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Virosomes-Hybrid Drug Delivery Systems

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Abstract

The development of safe and effective models for the delivery of any prophylactic or therapeutic agent remains an uphill task for pharmaceutical formulation developers. Drug molecules, nucleic acids, carbohydrates, proteins and a variety of other biological and chemical entities are used for attaining pharmacological benefits. However, the major challenge remains in the delivery of these agents to the specific site of action in a time-efficient manner. Among the many drug delivery systems developed, the nano scale technology of virosomes tends to present an established system of delivering the therapeutic agents to the site of pharmacological action. Virosomes are lipid bilayer, unilamellar structures that present viral protein on their surface. They are safe, biocompatible and biodegradable structures that can achieve ideal pharmacological profile once administered into the body. Tissue targeting, immune activation and potentiation are the chief advantages that make them efficient prophylactic and therapeutic agents. The review presents the biopharmaceutical applications of virosomes and the immunological and pharmaceutical considerations that make them efficient agents for targeting spatiotemporal parameters in the body.

Keywords: Virosome; Targeted drug delivery; Virus vaccine; Vaccine adjuvants; Virosome production

Abbreviations: APCs: Antigen Presenting Cells; F: Fusion protein; FDA: United States Food and Drug Administration; HA/ HN: Hemagglutinin/ Hemagglutinating protein; IgA/G/M: Immunoglobulin A/G/M; MHC: Major Histocompatibility Complex; NA: Neuraminidase

Introduction

Virosomes are lipid-based, synthetic vesicles comprising of viral surface glycoproteins. The presence of these viral peplomers assists the recognition and attachment of these entities specifically to their target cells. A number of properties of virosomes make them a promising candidate for targeted drug and antigen delivery. These vesicles have a central cavity that can incorporate a variety of therapeutic agents including drug molecules, nucleic acids and proteins. The lipid bilayer of the vesicle prevents these molecules from the physicochemical and biological adverse reactions in the body. Moreover, due to the presence of antigenic viral proteins on the surface, these vesicles can serve as safe and effective vaccine and adjuvant models. These characteristics can be employed for attaining clinical benefits in a variety of health conditions. The paper discusses the biopharmaceutical and immunological aspects of virosome technology.

Applications of Virosome Technology

Viruses are obligate intracellular parasites as they are necessarily dependent upon specific host cells for their survival. This principle leads to the development of a drug delivery system that mimics the viral pattern of cellular infection [1]. Virosomes are composed of a phospholipid bilayer with the viral surface glycoproteins protruding from the surface of these vesicles [2-4]. The composition of the vesicular membrane enables the virosomes to be biocompatible and biodegradable. They are efficiently absorbed and distributed to the target site without being altered by the physiological processes of the body. Moreover, the formulation and composition of virosomes is such that drug molecules of diverse nature can be incorporated in them. The lipid bilayer can easily integrate the hydrophobic drugs in it. Hydrophilic drugs, on the other hand, become a part of the central lacunae [5,6]. Figure 1 represents the basic assembly of a virosome [7]. In order to attain the efficient delivery of the virosomes, the size and surface properties of the virosomes can be altered [4]. Alteration in these properties can help in achieving varied, yet controllable, biopharmaceutical properties. Virosomes do not contain large quantities of preservatives and can be prepared without the involvement of any complicated techniques [8].

Virosomes can be coupled to an antibody to ensure the targeted delivery of a therapeutic agent in order to enhance the tissue specificity. These antibodies bind to the specific receptors of cells aiding the delivery of drug molecules to these targets. This property can, especially, be utilized for carrying the drug molecules with narrow safety profiles. Cancer chemotherapeutic agents, for instance, can be delivered specifically to the tumors by labeling the virosomes with antibodies [9,10]. Virosomes have shown to effectively transport macromolecules including drugs, nucleic acids and proteins to various cell types including hepatocytes, erythrocytes, immune cells and glioma cells [1,11-13].

