Review

Liposome-Encapsulated Antibiotics for the Therapy of Mycobacterial and Other Bacterial Infections, and Liposomal Vaccines against Tuberculosis

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Review

Liposome-Encapsulated Antibiotics for the Therapy of Mycobacterial and Other Bacterial Infections, and Liposomal Vaccines against Tuberculosis

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Abstract: About a quarter of the world's population is infected with *Mycobacterium tuberculosis*. Growing antibiotic resistance by the microorganism is a major problem in the therapy of the disease. *M. avium-M. intracellulare* that emerged as a major opportunistic infection of HIV/AIDS continues to afflict immunocompromised individuals. Here we briefly describe the various methods for liposome preparation, and the use of liposome-encapsulated antibiotics in the experimental and clinical therapy of mycobacterial and other bacterial infections. We also review recent experimental liposomal vaccines against tuberculosis.

Keywords: liposomes; tuberculosis; antibiotics; antimycobacterial agents; bacterial infections; tuberculosis vaccines

1. Mycobacteria

Mycobacteria are aerobic bacteria with a diameter of approximately 0.2–0.6 μm and they vary in length between 1–10 μm. Owing to the long-chain lipids, termed mycolic acids, in the bacterial membrane, the surface of the microorganism presents a hydrophobic interface, which resists the entry of many disinfectants as well as the Gram and Giemsa stains. A major characteristic of mycobacteria is that after incubation with the Ziehl-Neelsen stain, they are resistant to the decolorizing effect of an ethanol-hydrochloric acid solution (which can remove the stain from many other bacteria), and are thus termed acid-fast bacteria. Nevertheless, under some conditions, for example, after treatment with the mycolic acid synthesis inhibitor, isoniazid, or when it is in a metabolically inactive state in the host, *M. tuberculosis* may become acid-fast-negative [1]. Mycobacteria grow very slowly. The doubling time for *Mycobacterium tuberculosis* may be as long as 1676 h in the chronic infection phase in infected mice, and about 25 h during acute infection [2]. During the Industrial Revolution in the 18th and 19th centuries in Europe, tuberculosis became an epidemic, exacerbated by the crowding of urbanization and economic depression [3]. One quarter of the population of Europe in the 19th Century is thought to have died of "consumption," as tuberculosis was called at the time. The victims included Goethe, Rousseau, Thoreau, Keats, Paganini, and Chopin. It is estimated that even in the 21st Century, one in four people in the world are infected with *M. tuberculosis*. According to the World Health Organization, the bacterium infected 10.8 million people in 2023, and killed about 1.25 million people. Among these, 161,000 were HIV-1-infected people. It is estimated that between 5 and 10% of individuals infected with the mycobacterium will eventually develop tuberculosis. The incidence is highest in Southeast Asia (45%), Africa (24%) and the Western Pacific (17%). Tuberculosis is prevalent in areas with poverty, malnutrition and poor housing. Mycobacteria have a complex cell wall, composed of the peptidoglycan layer linked with arabinose-galactose-mycolic acid [3]. Long chain mycolic acids, in the range of 70 to 90 carbon

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atoms, are the major lipids in the mycobacterial cell wall. Free lipids are located on the outer layers, including waxes, mycosides, which are complex saturated glycolipids, and 6,6'-dimycolate of trehalose, known as cord factor, one of the virulence factors of mycobacteria. The tubercle bacillus is transmitted by via inhalation of infectious aerosols, generally involving person-to-person contact in close quarters, and occasionally by ingestion and skin trauma. Large aerosol particles are usually trapped by the lung mucosal surfaces and removed by the mucociliary escalator. Smaller aerosol particles with 1–3 mycobacteria can reach the alveoli and are phagocytosed by alveolar macrophages, where they replicate and may destroy their host cell. Infected macrophages may migrate to local lymph nodes, the bloodstream, bone marrow, spleen, kidneys, and the central nervous system [3]. According to American Thoracic Society recommendations, the initial treatment regimen involves isoniazid (and inhibitor of mycolic acid biosynthesis), rifampin (an inhibitor of bacterial RNA synthesis), pyrazinamide (an inhibitor of fatty acid synthase type I), and ethambutol (an inhibitor of arabinogalactan synthesis) for 2 months. This is followed by isoniazid and rifampin therapy for 4–6 months. Noncompliance is known to cause the emergence of multi-drug-resistant (MDR) strains; thus, in many countries, directly observed therapy (DOT) is employed to ensure compliance.

