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Review Article

VIROSOMES AS NEXT-GENERATION NANOCARRIERS: ADVANCES, CHALLENGES, AND FUTURE PROSPECTS

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ABSTRACT

Virosomes are virus-like nanocarriers that combine the structural advantages of viral envelopes with the safety of liposomes. By retaining viral glycoproteins such as hemagglutinin and neuraminidase but lacking genetic material, virosomes mimic natural viral entry while avoiding replication. This enables efficient, targeted delivery of vaccines, chemotherapeutics, and nucleic acids. Compared with conventional drug delivery systems, virosomes offer high biocompatibility, the ability to encapsulate both hydrophilic and hydrophobic drugs, and pHsensitive release. They have demonstrated clinical success in licensed vaccines such as Inflexal® V and Epaxal®, and ongoing research highlights applications in oncology, antimicrobial therapy, and gene delivery. Despite these advantages, challenges remain in largescale manufacturing, long-term stability, immune system recognition, and regulatory approval. Future directions include personalized medicine, combination therapies, CRISPR/Cas delivery, and integration with imaging agents for theranostic platforms. Overall, virosomes represent a versatile and promising next-generation nanocarrier technology with the potential to significantly advance targeted therapeutics and vaccination strategies.

Keywords: Virosomes, Targeted drug delivery, Vaccines, Nanocarriers, Oncology, CRISPR, Theranostics

1. INTRODUCTION

1.1 Brief overview of targeted drug delivery

Targeted drug delivery is a strategy designed to transport therapeutic agents specifically to diseased tissues or cells, thereby enhancing efficacy and minimizing systemic toxicity [1,3]. Carrier-based systems such as nanoparticles, liposomes, and virosomes can be engineered with specific physicochemical properties and ligands to recognize target cell receptors, enabling selective uptake and controlled release of the payload [1,3]. Nanovaccine platforms further improve delivery efficiency by optimizing particle size, surface characteristics, and adjuvant properties, thereby boosting antigen presentation and immune activation [3]. Such targeted approaches are particularly useful for drugs with poor bioavailability or requiring intracellular delivery [2,3].

1.2 Limitations of conventional delivery systems

Traditional drug delivery methods, including oral and parenteral routes, often suffer from poor target specificity, rapid clearance, enzymatic degradation, and first-pass metabolism [4,5]. These limitations result in reduced therapeutic efficiency and increased dosing frequency. Natural bioactive compounds like curcumin exhibit low water solubility and instability, limiting their clinical application unless incorporated into advanced delivery systems [2]. Similarly, crude plant extracts with demonstrated in vitro antiviral activity may show limited in vivo efficacy without strategies that enhance stability and site-specific delivery [4]. Certain small polar molecules, such as fructose-1,6-diphosphate, require large systemic doses to overcome metabolic consumption and achieve therapeutic levels at the target site [5].

1.3 Virosomes - definition and general mechanism

Virosomes are non-replicating, reconstituted viral envelopes composed of a phospholipid bilayer incorporating functional viral glycoproteins such as haemagglutinin and neuraminidase [1,2]. These glycoproteins enable receptor recognition, membrane fusion, and intracellular delivery of the encapsulated therapeutic payload [1,2]. Virosomes can carry hydrophilic agents within their aqueous core or lipophilic drugs within their membrane, offering versatility for drug and vaccine delivery. Their ability to mimic viral entry mechanisms allows efficient endosomal escape and cytoplasmic

release, making them valuable tools for targeted therapy and immunization [1,3].

1.4 Historical background & first uses

The concept of virosomes was introduced following the development of techniques to reconstitute viral envelopes while removing their genetic material, maintaining fusion capability but eliminating replication potential [1]. Influenza virus-derived virosomes were among the earliest to be studied and later used as vaccine carriers, leading to licensed formulations such as Epaxal® (hepatitis A) and Inflexal® V (influenza), which demonstrated high immunogenicity and safety [1]. Over time, virosomes have been adapted for targeted delivery of chemotherapeutics, natural compounds, and genetic material [2]. Modern innovations, such as hybrid virosomes containing nanocurcumin, show improved drug stability, bioavailability, and therapeutic performance [2,3].

1.5 Objective of this review

This review aims to summarize the principles, advantages, and applications of virosome-based drug delivery systems, discuss their mechanisms and historical development, and highlight recent advances that expand their potential in targeted therapy and vaccine delivery.