Apart from the targeted delivery of therapeutic agents, virosomes preserve the stability and activity of active agents. Therapeutic molecules get degraded by the endosome lysosomal degradation mechanisms before reaching the target cells in case conventional drug delivery procedures are utilized [14]. Virosomes can, however, deliver these agents to the target cells without being affected by the host defense mechanisms. Once the virosome-lysosome membranes fuse, the drug molecules are delivered to the cell to exhibit their therapeutic effects. Adequate localization of drugs, nucleic acids and proteins in various sub cellular compartments has been observed in a number of studies using the virosome technology.

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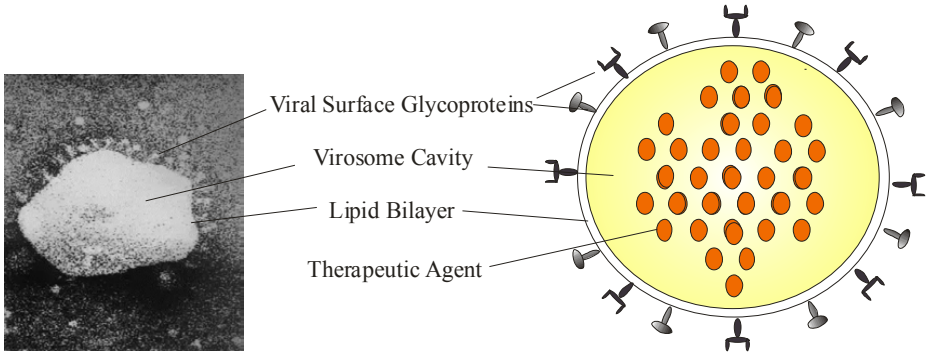

Figure 1: An overview of the structural composition of a virosome comprising of influenza virus surface glycoproteins in comparison to an electron micrograph (Glück and Metcalfe 2002).

A chief concern of the virosome based approach is the induction of immune response against the viral glycoproteins [15,3]. This property is detrimental as it can result in their rapid detection by the immune system resulting in the early clearance of the virosomes from systemic circulation [16]. However, these proteins can help in inducing a prophylactic response against the virus. This property establishes their candidature as vaccine and immunological adjuvants. Another issue associated with the virosome use is the rapid disintegration in the blood compartment [17]. However, if they can reach the site of action within a short time of administration in the body, efficient drug delivery can be predicted. Furthermore, once inside the cells, the virosomes do not replicate, ensuring their safety profile [18,14].

A number of virosome based products have been approved by the United States Food and Drug Administration (FDA) for human use [19,20]. Table 1 represents various therapeutic agents based upon virosome technology [21-27]. The surface glycoproteins of Influenza virus, hepatitis viruses and vesicular stomatitis virus have been successfully incorporated in a number of vaccine and drug delivery systems [28-31]. Virosomes containing cancer chemotherapeutic agents, antimalarial, antibacterial and antifungal agents have shown efficient release profiles *in vitro* and *in vivo* [32-35]. Based upon the same principle, bacterial ghosts have been developed [36]. These vesicles comprise of the outer shell or the envelop protein of various gram negative bacteria [37]. These bacterial ghosts mimic a similar pattern as is observed in case of a natural infection. The virosome based drug delivery is, however, rapid, safe and effective as opposed to other related systems.

Virosomes as Immunopotentiating Agents

Virosomes are the agents that can serve the function of delivering antigens and drugs to specific cell types. The chief property exploited by the virosome design is the interaction between the antigenic proteins of the virus with the cellular receptors [38]. Moreover, the identification, uptake and representation of the antigen incorporated in the virosome by the relevant antigen presenting cells helps in stimulating the immune system. As a result efficient regulatory and effector immune responses are generated [1,15]. They initiate both cell mediated and humoral arms of immune system [39,40]. Additionally, virosomes induce both cytotoxic and helper T-cell responses [41,42].