MDR strains that are resistant to at least isoniazid and rifampin have become a worldwide problem [4]. The resistance is attributed to one or more chromosomal mutations. One of these mutations is in a gene for mycolic acid synthesis, and another in a gene for catalase-peroxidase, an enzyme required to activate isoniazid within the bacterium [3]. Previous treatment for tuberculosis predisposes the patient to the selection of MDR organisms. In addition to MDR tuberculosis, extensively drug resistant (XDR) *M. tuberculosis* strains have emerged throughout the world [5]. These bacteria are MDR- *M. tuberculosis* strains that are resistant to fluoroqinolones and at least one of the second line drugs, such as kanamycin, capreomycin and amikacin.

M. avium and *M. intracellulare* (MAC) are difficult to differentiate physiologically, and cause identical diseases, and are thus grouped together as the *M. avium-M. intracellulare* Complex, abbreviated as MAC [3]. They cause disseminated disease, where tissue macrophages are inundated with the microorganisms, especially in advanced HIV/AIDS patients, and the blood contains large numbers of organisms. *M. abscessus has been recogized recently as a pathogen* that can colonize the lungs of patients with cystic fibrosis, chronic obstructive pulmonary disease, or bronchiectasis, and that can grow in macrophages and free-living amoebae [6]. The mycobacterium has intrinsic and acquire mechanisms of resistance to therapeutics [7].

2. Early Studies on Liposome-Encapsulated Antibiotics for Tuberculosis Therapy

The first English-language publications on the use of liposome-encapsulated antibiotics appeared in 1982. Vladimirsky and Ladigina [8] treated mice infected with *M. tuberculosis* strain H37Rv by intravenous injection of streptomycin sulfate encapsulated in liposomes composed of lecithin (phosphatidylcholine with various acyl chains). There was a statistically different decrease in mycobacterial counts in the spleen compared to an equivalent concentration free streptomycin, which translated into prolonged survival in the liposomal antibiotic group, and reduced antibiotic toxicity. In a subsequent study, Ladigina & Vladimirsky [9] showed that the total area under the serum concentration-time curve (“AUC”) of liposomal 3H-dihydrostreptomycin was 8.8 times higher in uninfected mice than the free antibiotic, and 5.9 times higher in mice with advanced tuberculosis. The total amounts of liposome-delivered antibiotic in the spleen and liver of infected mice were 9.2 and 7.3 times higher, respectively than that achieved with the free antibiotic. Orozco et al. reported that mice with severe tuberculosis treated with rifampicin and isoniazid in free and liposome-encapsulated form, together, had a higher survival rate (about 85%) after 30 days, the lowest colony-forming units (CFU) of the bacteria, and less inflammation in the lungs [10]. These early experiments were followed by the impressive results obtained by Agarwal et al. who employed rifampin-loaded phosphatidylcholine liposomes to which the macrophage activating tetrapeptide, tuftsin (Thr-Lys-Pro-Arg), was coupled covalently via its C-terminus, using an ethylene diamine spacer. The

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liposomes delivered twice weekly for 2 weeks were several orders of magnitude more effective effective than the free drug in lowering the CFU in the lungs, liver and spleen of mice that had been infected for 13-16 days with the H37Rv strain of *M. tuberculosis [11]*.

3. Early Studies on Liposomal Antibiotic Therapy of Mycobacterium Avium-Mycobacterium Intracellulare Infections

The first report on the use of liposome-encapsulated antibiotics against experimental MAC infections in beige mice employed amikacin in phosphatidylglycerol-phosphatidylcholine-cholesterol (1:1:1) liposomes prepared by reverse-phase evaporation followed by extrusion through polycarbonate membranes [12]. This formulation arrested the growth of MAC in the liver, and reduced the CFU counts by about 1,000-fold in the spleen and kidneys, compared with those of both untreated controls and free-drug-treated mice. Using a much higher dose of amikacin, either free or encapsulated in phosphatidylcholine liposomes, Cynamon et al. obtained similar results in the liver and spleen; however, they also found a significant reduction in CFU in the lungs with both free and liposomal amikacin [13]. Relatively low concentrations of amikacin and gentamicin in liposomes reduced significantly the MAC CFU in blood, liver, and spleen [14]. Klemens et al. found that both encapsulated and free gentamicin reduced viable MAC counts in the liver, spleen and lungs compared with no treatment [15]. Encapsulated gentamicin was more effective than the free antibiotic in reducing the viable cell counts in the spleen and liver. Liposomal amikacin was also more effective than the free drug when administered to MAC-infected murine peritoneal macrophages [16].

