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TRANSFORMING THERAPEUTICS WITH STEALTH LIPOSOMES

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ABSTRACT: This review discusses PEGylated liposomes, also known as stealth liposomes, as modern nanocarriers that overcome the limitations of traditional drug delivery systems and pave the way for precision therapeutics. The article highlights that PEGylation gives stealth properties that include a significant increase in blood circulation time, greater stability, reduced immunogenicity, and improved passive tumor accumulation due to better permeability and retention. It also allows to achieve active targeting through ligand conjugation. The main sections cover the composition and mechanism of PEGylated liposomes, and their successful applications in cancer treatment, antimicrobial therapy, vaccines and immunotherapy, gene therapy, fungal infections, and autoimmune diseases. The review also discusses novel advancements, such as RNA loaded liposomes, while acknowledging critical challenges like accelerated blood clearance. In conclusion, despite facing significant challenges, PEGylated liposomes remain a key element of nanomedicine. Continued improvement in formulation, targeting strategies, and manufacturability will expand the impact beyond oncology to infectious, autoimmune, and genetic diseases.

INTRODUCTION: The drug delivery systems are the technologies that provide the medicine to the specific sites in the body at a given time. It makes the medicine more effective and safer by increasing bioavailability, reducing the side effects, and allowing the release of the drug either in a sustained or targeted way. Different platforms like liposomes, micelles, nanoparticles, and hydrogels have been created to get rid of the disadvantages of conventional therapies and to facilitate chronic disease management and patient compliance, which in turn helps personalized medicine [1]. Among these, the major interest is gained by PEGylated liposomes.

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By combining the liposomes with polyethylene glycol (PEG), they provide advantages like improved pharmacokinetics, stability, and superior therapeutic effects. These features make them widely employable in cancer and antimicrobial therapy, establishing them as a versatile platform in precision medicine [2]. Liposomes are spherical vesicles consisting of lipid bilayers that can encapsulate both hydrophilic and hydrophobic drugs, protecting them from degradation and thus enabling their controlled release [3]. PEGylation provides a "stealth" feature, which decreases the immune clearance of liposomes [4].

This modification extends the circulation time considerably by improving the stability, reducing immunogenicity, preventing aggregation and increasing drug accumulation at disease sites due to reduced clearance [5, 6, 7]. This review focuses on PEGylated liposomes in terms of their structure, delivery mechanisms, key advances in

pharmacokinetics and targeting, existing limitations, and future outlook.

Advantages of PEGylated Liposomes: PEGylation makes liposomes stay in the circulation for a longer time, going from minutes to hours or even days, thus enabling prolonged release of the drug and better therapeutic effect [8]. It promotes passive targeting through the Enhanced Permeability and Retention (EPR) effect and can also allow active targeting if ligands such as antibodies or peptides are attached to the PEG chains [9]. Lowered immune recognition opens the door for repeated dosing, while increased stability prevents drug leakage to an extent and facilitates controlled release [10, 11]. Accumulating drugs at the sites of disease decrease their systemic toxicity, which is a key factor in cancer treatment [12]. Consequently, PEGylated liposomes are able to provide more efficient and safer drug delivery, especially in the case of cancer therapy [13].

Composition of PEGylated Liposomes: PEGylated liposomes are made up of phospholipids, cholesterol that enhances the strength of the layer and protects against leakage, and PEG-lipid conjugates, which are responsible for their stealth properties [2]. The latter consist of amphiphilic molecules with covalently attached lipid anchors, such as Di stearoyl phosphatidylethanolamine (DSPE), which allow them to be securely embedded in the liposome bilayer [48]. The circulation and targeting ability are determined by the length and density of the PEG chains; greater PEG density leads to longer circulation, whereas lower density allows surface modification for active targeting [2].

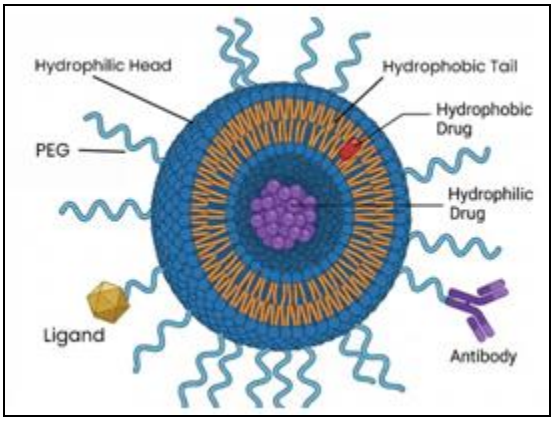

FIG. 1: REPRESENTATION OF PEGYLATED LIPOSOME STRUCTURE

Mechanism of Action: PEGylated liposomes are capable of drug delivery through both passive and active targeting mechanisms.

