayer of the virosome, as a result, the bioactive drug gets entry into the cytoplasm of the targeted cell (Nair *et al.*, 2020).

After penetration, the bioactive drug is delivered to the targeted cell. In the targeted cell, these bioactive drugs will act according to the function they perform. For instance, doxorubicin is known as an anticancerous drug and thus will act against human cancer cells.

Application of virosomes

Almost all types of drugs such as organic molecules, protein, peptides and nucleic acid have been successfully delivered through virosomal technology. Evidently, gelonin subunit A of diphtheria (a peptide chain) has been successfully delivered through a virosomal system (Bron *et al.*, 1994; Schoen, 1999; Bungener, 2002). Virosomes that are incorporated with malignant neoplastic drugs, malaria drugs, anti-bacterial drugs and anti-fungal drugs show promising effects in vivo and in vitro conditions (Cech, 2011; Krishnamachari, 2011; Cassone and Casadevall, 2012). Further, virosomes can be incorporated with different antibodies to enhance targeted cell delivery of drug due to the fact that antibody attaches to the particular target receptor.

The Virosomal system has shown promising results in the field of oncology since they deliver peptides that are corresponding to Tumour Associated Antigens (TAA). The peptide is obtained either from the parathyroid hormone-related protein (PTH-rP) or recombinant protein like HER-2/neu. Further, by combining anti-rat Neu (rat homologous of human HER2) monoclonal antibodies with virosomes has been selectively targeted rNeu overexpressing breast cancer.

Besides, enhancement can be made by combing anti-rat Neu mAb with doxorubicin (cytotoxic drug) that results in the formation of Fab'-Doxo-virosomes and thus merge the antiproliferation activities of monoclonal antibody and cytotoxic effect of doxorubicin (Bhattacharya and Mazumder, 2011).

A futuristic assembly of Decitabine (anti-cancerous drug) can be obtained by virosomal technology. Considering the fact that magnetic forces can be utilized to obtain virosomes, formulated anticancer drugs. Cinti and his team have successfully purified tailored erythrocytes (erythromagneto-hemagglutinin virosomes, EMHVs) incorporated with Decitabine, which resulted in the formation of a novel drug delivery system. EMHV drug delivery system has shown enhanced pharmacokinetics of decitabine and thus promotes a notable decrease in tumour mass in xenograft of prostate cancer at lesser concentration than the normal dose (Naldi *et al.*, 2014).

Virosomes can be incorporated with an antimalarial peptide which shows promising acceptability and is highly accepted by the human immune system. NPNA and AMA-1 are two peptide regions that can serve as the antigen for the formation of the vaccine (Miyanohara, 2012).

Ebola virosomes were formed with a recombinant baculovirus vector system in the cell of insects; further, its efficacy on Ebola virus disease (EVD) was investigated in mice.

Interestingly, Ebola virosomes shows promising result in infected mice with Ebola infection (Bengtsson *et al.*, 2016).

Virosomes can also perform adjuvant and carrier functions. The carrier function shows the conclusive effect of incorporating the antigen into virosomes. The adjuvant function of virosomes depends on the HA present on virosomes and shows enhanced immune properties through stimulation of innate immune cells and the release of cytokines.

Prospect of virosomes

Virosomes have shown a novel way for targeted delivery of drugs to a different part of the body. The tailoring of a virosomal viral envelope for targeting specific tissue or cell will certainly revolutionize the drug delivery system (Table 2). However, research is required for understanding their pharmacological profiles, clinical result and stability, further figuring out the way for long-term authenticity as a secure, efficient and cost-effective drug delivery product.

CONCLUSION

In the wake of developments in all fields of biotechnology, we have millions of drugs available, but the rising side effect and toxicity caused by many drugs poses a great problem. These side effects occur when the drugs are misdirected to some other organs they are not desired to reach. In other cases, there can be reduced efficacy of drugs if they do not reach their target but are degraded in the gut, in the liver or excreted out quickly. To overcome all these issues, a promising method of targeted virosomal drug delivery is under research and development. It is virtually an extremely useful method of delivering the drugs to its specific target while leaving them protected from degradation and at some time not being misdirected to other non-specific organs, thus reducing the side effects of drugs. Since viruses has been known to us for ages and can be cultivated in labs easily and thus, we can exploit their property of being specific to their target by some simple modifications of viruses as mentioned in the review. A variety of drugs can be delivered by this method for a large number of diseases, but the subject needs more research and data to emerge as a predominant choice of drug delivery system.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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REFERENCES

Almeida, J. D. 1975. Formation of Virosomes from Influenza Subunits and Liposomes. *The Lancet*, 306(7941):899–901.