Intraperitoneal administration of rifampin-incorporating multilamellar liposomes resulted in a larger reduction in bacterial growth in the lungs and spleen of infected ddY mice than did free rifampin [17]. Liposome-encapsulated streptomycin, administered intravenously in weekly doses (15 mg/kg) for 4 weeks, reduced the CFU in the liver and spleen of MAC-infected beige mice by an extent similar to that of a 50- to 100-fold higher dose of the free drug [18]. With this injection schedule, the CFU in the liver and spleen were lower by 2.4 and 2.9 log units, respectively, compared to untreated controls, even by the end of 12 weeks, suggesting that the liposomal formulation also increases the residual activity of the drug in these organs. In a parallel study, the effect of free streptomycin at 150 mg/kg given im five days a week for 8 weeks was compared with 15 mg/kg of streptomycin in unilamellar liposomes administered intravenously in 4 injections, with no further treatment up to 8 weeks [19]. The chemotherapeutic efficacy, expressed as the reduction in CFU/unit dose of the antibiotic, was several fold higher for the liposomal drug.

4. Encapsulation of Antibiotics in Liposomes

Various drug delivery systems, including metallic, polymeric, carbon-based, and dendrimeric carriers, as well as liposomes, have been utilized to overcome the limitations of antibiotics, such as low aqueous solubility, drug degradation, and antibiotic resistance, while simultaneously enhancing their bioavailability and minimizing adverse effects [20]. Liposomes offer advantages such as biocompatibility, capacity for self-assembly, low immunogenicity, passive and active targetability, prolonged half-life of the loaded drug, protection of sensitive molecules, and enhanced bioavailability [21].

Liposomes are generally classified based on their size, with small unilamellar vesicles (SUVs) being <100 nm and large unilamellar vesicles (LUVs) being >100 nm, as well as by the number of lamellae (unilamellar or multilamellar vesicles). In the preparation of liposomal formulations, the composition and charge—neutral, anionic, or cationic—can be tailored according to the therapeutic target [22]. This is particularly important for the interaction of liposomes with bacteria and their uptake by eukaryotic cells [23] . Additionally, liposomal formulations offer significant advantages in the optimal treatment of infections, as they can be administered through various routes, including intravenous [24], transdermal [25], oral [26], inhalational [27,28], and nasal delivery [29].

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The encapsulation of antibiotics in liposomes can be achieved through two main approaches: active and passive loading. Passive loading techniques include mechanical dispersion, solvent dispersion, and detergent removal methods, whereas active loading techniques involve approaches such as detergent dialysis and microfluidic methods. These techniques are used to load drugs into preformed liposomes, ensuring minimal drug loss during the loading process [30].

Hydrophilic antibiotics are incorporated into the aqueous core, whereas hydrophobic antibiotics are naturally embedded within the lipid bilayer. The development of antibiotic-loaded liposomes depends on multiple factors, including the physicochemical properties of the drug, formulation stability, drug leakage, and retention [31]. In general, liposomes are prepared by dissolving lipid components in an organic solvent, forming a lipid film, which is then redispersed in an aqueous medium, followed by sizing and purification. Various techniques have been utilized for liposome synthesis and antibiotic encapsulation, including sonication, thin-film hydration, freeze-thawing, micro-emulsification, solvent injection, reverse-phase evaporation, dehydration-rehydration, hydration in a packed bed of colloidal particles, pH jumping, detergent removal, and extrusion [32,33].

One of the distinguishing factors that set liposomes apart from other drug carriers in antibiotic delivery is the flexibility of their surface modification. The frequently employed polyethylene glycol (PEG)-conjugated lipids can be used to confer to liposomes incorporating them the property of prolonged circulation after intravenous administration [34,35]. These "PEGylated" liposomes can be further functionalized on their surface with antibodies, peptides, proteins, or carbohydrates [36]. This surface modification enhances drug efficacy while correspondingly improving therapeutic effectiveness [37-39].