Enhanced Permeability and Retention (EPR) Effect: Due to the permeable nature of the tumor's blood vessels, the EPR effect takes place in tumors, which are the main sites for the accumulation of PEGylated liposomes besides the blood circulation [14]. The main contributors are as follows:

Abnormal Blood Vessels: Abnormalities in tumor blood vessels such as large gaps in endothelial cells and immature vascular structures increase the permeability for nanoparticles and thus, PEGylated liposomes and other larger molecules can diffuse from the blood into the tumor extracellular space easily [15].

Poor Lymphatic Drainage: The lymphatic drainage system in normal tissues is very effective in getting rid of interstitial fluid and large molecules. In contrast, ineffective lymphatic drain in tumors will retain liposomes longer and hence, increase the drug concentration and therapeutic effect in the tumor area [16].

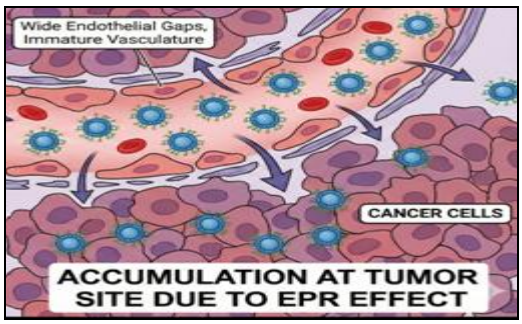

FIG. 2: ENHANCED PERMEABILITY AND RETENTION (EPR) EFFECT

Active Targeting: Active targeting alters the liposomal surface through the use of ligands that bind to receptors highly present on cancer cells thereby enhancing selectivity and cellular uptake [17]. Antibodies, peptides, and aptamers significantly increase the interaction with the tumor tissue.

Some examples are:

- Folate-conjugated PEGylated liposomes, which at the same time enhance delivery and are selectively taken by ovarian cancer cells with folate-receptor overexpression [18].
- Liposomes modified with transferrin have shown to be more efficient in drug uptake in the cancer cells due to receptors overexpression [9].

These methods facilitate precision targeting beyond passive EPR accumulation.

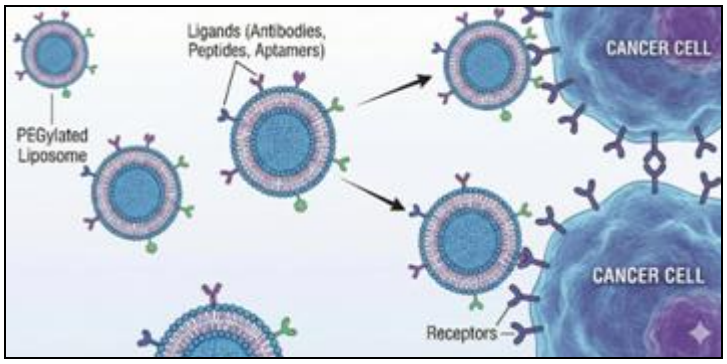

FIG. 3: ACTIVE TARGETING OF LIPOSOMES

Applications of PEGylated Liposomes:

Cancer Therapy: The delivery of cytotoxic chemotherapy agents, such as doxorubicin, has been greatly altered by PEGylated liposomes and, hence, cancer therapy has immensely benefitted from PEGylated liposomes. Doxil, the PEGylated liposomal drug that was the first to be approved by the U.S. Food and Drug Administration (FDA), showed a significant decrease in cardiotoxicity as compared to free doxorubicin [19]. Likewise, other drugs like irinotecan and paclitaxel have also shown better efficacy when formulated in PEGylated liposomes. The latest studies also seem to back this approach. For instance, the usage of PEGylated liposomes for palbociclib in triple-negative breast cancer allowed for a 2.5-fold increase in the drug level in the cancer area as well as a decrease in the side effects caused by the drug when compared to the free drug, all leading to better therapeutic results [20]. The liposomal formulation of 5-fluorouracil co-evaluated for increased pharmacokinetics and decreased cardiotoxicity, exhibiting the drug's greater effectiveness in animal tumor models [46].

Antimicrobial Therapy: The encapsulation of antibiotics like vancomycin and amphotericin B in PEGylated lipids improved their therapeutic index by concentrating on infected tissues and reducing off-target effects [21, 22]. Moreover, in new studies, targeted PEGylated liposomes with ciprofloxacin showed better drug retention and higher survival rates in *Pseudomonas aeruginosa*-infected mice [23]. Also, it was shown that PEGylated liposomes with endolysins were successful in disrupting the bacterial biofilms formed by *Staphylococcus pneumoniae* [47].