Asano, A., Asano, K. 1982. Viral proteins in cell fusion. *The Tokai journal of experimental and clinical medicine*, (7):193–196.

Beg, S. 2013. Colloidal Drug Delivery Systems in Vaccine Delivery. *Current Drug Targets*, 14(1):123–137.

Bengtsson, K. L., *et al.* 2016. Matrix-M adjuvant enhances antibody, cellular and protective immune responses of a Zaire Ebola/Makona virus glycoprotein (GP) nanoparticle vaccine in mice. *Vaccine*, 34(16):1927–1935.

Bhattacharya, S., Mazumder, B. 2011. Virosomes: A novel strategy for drug delivery and targeting. *Bio-Pharm International*, 24(1 Supple 1).

Blom, R. A. M. 2017. Pulmonary delivery of virosome-bound antigen enhances antigen-specific CD4+ T cell proliferation compared to liposome-bound or soluble antigen. *Frontiers in Immunology*, 8:359.

Bron, R., Ortiz, A., Wilschut, J. 1994. Cellular Cytoplasmic Delivery of a Polypeptide Toxin by Reconstituted Influenza Virus Envelopes (Virosomes). *Biochemistry*, 33(31):9110–9117.

Bungener, L. 2002. Virosome-mediated delivery of protein antigens to dendritic cells. *Vaccine*, 20(17-18):2287–2295.

Cassone, A., Casadevall, A. 2012. Recent progress in vaccines against fungal diseases. *Current Opinion in Microbiology*, 15(4):427–433.

Cech, P. G. 2011. Virosome-formulated plasmodium falciparum AMA-1 and CSP derived peptides as malaria vaccine: Randomized phase 1b trial in semi-immune adults and children. *PLoS ONE*, 6(7):e22273.

Chang, H. I., Yeh, M. K. 2012. Clinical development of liposome-based drugs: Formulation, characterization, and therapeutic efficacy. *International Journal of Nanomedicine*, 7:49–60.

Cornet, B., *et al.* 1990. Virosomes reconstituted from Human Immunodeficiency Virus proteins and lipids. *Biochemical and Biophysical Research Communications*, 167(1):222–231.

Curran, J., Kolakofsky, D. 1999. Replication of paramyxoviruses. *Advances in virus research*, 54:403–422.

Cusi, M. G. 2006. Applications of influenza virosomes as a delivery system. *Human Vaccines*, 2(1):1–7.

De Jonge, J. 2006. Use of a dialyzable short-chain phospholipid for efficient solubilization and reconstitution of influenza virus envelopes. *Biochimica et Biophysica Acta-Biomembranes*, 1758(4):527–536.

Felnerova, D., *et al.* 2004. Liposomes and virosomes as delivery systems for antigens, nucleic acids and drugs. *Current Opinion in Biotechnology*, 15(6):518–529.

Fukuyama, S., Kawaoka, Y. 2011. The pathogenesis of influenza virus infections: The contributions of virus and host factors. *Current Opinion in Immunology*, 23(4):481–486.

Glück, R. 1992. Immunopotentiating reconstituted influenza virus virosome vaccine delivery system for immunization against hepatitis A. *Journal of Clinical Investigation*, 90(6):2491–2495.

Harandi, A., Medaglini, D. 2010. Mucosal Adjuvants. *Current HIV research*, 8(4):330–335.

Hatz, C. 2011. Real-life versus package insert: A post-marketing study on adverse-event rates of the virosomal hepatitis A vaccine Epaxal® in healthy travellers. *Vaccine*, 29(31):5000–5006.

Huckriede, A., *et al.* 2005. The virosome concept for influenza vaccines. *Vaccine*, 23(Supple 1):26–38.

Jabbal-Gill, I. 2010. Nasal vaccine innovation. *Journal of Drug Targeting*, 18(10):771–786.

Kalra, N., *et al.* 2013. Virosomes: As a Drug Delivery Carrier. *American Journal of Advanced Drug Delivery*, 1(1):29–35.

Kammer, A. R. 2007. A new and versatile virosomal antigen delivery system to induce cellular and humoral immune responses. *Vaccine*, 25(41):7065–7074.

Kaneda, Y. 2002. Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system. *Molecular Therapy*, 6(2):219–226.

Kapczynski, D. R., Tumpey, T. M. 2003. Development of a virosome vaccine for Newcastle disease virus. *Avian Diseases*, 47(3):578–587.