2. Structure and Composition of Virosomes

Virosomes are reconstituted, non-replicating viral envelopes that preserve the morphology and functional surface architecture of the parent virus but lack genetic material, ensuring they cannot replicate [6–8]. They consist of a phospholipid bilayer embedded with viral glycoproteins, most commonly hemagglutinin (HA) and neuraminidase (NA) derived from influenza virus.

2.1 Lipid Bilayer

The virosomal membrane is composed of a **phospholipid bilayer** that mimics the viral envelope. Natural lipids such as phosphatidylcholine or synthetic analogues are often used, with cholesterol added to enhance membrane stability and rigidity [6]. The bilayer provides amphiphilic properties, enabling encapsulation of hydrophilic drugs or antigens within the aqueous core and incorporation of lipophilic molecules into the lipid phase [6,8]. Lipid composition can be tailored to adjust membrane fluidity, fusogenic potential, and stability during storage.

2.2 Viral Glycoproteins

Virosomes retain the surface glycoproteins of the parent virus in their native conformation, most commonly:

Hemagglutinin (HA) — Binds to sialic acid receptors on host cells and undergoes pHdependent conformational changes that facilitate membrane fusion within endosomes.

Neuraminidase (NA) — Cleaves sialic acids to promote particle release and prevent aggregation during production [6,8].

These glycoproteins are essential for receptor recognition and intracellular delivery, making virosomes highly efficient carriers for targeted delivery of drugs, vaccines, and genetic material.

2.3 Absence of Viral Genetic Material

A defining feature of virosomes is the **complete absence of viral nucleic acids**-either RNA or DNA-achieved through detergent solubilization and genome removal during the reconstitution process [6,7]. This ensures that virosomes are **non-infectious**, enhancing their safety profile for clinical applications in drug delivery and vaccination.

2.4 Particle Size and Morphology

Virosomes are typically spherical vesicles ranging from **150–200 nm**in diameter, closely resembling intact enveloped viruses under electron microscopy [7]. Their size and surface composition can be tuned depending on the source virus, lipid formulation, and intended therapeutic application.

Table no:1 Structural components of virosomes and their functions

Component	Source material origin	Function in virosome structure & delivery
Lipid bilayer	Natural (influenza envelope) or synthetic phospholipids (e.g., phosphatidylcholine)	Provides structural integrity; amphiphilic environment for dual drug loading (hydrophilic & lipophilic agents)
Cholesterol	Formulation additive	Increases membrane rigidity, stability, and resistance to leakage
Hemagglutinin (HA)	Influenza viral envelope	Binds to sialic acid receptors; mediates pH-dependent membrane fusion in endosomes
Neuraminidase (NA)	Influenza viral envelope	Cleaves sialic acid residues; prevents aggregation and facilitates particle mobility
Aqueous core	Formed during reconstitution	Encapsulates hydrophilic drugs, peptides, or antigens
Absence of genome	Achieved by detergent solubilization during preparation	Ensures virosomes are noninfectious and safe for therapeutic use

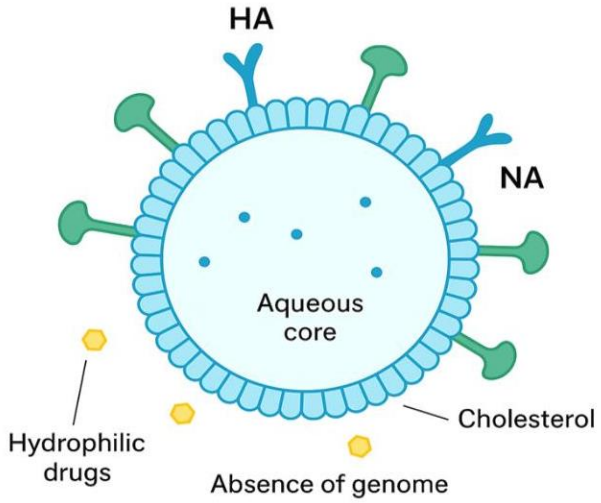

Figure No 1: Schematic representation of virosome structure

3. Preparation Methods

Various techniques have been developed for the preparation of virosomes, each differing in the way lipids and viral membrane proteins are assembled into bilayered vesicles. The three most widely used approaches are the detergent removal method, membrane reconstitution, and solvent fusion. The choice of method depends on

the target antigen, desired functional properties, and scale of production.