Bacteria can develop resistance mechanisms against drugs through various pathways, such as enzymatic inactivation of the drug and active efflux pumping, which expels the drug from the cell [40]. Numerous studies have demonstrated that liposome-based antibiotic formulations can be effective in treating antibiotic-resistant bacteria (Figure 1) [41].

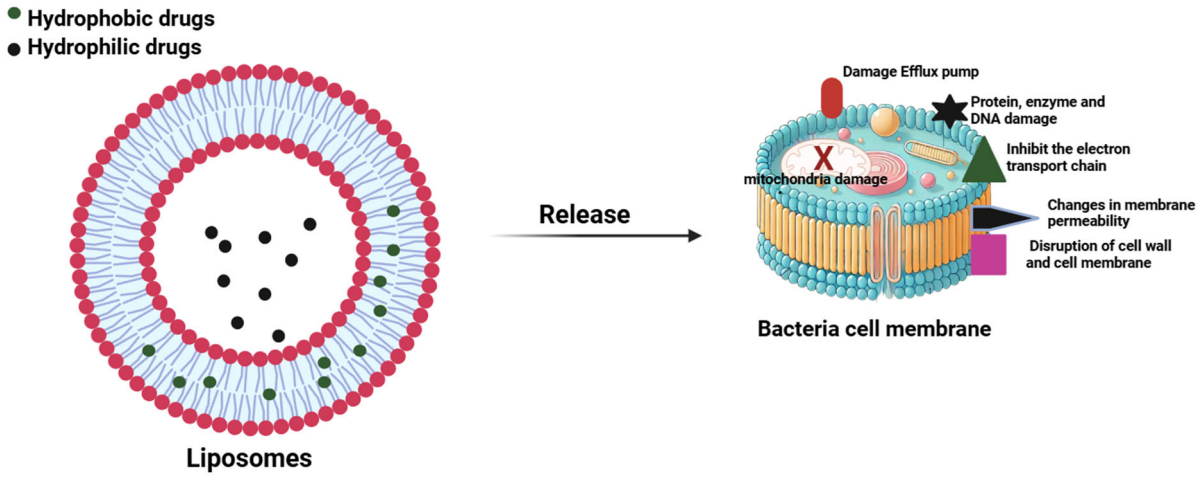

Figure 1. Treatment pathways of antibacterial drug-loaded liposomes against bacteria. (Reproduced from [42]).

Antibiotics encapsulated within liposomes can target both Gram-negative and Gram-positive bacteria, which often produce enzymes that degrade antimicrobial agents. Additionally, for an antibiotic to exert its effect, it must penetrate the bacterial membrane and enter the cell. However, mutations can lead to alterations in outer membrane porins, reducing permeability [42]. In Gram-negative bacteria, the complex structure of the outer membrane further hinders antibiotic penetration, contributing to resistance. Moreover, active efflux pump proteins, which play a crucial role in bacterial physiology, can also contribute to resistance by expelling antibiotics before they reach their target. During the formulation of liposomes, the use of potentially fusogenic phospholipids may help overcome this challenge by facilitating membrane penetration. These phospholipids enhance

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the fusion of liposomes with bacterial membranes, improving drug delivery and increasing the intracellular uptake of antibiotics, thereby enhancing their therapeutic efficacy against resistant bacterial strains [43].

Given these challenges, liposomal antibiotic formulations have been widely explored as a potential strategy to enhance the efficacy of antibiotics against resistant bacterial strains (Table 1). In a study targeting *Staphylococcus aureus* biofilms, negatively charged liposomes encapsulating Levofloxacin and Vancomycin were prepared using the dehydration-rehydration technique, enabling in situ antibiotic release within the biofilm [44]. Additionally, Nafcillin-loaded PEG-grafted liposomes, prepared via the reverse-phase evaporation method with an average size of 253 nm, exhibited a fourfold reduction in the minimum inhibitory concentration (MIC) against Methicillin-susceptible *Staphylococcus aureus* (MSSA) compared to free Nafcillin [45].