Vaccines and Immunotherapy: PEGylated liposomes are utilized as antigen and immune adjuvant carriers to increase immunogenicity and at the same time, reduce systemic toxicity. Such systems have been tested for the treatment of diseases like influenza and cancer [24]. Recent studies reveal that PEGylated cationic liposome vaccines have better antigen drainage to lymph nodes and induce strong immune responses against influenza in murine models [25].

Moreover, liposomal delivery of tumor-associated antigens resulted in T-cell activation and reduced systemic toxicity in cancer immunotherapy [26]. Research findings further show that PEGylation in liposomal adjuvant systems enhances vaccine efficacy by promoting dendritic cell uptake and antigen presentation [27].

Gene Therapy: The diversity of PEGylated liposomes is also applied to the delivery of genetic material, for example, plasmid DNA or interfering RNA (siRNA). PEGylation plays a significant role in the sustaining of nucleic acids' stability in circulation and, consequently, their delivery to the exact sites of action [28]. Numerous studies pointed out the benefit of use of such liposomes with folic acid conjugated PEGylated liposomes that deliver siRNA with cancer cell selectivity, thus increasing the uptake of the siRNA and the silencing of the gene both *in-vitro* and *in-vivo* [45]. PEGylated liposomes with plasmid DNA showed better circulation and better gene expression in the target tissues. The PEGylated liposomal system came up with a longer circulation which could be helpful in the gene-based therapy of diseases [29].

Fungal Infections: Liposomal amphotericin B (AmBisome®) has been very successful in the treatment of systemic fungal infections and at the same time has been less nephrotoxic than the traditional formulations. Moreover, liposomal amphotericin B was favored over the conventional treatment of invasive fungal infections in terms of good safety and effectiveness [21]. Latest researches indicate that PEGylated liposomes containing Ciclopirox olamine led to a significant reduction in fungal burden, which was accompanied by no toxicity in the immunosuppressed mice that had been infected with *Cryptococcus neoformans* [30]. Further research showed that PEGylated liposomal formulations also enhanced antifungal activity against *Candida auris* [31].

Autoimmune Diseases: Dexamethasone and other anti-inflammatory agents in liposome formulations have proven to be efficacious in the treatment of rheumatoid arthritis [32]. It has been demonstrated that PEGylated liposomal formulations of such anti-inflammatory agents can both lower toxicity and increase the effectiveness of treatment in models of autoimmune diseases [33].

TABLE 1: KEY FINDINGS OF PEGYLATED LIPOSOMES APPLICATIONS

Sr. no.	Application Area	Key Findings
1	Cancer Therapy	PEGylated liposomal doxorubicin (Doxil) shows reduced cardiotoxicity vs. free doxorubicin; paclitaxel and irinotecan show improved therapeutic efficacy; palbociclib-loaded stealth liposomes increase drug exposure (2.5-fold) with reduced toxicity.
2	Antimicrobial Therapy	PEGylated liposomal vancomycin and amphotericin B demonstrate improved localization to infected tissues and lower systemic toxicity; ciprofloxacin-loaded PEGylated liposomes enhance survival in *P. aeruginosa* infection; PEGylated liposomes disrupt *Staphylococcus pneumoniae* biofilms.
3	Vaccines & Immunotherapy	PEGylated liposomal vaccines enhance antigen delivery to lymph nodes, increase dendritic cell uptake, and improve immune responses in influenza and cancer models; reduced systemic toxicity.
4	Gene Therapy	PEGylated liposomes improve siRNA/plasmid stability in circulation; folic acid-PEGylated systems increase cellular uptake and gene silencing; PEGylated liposomes support efficient transgene expression.
5	Fungal Infections	Liposomal amphotericin B (AmBisome®) lowers nephrotoxicity vs. conventional formulation; improved antifungal outcomes for *Cryptococcus neoformans* and *Candida auris* with PEGylated liposomes.
6	Autoimmune Diseases	PEGylated liposomal dexamethasone and similar anti-inflammatory systems reduce systemic toxicity and improve therapeutic effectiveness in rheumatoid arthritis models.

Emerging Innovations in Liposome Technology:

Stimuli Responsive Liposomes: Stimuli-responsive liposomes release their drug in response to external stimuli, e.g., pH, temperature, or light. For example, pH-sensitive PEGylated liposomes are made to release drugs in acidic tumor microenvironments to increase drug accumulation at the tumor site [22]. In tumor environments, pH-sensitive and thermosensitive liposomal nanoformulations allow for highly selective drug release, increasing drug accumulation and therapeutic efficacy, according to recent research [34]. In models of hepatocellular carcinoma, multifunctional liposomes that combine phototherapy and gene-drug delivery have demonstrated potent tumor suppression when activated by external stimuli [35].