The first exposure of the virosomes to the immune system occurs at the site of administration. In case they are administered into the body through a mucosal route (oral, respiratory, vaginal or rectal) the localized immune system is activated [43]. The antigen is taken up and processed by the follicular dendritic cells and other antigen presenting cells (APCs) in the surrounding tissues [40,44]. The B and T lymphocytes in the mucosal tissue are directed to produce antigen specific immunity. In addition to this, the humoral arm utilizing the immunoglobulins IgG, IgM and IgA is also activated [45]. If, however, administered into the systemic circulation, virosomes are primarily exposed to the process of phagocytosis [39]. After the antigen processing, it is presented on the surface of the antigen presenting cells and is, then, available for generation of cell-mediated and humoral immune response. The proteins incorporated into the virosomal assembly have been associated with the induction of a number of inflammatory cytokines [46]. An increase in the inflammatory response ensures the rapid presentation and processing by the required immune machinery. However, if the antigen is incorporated into the virosome body, it is released inside the cell. The same essential steps of immune activation follow the antigen processing.

Virosomes cannot only serve as a means to transfer the immunogen to the body but can also act as adjuvants for directing the immune response to the particular antigen [47]. They, being of particulate nature, can easily attract the dendritic cells and other antigen presenting cells for attaining immunological benefits [48]. The composition of the virosome ensures that the antigen, whether intercalated into the lipid bilayer, conjugated to the surface proteins or present in the central cavity, is delivered continuously in a sustained manner to the immune system [49]. This delay in the release of the antigen can act as a tool for focusing the immune response to the particular antigen in order to gain a depot-like effect [50]. Furthermore, the combined delivery of the antigen and the adjuvant can help in the attainment of an exaggerated immune protection against various diseases. Recent studies on murine models have exhibited up to four-fold improved humoral response in case of virosome based product in comparison to that observed on the delivery of nascent antigen [51].

Virosomes as Agents of Targeted Drug Delivery

One of the important prerequisites of a drug delivery system is to transport a therapeutic agent effectively to the target site in a timely manner. In order to aid the targeted drug delivery, drugs need to be either modified or packaged in such a manner that therapeutically effective quantities of drug molecules reach the site of action. Modification might involve the alteration of physical and/or chemical parameters of the drug resulting in the production of new chemical entities, mixing with other chemical constituents to modify their *in vivo* release profiles or alteration of physical structures of the drug molecules [52]. These processes, ultimately, necessitate the involvement of certain biological

Therapeutic/ Prophylactic Purpose	References
Influenza virus vaccine	[21]
Hepatitis A virus vaccine	[22]
Hepatitis B virus vaccine	[23]
Hepatitis C virus vaccine	[24]
Antifungal agents	[25]
Cancer chemotherapy	[26]
Antiparasitic agents	[27]

Table 1: Virosome Based Products Approved and under review by regulatory authorities.

parameters for exhibiting the drug action in the body. On the contrary, packaging of molecules in suitable vesicles can result in the transport of the parent drug to the intended site of action without involving any biological process [53].

Virosomes can package drugs of a variety of nature in themselves [40]. They can serve as excellent means to deliver hydrophilic and hydrophobic drug molecules to a specific type of tissue [13]. The water-loving or hydrophilic drugs are encapsulated in the central compartment during the virosome production process. The lipophilic drugs, on the other hand, cannot be encapsulated in this manner and are, therefore, embedded in the lipid bilayer. The slow disintegration and dissolution of the virosomes within the cell can serve as a means of delivering these drug molecules to the intended site of action. The encapsulation of various forms of genetic material in the virosome, to be used for prophylactic or therapeutic purposes, has been achieved in a number of studies [54]. The lipid bilayer of the virosome helps in the protection of these therapeutic agents from various nucleic acid degrading enzymes including DNAases and RNAases [55]. The viral glycoproteins after recognizing the specific cell types help in the fusion of the membranes. The genetic material once delivered can, then, be utilized by the cellular machinery for the production of the encoded genes [56].