3.1 Detergent Removal Method

The detergent removal method is one of the earliest and most extensively used techniques for virosome assembly. In this approach, viral membrane proteins and phospholipids are first solubilized using mild detergents such as octylglucoside or Triton X-100. These detergents disrupt the bilayer but generally preserve protein conformation and activity. Removal of the detergent, commonly by dialysis, gel filtration, or hydrophobic adsorption, facilitates the selfassembly of lipid bilayers with embedded viral proteins in an orientation similar to that in native viruses [9–12]. This method is widely applied in influenza virosome production because it retains haemagglutinin and neuraminidase activities, essential for fusion and immunogenicity [13]. Similar detergent-based strategies have also been adapted for other viral systems, demonstrating the flexibility of this approach [10]. While the process is well-established and scalable, it is time-intensive, and incomplete detergent removal can compromise vesicle stability.

3.2 Membrane Reconstitution

Membrane reconstitution relies on the use of empty viral envelopes obtained after removal of the viral genome. These envelopes, which retain viral glycoproteins, are subsequently combined with additional phospholipids to restore membrane integrity. This can be achieved directly or after temporary solubilization with detergents to allow lipid mixing [14,15]. The method closely mimics the structure of the original virus, preserving native membrane fusion properties and antigen presentation [14,15]. Such virosomes have been used successfully for vaccine delivery and in experimental nucleic acid delivery systems [16,17]. However, this method requires access to viral material and must be performed under appropriate biosafety conditions.

3.3 Solvent Fusion

In the solvent fusion method, lipids and viral membrane proteins are co-dissolved in organic solvents such as ethanol or chloroform–methanol mixtures. Following solvent removal under reduced pressure, a thin lipid–protein film is formed, which is then hydrated to yield vesicles [9,18]. This method, as reported in virosome formulation studies, is technically straightforward and can be adapted for large-scale processes [18]. However, the risk of residual solvent and potential protein denaturation limits its application, especially for sensitive antigens.

3.4 Factors Affecting Efficiency

Regardless of the preparation method, certain physicochemical factors influence virosome yield, stability, and functionality:

Lipid composition: The type and ratio of phospholipids affect membrane fluidity, antigen exposure, and fusion capacity. Unsaturated lipids generally promote flexibility and fusion, while saturated lipids increase rigidity and stability [9,14].

- pH: pH during assembly influences protein charge, folding, and membrane interactions, which in turn affect fusion efficiency [14,17].

Temperature: Preparation at temperatures close to physiological conditions often results in optimal protein–lipid interactions, whereas extreme temperatures can cause protein inactivation [9,17].

Careful optimisation of these parameters is essential to achieve reproducible and stable formulations.

3.5 Comparative Summary

Based on literature analysis, the advantages and limitations of each preparation method are summarised in **Table no:1**.

Table no:1 Summary of virosome preparation methods, with their advantages and limitations as reported in the literature.

Method	Advantages	Limitations

Detergent Removal	Maintains protein activity; established protocols; scalable	Time-consuming; incomplete detergent removal may destabilise vesicles
Membrane Reconstitution	High antigenic fidelity; preserves native fusion	Requires viral source; biosafety constraints
Solvent Fusion	Simple; suitable for large batches	Solvent residues; possible protein denaturation

3. Mechanisms of Cellular Uptake and Intracellular Delivery in Virosomes

3.1 Mechanism of Cellular Uptake

Virosomes are specially designed delivery systems that work by imitating how certain viruses get into cells. In the case of influenza-based virosomes, the key player is the viral protein hemagglutinin (HA), which sits on the surface of the virosome. HA recognises and binds to sugar molecules called **sialic acids** that are naturally present on cell surfaces. Once this binding happens, the cell "swallows" the virosome through a process called **receptor-mediated endocytosis**. The virosome ends up inside a small bubble-like compartment in the cell called an endosome, where it stays intact until it is ready to release its contents (19,20).

3.2 HA-Mediated Receptor Binding

HA is shaped like a tripod, with three identical parts (a "homotrimer"). Each part has two sections:

HA1 – the top part, which actually recognises and sticks to the sialic acids on the cell.

HA2 – the lower part, which is anchored in the virosome's membrane and contains a hidden "fusion peptide."

At normal pH, this fusion peptide stays tucked away. This arrangement allows HA1 to do the job of sticking to the cell first, and then HA2 steps in later to help the virosome fuse with the cell's membrane (19,20).