For the treatment both Gram-positive and Gram-negative bacterial infections, amoxicillin, β-lactam antibiotic, was successfully encapsulated into liposomes (~200 nm in size) using the Supercritical Assisted Liposome Formation (SuperLip) method. In this technique, liposomes are prepared by spraying water droplets into an expanded phase composed of phospholipids, ethanol, and carbon dioxide (CO_2) under high pressure. During the process, the water droplets are rapidly surrounded by a lipid layer, and upon falling into the water pool located at the bottom of the vessel, liposomes are formed. Using this approach, an encapsulation efficiency of 84% was achieved [46]. Furthermore, pH-sensitive liposomes loaded with a silver-tinidazole complex effectively eradicated tumor-associated bacteria from primary tumors.

Table 1. Overview of the Effect of Antibiotic-Loaded Liposomes Against Bacterial Infections.

Drug	Method	Size	Target	Remarks	References
Rifabutin	Dehydration-rehydration	100–115 nm	MRSA biofilm	Rifabutin-loaded liposomal formulations demonstrated superior efficacy compared to free vancomycin	[47]
Tetracycline, Amoxicillin	-	270–340 nm	MRSA	Drug-loaded liposomes enhance the cellular uptake of antibiotics, thereby providing more effective treatment compared to their free forms.	[48]
Colistin	Thin layer hydration	73–217 nm	*Pseudomonas aeruginosa* infection	Colistin-loaded cationic liposomes, with an encapsulation efficiency of 77%, had low MIC values against *Pseudomonas aeruginosa*.	[49]
Amoxicillin	Film hydration method	210 nm	*Staphylococcus aureus* infection	Amoxicillin-loaded PEG-◎-cyclodextrin- acrylamide-liposomes were incorporated into biocompatible hydrogels to prepare a wound dressing. The formulation demonstrated controlled drug release and effective antibacterial activity.	[50]
Piperacillin sodium	Film hydration method	94.49 nm	Antibiotic resistance of clinical isolates of *Pseudomonas aeruginosa*	Liposome-loaded Piperacillin exhibited superior antibacterial activity at a lower MIC value compared to its free form.	[51]

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Vancomycin	Freeze–thaw method	157 nm	MRSA	At a 1:10^2 dilution, free vancomycin failed to inhibit bacterial growth, whereas the liposome-loaded form achieved 100% inhibition.	[52]
Ampicillin	SuperLip	200 nm	-	Ampicillin-loaded liposomes were entrapped in alginate gels. This method resulted in enhanced encapsulation efficiency and improved polydispersity index values, indicating a more effective formulation.	[53]
Azithromycin	Proliposome	164- 187 nm	*Chlamydia trachomatis*	The formulations exhibited at least twofold higher activity compared to the free form against both clinical isolates and bacterial strains.	[54]
Ciprofloxacin and colistin	Thin film evaporation and sonication	102.1-119.7 nm	Clinical isolates of *Pseudomonas aeruginosa* H131300444 and *P. aeruginosa* H133880624	The formulation exhibited effective antibacterial properties against *Pseudomonas aeruginosa*, a multidrug-resistant Gram-negative bacterium responsible for pulmonary infections, while showing no cytotoxic effects on A549 cells. However, the encapsulation efficiency for both drugs remained below 50%.	[55]
Levofloxacin	Film hydration method	127.6 nm	*Staphylococcus aureus*	Levofloxacin-loaded liposome formulations were coated with chitosan (CS). Following CS coating, an increase in particle size was observed, along with an enhancement in antibacterial activity.	[56]

5. Therapy of *Mycobacterium tuberculosis* Infection

Airborne and primarily affecting the lungs, tuberculosis is a highly lethal disease caused by *Mycobacterium tuberculosis*. According to WHO data, tuberculosis affects approximately 10 million people annually and results in about 1.3 million deaths worldwide [57]. The treatment of this disease commonly involves Rifampicin (RIF), Isoniazid (INH), Pyrazinamide (PZA), and Ethambutol (EMB). However, due to the potential development of resistance to these drugs, novel and more effective therapeutic strategies are needed urgently [58].

Formulations of various drugs prepared using liposomal delivery systems not only enhance therapeutic efficacy but also serve as promising platforms for the development of vaccines against tuberculosis infections [59]. A novel vaccine strategy has been explored by combining Poly:IC adjuvant with liposomes containing the Ag85B and ESAT-6 antigens [60]. To induce a stronger immune response, fusion proteins such as Hspx, PPE44, and EsxV have been loaded into liposomes for the development of a tubeculosis vaccine [61].