A combination of proteins is utilized in order to enhance the targeted drug delivery of the virosome. In general one of the viral protein aims at the attachment of the virosome to the target cell while the other viral protein helps in the fusion of the virosome membrane with the cellular membrane. For instance, in case of influenza virosomes, hemagglutinin is involved in the very specific interaction of the viral assembly to the host-cell membrane [57,48]. An additional protein, neuraminidase is also incorporated to help in the release of the virus from the cell and aiding its entry into the cell. Similarly, in case of Hemagglutinating virus of Japan (HVJ) based virosomes, two proteins, HN and F, are incorporated into the surface [58]. HN or the Hemagglutinating protein is involved in the cell recognition and attachment mechanism while the F or fusion protein induces the fusion of the two membranes. An important consideration is the amount of the drug that can be delivered to the target cells. Virosomes of varied sizes can be produced to incorporate a variety of doses. The ultimate size of the virosome produced depends upon the nature of the viral proteins and the composition of the phospholipid bilayer. Influenza virosomes generally measure 150-200 nm while the HVJ based virosomes have a mean diameter of 400-500 nm [59,60]. This variation in size of various virosomal preparations can act as a possible avenue for the delivery of a specific quantity of a macromolecule to the target cell. Moreover, these molecules can be protected from the endosome lysosomal system from degradation, ultimately, providing a means to safely deliver the therapeutic agent to the intended site of action. It has been demonstrated that the virosome based product can be delivered, intracellularly, with up to three time's greater efficiency as compared to that delivered by using the conventional methods [61].

Virosome-Cell Interaction

The chief advantage of the virosome technology is their capability to simulate an *in vivo* infection state that can be helpful in attracting the immune players and the provision of macromolecules to the respective site of action. Virosomes recognize and bind to the same receptors that are utilized in case of a natural viral infection. Sialic acid receptors, for instance, are utilized by the influenza virosomes [62]. After the cell receptor recognition by the virus, fusion of viral and endosomal membrane is observed [38]. In case of influenza virosomes, for example, the hemagglutinin (HA) viral protein utilizes its dipartite assembly for the same purpose [63]. In addition, the neuraminidase (NA) is also included in the virosome assembly as it can enhance the immunogenicity and targeting of the virosome to a particular tissue. Figure 2 represents the mechanism of virosome interaction with the cell surface receptors and its fate in the cell.

Apart from specificity of cell surface interaction, a major advantage of virosomes is their capability to enhance antigen presentation. Once the virosomes have been administered into the body, both the classes of major histocompatability complex, MHC I and MHC II can be stimulated [48]. However, the exact choice of the MHC to be activated depends upon the nature of the antigen associated with the virosome and its ultimate interaction with the Antigen Presenting Cells (APCs). Subsequently, the antigen interacts with the endosome, activating the helper T-cell mediated immune response. To the contrary, exogenous antigens are transported to APCs for the activation of cytotoxic T-cell response. B cells are also induced by the APCs through the activation of interleukins and other chemokines. The virosomes can, therefore, activate both the humoral and cellular arms of the immune system of the body. Vesicles containing the surface glycoprotein of the Hemagglutinating Virus of Japan (HVJ), for instance, have been shown to exhibit sustainable patterns of immune response on repeated administration of these virosomal units [64].

The virosome-receptor interaction has been investigated for the treatment of a number of diseases including parasitic diseases, viral diseases, neurological disorders and many other metabolic disorders [11,65]. In all the cases, the main aim is the provision of a nano-sized protein, nucleic acid or a drug molecule to the intended site of action. Peptides and proteins have been very successfully conjugated with the virosome-surface glycoproteins. Vaccines have been developed against the Respiratory Syncytial Virus (RSV) using the influenza virosomes [14]. By fusing the conserved proteins of the Hepatitis C virus surface proteins with the virosomal proteins positive induction of cytotoxic T-cell immune response. Similarly, epitopic regions of B-cell have been devised using influenza virosomes specifically against malaria [66]. Additionally, multiple pathogens have been targeted using the virosome system. This mechanism, therefore, helps in avoiding repeated and multiple dosing for immunization purposes.