3.3 Endocytosis and pH-Triggered Fusion

Once the virosome is inside the endosome, the environment becomes acidic. This change in pH acts like a signal for HA2 to change its shape. The hidden fusion peptide pops out and inserts itself into the endosomal membrane. This starts to destabilise the membrane, eventually causing it to merge (or "fuse") with the virosome's membrane. This fusion step is important because it helps the virosome avoid ending up in the cell's waste-disposal pathway (lysosomes) and instead deliver its cargo right where it's needed (19,20).

3.4 Release of Encapsulated Drug

After the membranes fuse, the virosome can release whatever it is carrying—whether that's a vaccine antigen, a small-molecule drug, or genetic material—directly into the watery inside of the cell (the cytosol). For vaccines, this means the antigens can be shown to the immune system through both **MHC class I** and **MHC class II** pathways, which helps create strong and balanced immune responses. For therapies, it means the drug can reach its target inside the cell without being broken down first (19,20).

3.5 Targeting Ligands (Antibodies, Peptides, Aptamers)

By default, HA allows virosomes to attach to many types of cells, but sometimes we want them to focus only on specific cells—like tumour cells or cells infected by a virus. To do this, scientists can decorate the outside of virosomes with **targeting ligands**:

Antibodies or antibody fragments that recognise a unique marker on a target cell (20,21).

Peptides such as RGD, which bind to integrins often found in tumour blood vessels (21).

Aptamers, which are short DNA or RNA sequences that can be designed to attach to specific molecules on the cell surface, with the advantage of being non-immunogenic (22).

These ligands are usually attached to special lipids or PEG "spacers" built into the virosome membrane. This gives a **dual targeting effect**—the ligand guides the virosome to the right cell,

and HA ensures the payload gets inside efficiently (20–22).

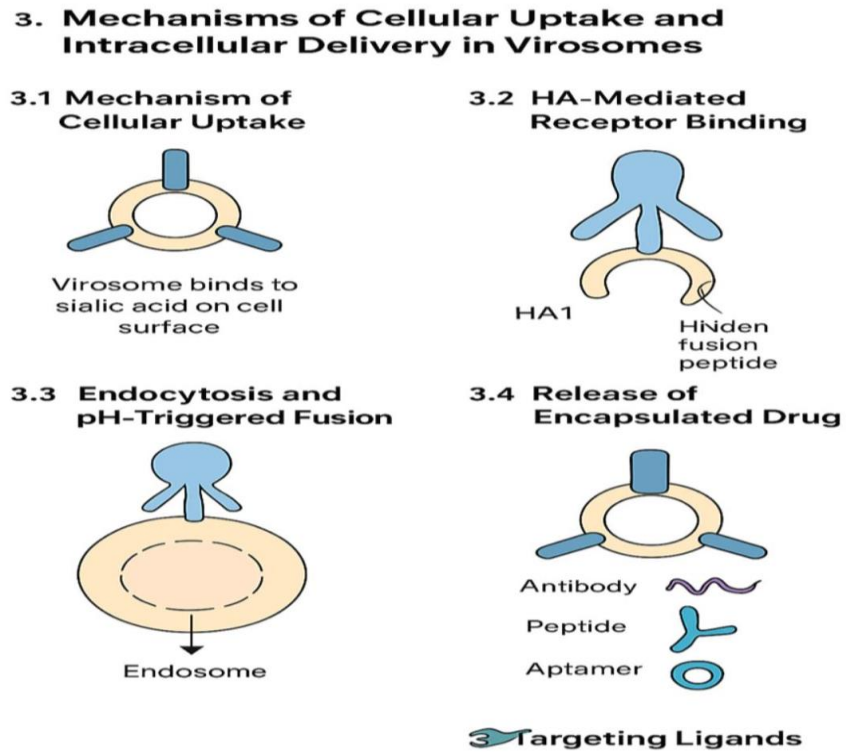

Figure no :2. Schematic representation of the mechanism of cellular uptake and intracellular delivery of virosomes.

5. Therapeutic Applications

5.1 Oncology

Virosomes are used in cancer therapy to carry anticancer drugs such as doxorubicin and methotrexate. They allow drugs to enter tumor cells more effectively and reduce side effects compared to free drugs. [23,24].

A more recent example is nanocurcumin virosomes, which are ~60–90 nm in size and showed strong anticancer activity in breast cancer cells. These virosomes had higher encapsulation efficiency and produced lower IC_{50} values (54.23 µg/mL vs. 79.49 µg/mL for free nanocurcumin), indicating improved potency and safety [25].