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The FDA approval of liposome-based drug formulations and the widespread utilization of this nanocarrir system highlight its significance in advancing next-generation anti-tuberculosis treatment approaches [62]. Thanks to remarkable advancements in liposome technology, the development of inhalable antibiotic-loaded liposomes, in addition to those administered via the intravenous [63] or oral route [37], has been made possible. Additionally, targeted drug delivery systems help reduce the degradation of drugs in the body compared to their free forms, allowing for the administration of lower doses while maintaining therapeutic efficacy. As a result, the toxic effects of the drugs are minimized, leading to a more effective treatment.

These formulations hold potential for clinical applications and patent acquisition, ultimately making them available for human use [64]. This characteristic provides a significant advantage in the treatment of tuberculosis, which is primarily localized in the lungs [65]. Although oral administration of rutin-based drugs is more frequent and cost-effective, their low gastrointestinal absorption and rapid hepatic first-pass metabolism necessitate high-dose administration. Therefore, parenteral and pulmonary delivery of rutin-based drugs offers a higher bioavailability, as they bypass the first-pass metabolism. *M. tuberculosis* evades macrophage-mediated bactericidal mechanisms by inhibiting the formation of phagolysosomes [66]. Liposomes can be administered via inhalation depending on their size. Liposomes with a particle size ranging from 0.1 to 2 μm can reach the alveoli, whereas those larger than 15 μm cannot penetrate the respiratory barrier [67].

For the treatment of tuberculosis, liposomes have been designed to encapsulate antibiotics such as RIF [68], INH [69], PZA [70], and EMB [71], either individually or in combination [72,73]. Additionally, potential compounds with anti- tuberculosis activity [19] have also been loaded into liposomes for therapeutic applications, including theranostic approaches. Furthermore, increasing the particle size can influence the therapeutic effect. (Figure 2).

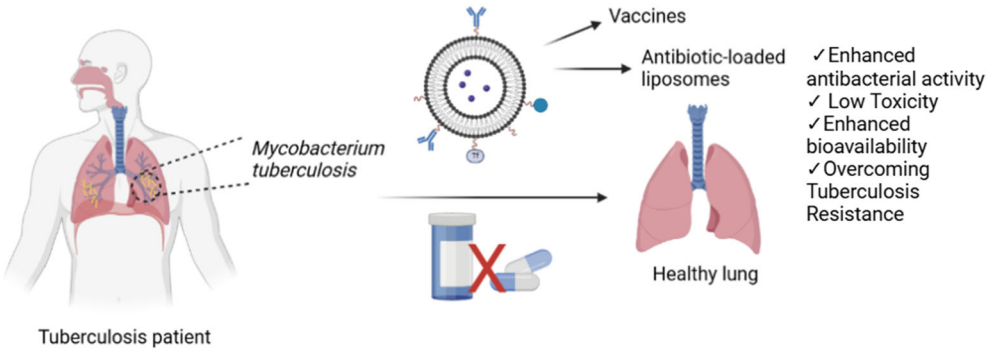

Figure 2. Antibiotic-loaded liposomes as a strategy to overcome tuberculosis resistance and enhance treatment efficacy.

For targeted applications, if the aim is to reduce the immunogenicity of liposomes and decrease their uptake by the mononuclear phagocyte system, their surface can be modified with PEG [74]. PEGylated liposomes have been developed for the co-delivery of antitubercular drugs and TGF-β1 siRNA for tuberculosis treatment. The formulated liposomes exhibited minimal toxicity to human macrophages while demonstrating good selectivity [75].

The conjugation of various polysaccharides, including chitosan, dextran, and fucoidan, to the surface of liposomes can enhance their stability while also imparting mucoadhesive properties. Additionally, these formulations not only improve cellular uptake but also interact with carbohydrate receptors expressed on the surface of macrophages [76]. Fucoidan-based surface modifications have been applied to enhance the activity of usnic acid-loaded liposomes in tuberculosis treatment [77]. The use of cationic pH-sensitive liposome-based delivery systems facilitates the passage through the endosomal membrane under low pH conditions, enabling the release of the encapsulated content into the cytosol. This prevents the degradation of delivered

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antigens and, consequently, enhances cytotoxic CD8+ T-cell responses [78]. In tuberculosis treatment, to overcome local and systemic toxicity, liposomes encapsulating anti- tuberculosis drugs can be surface-modified with macrophage-specific ligands. To facilitate the delivery of RIF-loaded liposomes to alveolar macrophages, liposomal formulations coated with maleylated bovine serum albumin and O-stearoyl amylopectin were developed. Based on drug localization index data, ligand-functionalized drug-loaded liposomes exhibited a 1.4- to 3.5-fold higher localization compared to non-ligand-modified counterparts. These findings indicate that the ligand-modified liposomes enable rapid drug delivery to the lungs and achieve high drug concentrations at the target site [79].