Kára, J., Mach, O., Černá, H. 1971. Replication of rous sarcoma virus and the biosynthesis of the oncogenic subviral ribonucleoprotein particles ("virosomes") in the mitochondria isolated from rous sarcoma tissue. *Biochemical and Biophysical Research Communications*, 44(1):162–169.

Krishnamachari, Y. 2011. Nanoparticle delivery systems in cancer vaccines. *Pharmaceutical Research*, 28(2):215–236.

Liu, H. 2015. Virosome, a hybrid vehicle for efficient and safe drug delivery and its emerging application in cancer treatment. *Acta Pharmaceutica*, 65(2):105–116.

Lund, P. E., *et al.* 2010. Pseudovirions as vehicles for the delivery of siRNA. *Pharmaceutical Research*, 27(3):400–420.

Mastrobattista, E. 2001. Targeting influenza virosomes to ovarian carcinoma cells. *FEBS Letters*, 509(1):71–76.

Mischler, R., Metcalfe, I. C. 2002. Inflexal® V a trivalent virosome subunit influenza vaccine: production. *Vaccine*, 20(Supple 5):17–23.

Miyanohara, A. 2012. Preparation of vesicular stomatitis Virus-G (VSV-G) conjugate and its use in gene transfer. *Cold Spring Harbor Protocols*, 7(4).

Moghimi, S. M., Szebeni, J. 2003. Stealth liposomes and long circulating nanoparticles: Critical issues in pharmacokinetics, opsonization and protein-binding properties. *Progress in Lipid Research*, 42(6):463–478.

Morein, B., *et al.* 1978. Effective subunit vaccines against an enveloped animal virus. *Nature*, 276(5689):715–718.

Nair, C. S., Sreelekshmi, A. S., Rahiman, C. A. A. A., Krishnan, K., Gopika, G., Nair, T. P., Ajit, U. G. 2020. Virosomes: A groundbreaking revolution in novel drug delivery. *International Journal of Research in Pharmaceutical Sciences*, 11(4):5809–5820.

Naldi, I., *et al.* 2014. Novel epigenetic target therapy for prostate cancer: A preclinical study. *PLoS ONE*, 9(5):1–13.

Nishimura, Y., *et al.* 2013. Targeting cancer cell-specific RNA interference by siRNA delivery using a complex carrier of affibody-displaying bio-nanocapsules and liposomes. *Journal of Nanobiotechnology*, 11(1):19.

Noda, T., Kawaoka, Y. 2010. Structure of influenza virus ribonucleoprotein complexes and their packaging into virions. *Reviews in Medical Virology*, 20(6):380–391.

Okada, Y. 1993. Sendai Virus-Induced Cell Fusion. *Methods in Enzymology*, 221(C):18–41.

Schoen, P. 1999. Gene transfer mediated by fusion protein hemagglutinin reconstituted in cationic lipid vesicles. *Gene Therapy*, 6(5):823–832.

Shaikh, S. N., *et al.* 2019. Overview on virosomes as a novel carrier for drug delivery. *Journal of Drug Delivery and Therapeutics*, 8(6-s):429–434.

Sharma, R., Yasir, M. 2010. Virosomes: A novel carrier for drug delivery. *International Journal of PharmTech Research*, 2(4):2327–2339.

Skehel, J. J., Wiley, D. C. 2000. Receptor binding and membrane fusion in virus entry: The influenza hemagglutinin. *Annual Review of Biochemistry*,

69:531–569.

Stegmann, T. 1987. Functional reconstitution of influenza virus envelopes. *The EMBO journal*, 6(9):2651–2659.

Suarez, D. L., Schultz-Cherry, S. 2000. Immunology of avian influenza virus: A review. *Developmental and Comparative Immunology*, 24(2-3):269–283.

Takimoto, T. 2002. Role of the Hemagglutinin-Neuraminidase Protein in the Mechanism of Paramyxovirus-Cell Membrane Fusion. *Journal of Virology*, 76(24):13028–13033.

Uchida, T. 1979. Reconstitution of lipid vesicles associated with HVJ (Sendai virus) spikes. Purification and some properties of vesicles containing nontoxic fragment A of diphtheria toxin. *Journal of Cell Biology*, 80(1):10–20.

Wegmann, N. 2002. Targeting HER-2/neu with anti-rat neu virosomes for cancer therapy. *Cancer Research*, 62(2):437–444.

Yamada, T., *et al.* 2003. Nanoparticles for the delivery of genes and drugs to human hepatocytes. *Nature Biotechnology*, 21(8):885–890.

Zurbriggen, R. 2003. Immunostimulating reconstituted influenza virosomes. *Vaccine*, 21(9):921–924.