5.2 Vaccines

Licensed virosomal vaccines such as Inflexal® V (influenza) and Epaxal® (hepatitis A) have been widely used and proven safe [26]. Research has also developed virosomal vaccines against malaria and HPV, where antigens are displayed on virosome surfaces to trigger strong immune responses [27,28].

Bungener et al. showed that fuson-active virosomes are highly efficient in delivering protein antigens to the immune system. Compared to liposomes and ISCOMs, virosomes triggered stronger immune responses because of their ability to fuse with cell membranes and deliver antigens directly to the cytoplasm [30]. This explains why virosomes are considered superior antigen carriers.

5.3 Antimicrobial and Antiviral Therapies

Virosomes can be used to deliver antimicrobial peptides in a protected and efficient way [24]. For antiviral applications, HIV-1 virosomes displaying gp41 peptides have been tested as vaccine candidates to generate mucosal and systemic immunity [27,29].

Similarly, virosomes carrying influenza or RSV proteins induced robust antibody and T-cell responses in preclinical studies [28,30]. These results suggest that virosomes are versatile carriers for multiple viral antigens and can

improve vaccine efficacy against difficult pathogens.

5.4 Gene and Nucleic Acid Delivery

Virosomes are effective in delivering nucleic acids such as DNA, siRNA, and mRNA. Their fusogenic properties allow them to release genetic material into the cytoplasm, bypassing degradation in lysosomes [23].

Studies demonstrate that DNA-loaded virosomes can activate cytotoxic T-cells, and siRNAvirosomes can silence target genes efficiently [24,29]. This makes them promising for future gene therapies and nucleic acid vaccines.

Table no:.1 Therapeutic applications of Virosomes

Application Area	Example Drugs / Antigens	Key Findings
Oncology	Doxorubicin, Methotrexate	Improved intracellular delivery, reduced systemic toxicity
	Nanocurcumin	Hybrid nanocurcumin virosomes showed higher encapsulation efficiency and lower IC_{50} (54.23 µg/mL vs. 79.49 µg/mL) in breast cancer cells
Vaccines	Influenza (Inflexal® V), Hepatitis A (Epaxal®)	Licensed virosomal vaccines with proven safety and immunogenicity in humans
	Malaria, HPV	Virosomal formulations induced strong immune responses in clinical/preclinical studies
	Fusion-active virosomes	Superior antigen delivery compared to liposomes/ISCOMs due to membrane fusion activity
Antimicrobial & Antiviral	Antimicrobial peptides	Protected from degradation, enhanced cellular uptake
	HIV-1 gp41 peptides	Induced mucosal and systemic immune responses, potential HIV vaccine candidate
	RSV, Influenza antigens	Virosomes stimulated neutralizing antibodies and T-cell responses
Gene & Nucleic Acid Delivery	siRNA, DNA, mRNA	Efficient cytoplasmic delivery by HAmediated fusion, gene silencing and immune activation demonstrated

6. Advantages of Virosomes Over Other Nanocarriers

Virosomes provide several unique benefits compared to other nanoparticle-based delivery systems such as liposomes, polymeric nanoparticles, or ISCOMs.

6.1 Target Specificity

The presence of influenza hemagglutinin (HA) on the virosome surface enables receptormediated binding to sialic acid residues on host cells, followed by efficient endocytosis and fusion. When further decorated with ligands such as antibodies or peptides, virosomes achieve high levels of cell-specific targeting, particularly useful in oncology and antiviral therapies [23–25,27].

6.2 Biocompatibility

Unlike synthetic carriers, virosomes are composed of natural phospholipids and viral envelope proteins, making them highly biocompatible. Licensed virosome-based

vaccines such as Inflexal® V (influenza) and Epaxal® (hepatitis A) have been administered to millions of people worldwide with an excellent safety profile, confirming their clinical tolerability [26–28].

6.3 Dual Drug Loading (Hydrophilic & Hydrophobic Compounds)

The virosomal lipid bilayer allows the incorporation of lipophilic drugs within the membrane, while the aqueous core can encapsulate hydrophilic molecules. This dual-loading ability makes virosomes versatile carriers for diverse therapeutic agents, including small molecules, peptides, and nucleic acids [24,25,29].

6.4 pH-Sensitive Release

The acidic environment of the endosome triggers conformational changes in HA, exposing its fusion peptide. This leads to membrane fusion and pH-sensitive release of the payload directly into the cytoplasm, preventing degradation in lysosomes [23,30].