A new therapeutic strategy has emerged in which mycobacteriophages were loaded into liposomes for the treatment of tuberculosis treatment, presenting an innovative approach to combat the disease [80] (Table 2).

Table 2. New Liposomal Strategies Against Tuberculosis.

Drug	Method	Size	Remarks	References
Cationic pH-sensitive liposome	Thin-film hydration	164.6 nm	pH-sensitive cationic liposomes formulated with the Ag85B-ESAT6-Rv2034 fusion antigen and CpG and MPLA adjuvants have been shown to induce potent polyfunctional $CD4^+$ and $CD8^+$ T-cell responses. Additionally, an increase in $CD69^+$ B-cell sub-populations was observed.	[78]
Anionic and neutral liposomes		-	For improved pulmonary TB treatment, ID93 plus GLA-containing liposomal adjuvant formulations were developed. However, the anionic or neutral liposome + QS-21 liposomal formulations did not result in a significant reduction in *M. tuberculosis* bacterial load. Nevertheless, these formulations were observed to induce distinct immune responses.	[81]
Moxifloxacin loaded liposome-siderophore conjugates	Film hydration technique	200 nm	Liposome formulations with a spherical shape had an encapsulation efficiency of 46 % and demonstrated anti-TB activity with a MIC value of 0.32 μg/mL.	[82]
Saquinavir	Thin-film hydration	116 nm	In the treatment of multidrug- and extensively drug-resistant *M. tuberculosis* strains, negatively charged Saquinavir-loaded liposomes were shown to enhance intracellular killing activity by human macrophages.	[83]
Rifampicin	Thin-Layer Evaporation	117 nm	The prepared formulation demonstrated a greater reduction in intracellular *M. abscessus* viability compared to the free form of the drug.	[84]
Oral liposomal glutathione supplementation	-	-	Commercially available liposomal glutathione supplementation (L-GSH) has been shown to reduce oxidative stress in patients with type 2 diabetes mellitus (T2DM). *In vitro* models have	[85]

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			demonstrated its ability to decrease intracellular mycobacteria.	
Rifampicin and isoniazid	Lipid film hydration, sonication and extrusion	-	Antibiotic loaded, polyorganophosphazene-arginine-grafted liposomes exhibited a 73% RIF and 80% IZN release at endosomal pH. The liposomes demonstrated a dose-dependent inhibition of *M. tuberculosis* growth in culture medium.	[86]
N′-Dodecanoylisonicotinohydrazide		~130 nm	For use in localized tuberculosis treatment, the Isoniazid derivative *N′dodecanoylisonicotinohydrazide*, a commonly used agent in tuberculosis therapy, was loaded into liposomes. PLGA-PEG-PLGA systems were incorporated to develop thermosensitive and self-healing hydrogel systems. Data obtained from *in vivo* microdialysis studies demonstrated the rapid release of the drug into the synovial fluid.	[87]
Coumaran (2,3-dihydrobenzofuran) derivatives—TB501 and TB515—	Thin film hydration	~60 nm	The liposome formulation prepared with TB515 exhibited high encapsulation efficiency. Multicomponent pH-sensitive stealth liposomes encapsulating TB501 were highly effective against *M. tuberculosis* in macrophage cell lines.	[74]
Zn-phthalocyanine	Ethanol injection	134 nm	ZnPC-loaded liposomes, prepared for the treatment of Rifampin-Isoniazid-resistant *M. tuberculosis* strains, achieved a 99.9% cell death rate in vitro through photodynamic therapy (PDT).	[88]
Isoniazid	Thin-film hydration	37–45 nm	Biocompatible hydrogenated soy phosphatidylcholine-phosphatidylglycerol liposomes were developed as isoniazid carriers. The encapsulation efficiency was determined using UV and Laser Transmission Spectroscopy.	[89]
Glucopyranosyl lipid adjuvant (GLA) and the experimental tuberculosis vaccine, ID93, composed of four *M. tuberculosis* antigens	Thin-film hydration, sonication, homogenization	50–87 nm	For use as a vaccine in the treatment of *Mycobacterium tuberculosis*, formulations containing a TLR4 agonist (GLA) and QS21, in combination with ID93, were developed. In an *in vivo* model, these formulations demonstrated a reduction in bacterial load in the lungs of mice infected with *M. tuberculosis*. Clinical studies involving human participants are ongoing to evaluate the safety,	[90]