The interaction of the virosome with a particular cell is dependent upon a number of physical and chemical factors. The size of the virosomes is of prime importance in this respect. It can be adjusted by altering the phospholipid and protein composition of the vesicle. The size attained by the virosome should be ideal for uptake by the receptor mediated endocytosis process [38]. They can only then be involved in the activation of humoral and cell-mediated arms of the immune system. After reaching a particular tissue, the virosome can provide prolonged residence time in a particular organ or tissue before being,

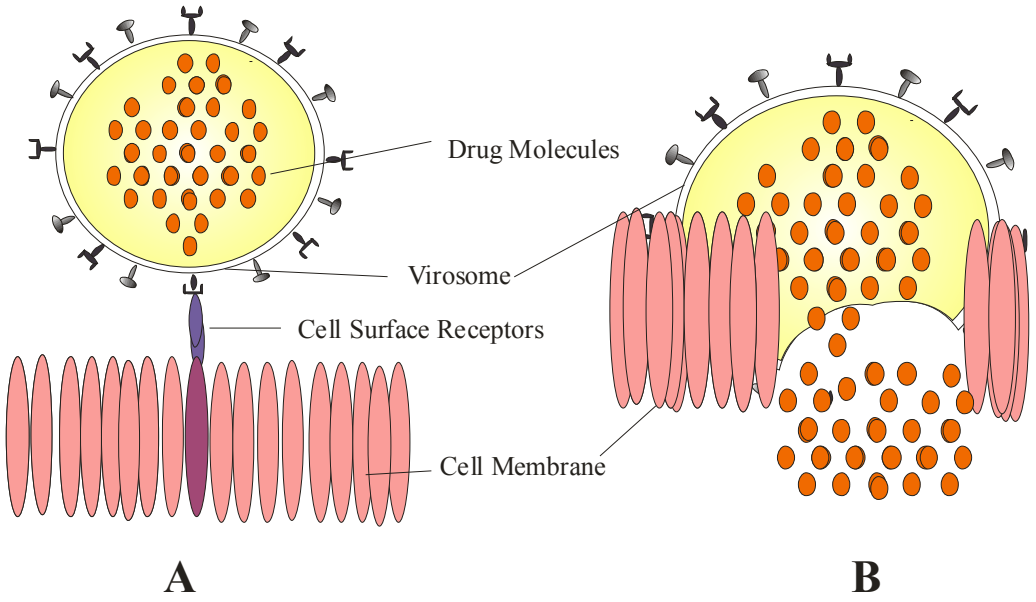

Figure 2: Mechanism of drug and genetic material delivery to the target cell using virosome technology. A. Interaction of the virosomes with cell surface receptors. B. Release of the encapsulated drug molecules in the target cell.

ultimately, decreased in size and draining to the associated lymphoid tissue [67,68].

Synthesis and Bioprocessing of Virosomes

The general composition of liposomes and virosomes is essentially the same. Both are composed of a lipid bilayer enclosing a cavity that can be utilized for carrying a drug, nucleic acid or other similar entity to the target site. The main problem associated with the liposomes is their rapid clearance by the reticuloendothelial system of the body [34]. A number of modifications have been proposed by the researchers in order to deal with these issues including the coating of liposomes with a chemical that can help the liposome to act as a stealth vesicle, thereby, preventing the detection from the immune system of the body. Another more useful method is the labeling of the synthetically prepared vesicle by a ligand or an antibody that can function as a marker for the detection and ultimate transport to the target site [69]. Virosomes, exploit this mechanism for demonstrating their function. The composition of the virosomes is such that it incorporates a lipid bilayer, much similar to the liposomal membrane, to enhance the interaction and passage through the biological membranes. However, a major step prior to ensuring the interaction between the virosome and the cell membrane is the delivery to the target tissue or organ. In order to achieve this target, viral proteins that are involved in the cell fusion process are incorporated into the lipid bilayer. The drug or the nucleic acid to be delivered to a specific site is added into the formulation resulting in the transfer of the molecule to the respective site of action. Moreover, virosomes tend to exhibit the viral glycoproteins on their surface; this further enhances the presentation of the viral proteins to the immune system resulting in their easy detection and presentation for enhancing the immunization against the encoded viruses.