6.5 Low Toxicity

Virosomes lack viral genetic material, which eliminates the risk of replication or infection. Preclinical and clinical studies consistently show low systemic toxicity compared to other nanocarriers, while maintaining high immunogenicity and therapeutic efficacy [24–27].

7. Challenges and Limitations of Virosomes

7.1 Large-Scale Manufacturing

Scaling up virosome production remains a challenge. Manufacturing requires strict control of viral protein integrity (especially hemagglutinin, HA) and lipid composition. Maintaining fusogenic activity while ensuring reproducibility and sterility increases production costs. Current approaches such as bioreactor-based protein expression and improved purification are promising but not yet widely standardized [31,34].

7.2 Stability During Storage

Virosomes are sensitive to temperature, pH, and freeze–thaw cycles, leading to aggregation or reduced fusion activity. Recent studies highlight the need for optimized formulations, including cryoprotectants and lyophilization, but these methods can reduce biological activity. Compared with synthetic lipid nanoparticles, virosomes require stricter cold chain logistics for long-term stability [31,32].

7.3 Immune System Recognition

Since virosomes mimic viral particles, they are rapidly recognized by the immune system. While this is useful for vaccines, it may lead to faster clearance after repeated administration, reducing therapeutic potential for chronic diseases like cancer. Repeated dosing can also induce neutralizing antibodies against HA, limiting drug delivery efficiency [31,33].

7.4 Regulatory Hurdles

Although virosome-based vaccines such as Inflexal® V and Epaxal® were successfully licensed, extending virosomes into areas such as gene delivery or oncology faces additional regulatory scrutiny. Authorities require extensive data on immunogenicity, long-term stability, and large-scale reproducibility, which lengthens approval timelines compared to simpler nanocarriers [33,34].

8. Future Prospects of Virosomes

8.1 Personalized Medicine Applications

Virosomes hold strong potential in personalized medicine, where therapies are tailored to individual patient profiles. By incorporating targeting ligands such as antibodies, peptides, or aptamers, virosomes can be engineered to deliver drugs or vaccines specifically to cells expressing unique biomarkers. This opens opportunities in oncology and infectious disease therapy, where patient-specific molecular signatures guide treatment [23,24,29].

8.2 Combination Therapy

One of the major advantages of virosomes is their ability to carry both hydrophilic and hydrophobic agents simultaneously. This makes them promising for combination therapy, where multiple drugs (e.g., chemotherapy agents plus immunomodulators) can be delivered together for synergistic effects. Studies on doxorubicin- and methotrexate-loaded virosomes, as well as hybrid nanocurcumin virosomes, show

significant potential in improving efficacy while reducing systemic toxicity [25,27].

8.3 Virosomes for CRISPR/Cas Delivery

Emerging applications explore virosomes as carriers for gene-editing tools such as CRISPR/Cas9. Their ability to encapsulate nucleic acids and fuse directly with the endosomal membrane makes them suitable for efficient cytoplasmic delivery of gene-editing complexes. While still experimental, this approach could overcome many limitations of viral vectors, including safety and insertional mutagenesis [24,28].

8.4 Integration with Imaging Agents (Theranostics)

Future research also envisions the integration of imaging agents into virosomes, enabling theranostic platforms that combine therapy and diagnostics in one system. By incorporating fluorescent dyes, MRI contrast agents, or radionuclides into the virosomal bilayer, researchers can track biodistribution in real time while simultaneously delivering therapeutic payloads. This dual functionality would be particularly useful in cancer treatment and targeted delivery research [26,30].

9. CONCLUSION

Virosomes represent a hybrid platform that successfully integrates the safety of synthetic nanocarriers with the natural efficiency of viral entry mechanisms. Their ability to encapsulate diverse therapeutic agents, deliver them directly into cells via receptor-mediated endocytosis, and stimulate strong immune responses underscores their clinical and translational value. Licensed virosome-based vaccines such as Inflexal® V and Epaxal® already validate their safety and efficacy.

Nevertheless, limitations persist in areas such as scalable production, long-term stability during storage, immune recognition after repeated administration, and complex regulatory pathways.

Addressing these challenges through optimized formulations, stabilization strategies, and harmonized regulatory frameworks will be critical to broadening their applications.

Looking forward, research gaps remain in chronic disease therapy, advanced nucleic acid and CRISPR/Cas delivery, and theranostic integration. Personalized and combination therapy approaches offer further opportunities to exploit the full potential of virosomes. With continued innovation, virosomes are positioned to become a cornerstone of next-generation drug delivery and vaccination technologies..

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