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			tolerability, and immunogenicity of the developed formulations.	
Artemisone, Clofazimine and Decoquinate	Thin-film hydration	147, 482, 253 nm	Drug-loaded liposomes, synthesized in various sizes, exhibited 32–42% inhibition of *M. tuberculosis* growth in culture medium. By contrast, drug-free liposomes induced only 12% inhibition.	[91]
Isoniazid-conjugated Phthalocyanine	"Heating Method"	150–650 nm	A complex of γ-Cyclodextrin with Isoniazid-conjugated Phthalocyanine, was incorporated into crude soybean lecithin liposomes using a simple and measurable heating method. This pH-sensitive formulation exhibited 100% drug release at pH 4.4, while releasing only 40% at pH 7.4, demonstrating its potential applicability in targeted therapies.	[92]
Isoniazid and Rifampicin	Reverse Phase Evaporation	332 - 361 nm	Liposomal formulations loaded with anti-TB drugs Isoniazid, Rifampicin, and their combination were developed for inhaled therapy. Isoniazid formulations exhibited a faster release compared to Rifampicin formulations, while their encapsulation efficiencies were found to be similar.	[93]

6. Therapy of Non-Tuberculous Mycobacterial Infections

The most common appearance of non-tuberculous mycobacteria (NTM) infections is lung disease [94]. These bacteria, which include *M. avium, M. intracellulare and M. chimaera*, are present in the environment and can cause pulmonary infections in immunocompromised individuals or persons who have lung damage. NTM may present as biofilms attached to the alveolar wall or intracellularly in monocytes and macrophages. Ehlers et al. [95] found that long-term intravenous administration of liposomal amikacin was not effective against lung infections in *M. avium*-infected mice. As an approach to this problem Zhang et al. [96] aerosolized amikacin-liposomes (dipalmitoylphosphatidylcholine-choesterol (2:1)) into rats infected with NTM, and observed a large increase in the mean area under the concentration-time curve in the lungs and lung macrophages compared to intravenous free amikacin .

A retrospective analysis of 17 patients undergoing treatment with amikacin-liposomes ("Amikacin Liposomal Inhalation Suspension") for NTM lung infection showed that at 6 months, 86% of the patients had clinical, microbiological, and radiological improvement. Twenty five percent of the treated patients, some of whom were coinfected with *M. abscessus*, relapsed after the therapy was completed [97].

Rifampicin embedded in the membrane of hydrogenated soy phosphatidylcholine-dipalmitoylphosphatidylcholine (1:1) liposomes reduced the viability of *M. abscessus* in infected, differentiated THP-1 human monocytic leukemia cells, significantly more than the free drug [84].

7. Future Directions, Challenges and Limitations

Liposomes, in addition to being biocompatible and undergoing natural degradation, offer the advantages of easy synthesis and modification with various agents. Their ability to encapsulate both hydrophilic and hydrophobic first-line anti-tuberculosis drugs, as well as other potential therapeutic

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compounds, further enhances their versatility. Additionally, liposomes can be utilized as vaccine carriers against tuberculosis. In the near future, formulations developed with liposomal technology hold promise for patentability, while providing effective solutions in tuberculosis treatment, ultimately improving patients' quality of life and minimizing the adverse impacts of the disease on public health. One of the major challenges associated with liposomes is drug leakage, which can significantly impact their efficacy. The high production cost of this carrier system poses another limitation. Moreover, liposomes are prone to lipid oxidation and hydrolysis, leading to potential instability. To enhance their stability and prolong their therapeutic effect, liposomal formulations often require polymeric coating. However, this coating process further increases the complexity of synthesis and production costs, making large-scale application more challenging.

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