Hybrid Liposomes: Hybrid liposomes merge lipids with polymers or inorganic nanoparticles for multifunctional use. PEGylated hybrid liposomes containing gold nanoparticles have shown

theranostic capability by allowing drug delivery and imaging at the same time [11]. Drug delivery and real-time tracking in cancer treatments are made possible by core-shell hybrid liposomes that incorporate inorganic nanoparticles like gold. For the best theranostic results in tumor models, a different study showed hybrid liposome–gold nanostructures that enable protected chemotherapeutic delivery and bioimaging at the same time [36].

Liposomal RNA Delivery: Emerging progress with RNA therapeutics, such as siRNA and messenger RNA (mRNA), has made PEGylated liposomes an attractive delivery system. mRNA-based COVID-19 vaccines, for example, by Moderna and Pfizer, delivered *via* lipid nanoparticles have shown the flexibility of this system to tackle public health issues [28].

In ovarian cancer models, folate-modified PEGylated liposomes have demonstrated efficacy in delivering CRISPR plasmid DNA, resulting in potent anti-tumor effects and improved gene transfer [37]. The stabilization of siRNA and mRNA is emphasized in reviews of PEGylated lipid-based RNA delivery systems [38].

Immunoliposomes: Immunoliposomes, one of the target liposomes, are designed through the conjugation of antibodies or antibody fragments to the surface of liposomes. These liposomes have increased affinity for particular antigens. As an example, trastuzumab-conjugated liposomes target HER2-positive (human epidermal growth factor receptor-2) carcinoma cells with exceptional specificity, bringing about enhanced therapeutic efficacy in treating breast cancer [2]. In cervical cancer-on-a-chip experiments, Anti-programmed death-ligand1 (PD-L1) immunoliposomes demonstrated enhanced drug delivery and cancer cell targeting, proving their usefulness for cancer immunotherapy [39]. In lymphoma models, valrubicin-loaded immunoliposomes with dual targeting markers markedly increased immunological modulation and antitumor efficacy [40]. However, PEGylation can interfere with antibody binding due to steric hindrance. Researchers are exploring optimized PEG chain lengths and densities to balance circulation time and targeting efficacy [24]. Immunoliposomes represent a promising innovation for treating cancers and autoimmune diseases where specific targeting is essential.

TABLE 2: KEY FINDINGS OF EMERGING INNOVATIONS

Sr. no.	Innovation Type	Key Findings
1	Stimuli-Responsive Liposomes	Triggered drug release under tumor-specific stimuli (pH, heat, or light); increased intratumoral accumulation; enhanced tumor suppression with combined phototherapy and gene delivery.
2	Hybrid Liposomes	Incorporation of gold or inorganic nanoparticles enables dual functionality (drug delivery and imaging); improved tumor targeting and controlled drug release.
3	Liposomal RNA Delivery	PEGylated liposomes stabilize siRNA/mRNA in circulation; enhanced gene transfer and antitumor efficiency; success demonstrated in vaccine.
4	Immunoliposomes	Antibody-linked PEGylated liposomes improve cancer-cell specificity and immune modulation; effective in HER2-positive cancers and lymphoma therapy.

Challenges in PEGylated Liposomes: Despite the therapeutic benefits, some challenges are encountered in the development of PEGylated liposomes. One of the main hindrances is the occurrence of the ABC phenomenon during the recurring use of PEGylated liposomes, which is caused by the production of anti-PEG antibodies, finally resulting in reduced circulation time and thus limiting the effectiveness of long-term treatments [41]. The manufacturing process has also remained very complicated, as there is a need for an exact control over the lipid composition, PEG density, and particle size in order to achieve consistency from one batch to another [42]. In addition, large-scale production involves not only the high-purity lipids and PEG derivatives but also the specialized processing equipment, which raises the price and thus limits the availability of the product [43]. Apart from these issues, storage instability has also been a challenge as the lipids in PEGylated liposomes. They can undergo oxidation and hydrolysis, which in turn, causes them to either destabilize or degrade. Freeze-drying with an appropriate cryoprotectant is commonly employed to enhance the long-term stability [44, 19].

CONCLUSION: PEGylated liposomes have proven to be one of the efficient drug-delivery platforms by improving the circulation time and stability, as well as targeted accumulation, with minimum systemic toxicity. Its successful application in oncology, antimicrobial therapy, vaccines, gene delivery, and autoimmune diseases demonstrates strong translational and clinical relevance supported by approved formulations.

However, accelerated blood clearance and manufacturing complexity have resulted in continuous formulation optimization to address such limitations. Promising strategies, including stimuli-responsive system hybrid liposome, RNA delivery platform, and immunoliposomes, are foreseen to further improve targeting precision and therapeutic performances.

With continued advancements, PEGylated liposomes will play a crucial role in advanced nanomedicine by enabling safer and more effective treatment strategies in future clinical applications.

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