During the formulation of virosomes, the exact nature and quantity of the individual components has to be optimized according to the intended purpose of use. In general, lecithin, phosphatidyl choline and phosphatidyl ethanolamine are considered the basic components for the production of stable virosome structures [70-72]. The viral proteins usually intercalated into the lipid bilayer structure are the surface glycoproteins of viruses. These proteins are generally involved in the fusion of the viral envelop with the host-cell membrane, thereby providing the virosome an opportunity to deliver the drug molecule or any other ligand into the cell. The complete virosome assembly is generally spherical in shape, with viral proteins protruding out of the surface. In order to produce the virosomes, virus is inactivated using various membrane degrading agents. This results in the extraction of viral glycoproteins [49]. At this stage detergents are added into the viral protein-phospholipid mixture in order to enhance the interaction between the two, otherwise, immiscible agents [57]. The lipid bilayer is then self-assembled resulting in the production of virosomes. This pattern not only helps in the formation of vesicular entities but also provides an opportunity for the incorporation of drugs of both hydrophilic and lipophilic nature into the system [70]. The hydrophilic drugs are added in to the solvent system while the hydrophobic drugs are added into the phospholipid mixture to ensure the solubility of these compounds.

The incorporation of the viral glycoproteins not only helps in the targeting of certain tissues but also aid the stabilization of the virosomal assembly. This property, therefore, enhances the ease of detection and, hence, the antigen presentation to the immune system. Likewise, the exact quality and quanitity of each of the different phospholipids incorporated into the virosomal preparation is evaluated. The trapping efficiency is increased by making larger virosomes that can help in entrapment of larger quantities of drugs and other macromolecular structures. A number of preparations of virosomes intended for administration through oral, parenteral, topical and respiratory routes [73-76]. The main target after the administration of a virosomal preparation is to attain therapeutically effective levels of the preparation in the blood. Once these vesicles are in systemic circulation, they can reach the specific tissue and help in yielding the desired therapeutic effects. Additionally, virosomes can be used for the transfer of DNA fragments of up to 100kb [77]. Antibodies labeling using various families of immunoglobulin (IgG and IgM) have also been exhibited

in various virosomal assemblies. Virosomes have also been labeled using monoclonal antibodies (mAbs) and tissue specific monoclonal antibody fragments (F_{ab}). Virosomes delivered after labeling have been observed to attain efficient pharmacological profile. Apart from their stability, a major factor is the distribution to various tissues that are to be targeted by any therapeutic agent. Erythrocytes, hepatocytes, tumor cells and cells of the respiratory and gastrointestinal system have been targeted using the technique [41,71,72].

Large scale production of virosomes follow batch processing phenomenon. The viral antigens after extraction are treated with phospholipids in the presence of a detergent. The product is, then, subjected to various purification and sterilization mechanisms. The finished product is usually suspended in a buffer solution in order to stabilize the formulation. Sodium chloride, potassium chloride and other salt formulations are generally used for the purpose [78,79]. This process makes the virosome not only stable but also safe for administration into the body. In order to evaluate the virosomes in a preparation, simple quantitative analysis is performed that are specific to the viral proteins. Electron microscope based analysis can be performed for determining the structure and size of the virosome. The individual tests performed for the analysis of the virosome vary depending upon the nature of the substance to be evaluated. Viral proteins are detected by relatively simpler tests including sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE). Fusion activity can be determined using the fluorescent resonance energy transfer assay (FRET) and other cell binding assay procedures [29,80,81]. The product, after being characterized and tested, is available for administration through an appropriate route.

Future Directions

Virosomes tend to present a novel, yet established, drug delivery system. A number of virosome based products are currently available in the market to fulfill the prophylactic, therapeutic and diagnostic functions. Virosome based vaccines can serve as the function of actively inducing an immune response. Similarly, they can serve as ideal candidates for serving as an adjuvant. Possessing the capability to carry macromolecules, virosomes are ideal agents to serve as drug delivery systems. These drug delivery platforms have ideal pharmacokinetic and pharmacodynamics properties ensuring the safe and effective means to exploit the therapeutic properties of a drug molecule. However, variation in the therapeutic response observed in case of a virosome based therapy hinders its acceptance as a mainstream drug delivery system. The fusion capability of the virosome varies with the number of viral proteins available on the surface of the virosomes. Moreover, the batch processing and complicated assay procedures impede the usefulness of virosomes. Efforts, therefore, need to be made in this respect to develop easy assay procedures. Addressing these concerns would certainly ensure the approval and availability of a greater number of virosome-based biopharmaceuticals.

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