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**Review Article**

# Nanoparticles for Targeting the Hydrophobic EPS Matrix of Mycobacterial Biofilms: Recent Advancements

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**Abstract:** Tuberculosis (TB) is said to be leading cause among the health problems in the world owing to its high morbidity and mortality, which is again due to antibiotic resistance. The bacteria's thick, lipid-rich cell wall makes the lipid layer permeable and limits the drug entry, and loosing hope in therapeutics. New methods are being developed in nanomedicine which boosts the drug solubility into the mycobacterial membranes. Enzyme-based nano delivery systems increase selectivity and reduce systemic toxicity by producing localized medication release via pathogen-specific enzyme activity. Carbohydrate-based carriers, which are engineered to resemble host glycoconjugates and allow for selective uptake and extended release, are a potential method of breaching the cell wall. Together, these innovative delivery strategies represent a paradigm shift in TB therapy, addressing the shortcomings of traditional treatment and opening the way to more successful management of drug-resistant Mycobacterium tuberculosis. The introduction of nanotechnology to antimicrobial therapy underscores the critical need for translational research to address the global tuberculosis load.

**Keywords:** Mycobacterium Tuberculosis, Lipid Coated Nanocarriers, Enzyme Based Nano Delivery Systems, Nanotechnology, Anti-Microbial Peptides.

## INTRODUCTION

Mycobacterium tuberculosis complex (MTBC) is the cause of mycobacterial diseases in humans and animals. Mycobacterium tuberculosis, which causes tuberculosis in humans, is one of the most adaptable human infections. Many virulence traits of M. tuberculosis help the bacteria overcome mucosal barriers. The M. tuberculosis infection is caused by an interaction between microbe and the host immune system. Prompt and effective therapy is necessary in prevent the development of drug-resistant types of this illness. This implies that prompt and precise diagnostic methods must be accessible in order to handle affected cases.

### 1.1 Pathogenesis

In order to infect macrophages, M. tuberculosis, must cling to them and then proliferate inside of them. The primary stage of entry is the host-pathogen interaction, which shows the specific adhesion moleucles that the pathogen uses to allow bacterial entry. Many bacteria evolved particular adhesion proteins that cling to components frequently found on the surface of host cells (Zhang Y., 2018). Breathing is the primary way that M. tuberculosis enters the body.

Because M. tuberculosis can enter the lungs' alveolar airways and interact with immune cells called resident macrophages, breathing in airborne particles harboring the bacteria poses a serious risk to susceptible people. M.

tuberculosis can resist the immune response after being ingested by macrophages, which can result in infection (Meena P. R. 2016). There are two sizes of M. tuberculosis particles: bigger particles are typically retained in the airways, while small particles can enter the lungs more deeply. There are other avenues for tuberculosis infection through mucosa-associated lymphoid tissue (MALT) or epithelial cells, illustrating the variety of transmission channels, despite the prevailing belief that the initial infection occurs in alveolar macrophages (AMs).

Macrophages are crucial to the primary response to *Mycobacterium* infection because they either confine the bacterium or serve as a venue for bacterial multiplication. This bacterium can change the metabolism and signaling of host cells to improve its chances of surviving (Chandra P., 2022). Understanding how M. tuberculosis enters the body is crucial to combating tuberculosis. The bacteria enter into host macrophages, is the frist step in the development of infection. Once inside host cells, *M. tuberculosis* employs a number of survival strategies, such as immunological evasion and phagosomal maturation prevention, to survive and multiply. To fight this global pandemic that kills millions of people annually, it is essential to comprehend these pathways in order to develop new drugs, vaccines, and anti-tuberculosis agents (Gong W. 2021).

### 1.2 Mycobacterium Cell Wall

*Mycobacterium tuberculosis* (Mtb) is gifted with most complex of cell wall than any other bacteria. Major core is made of peptidoglycans which are linked to galactofuran and other mycolic acids via a linker unit. This mycolic acid, which forms the crosslinking makes the lipid barrier. The mycobacterium cell wall is divided into three segments namely membrane, outermost layer and the core cell wall. Since most of the cell wall is made of lipids (60%), the cell wall is hydrophobic (Ghazaei C. 2018). Arabinogalactan (AG) and the heavily cross-linked peptidoglycan (PG) layer that surrounds the plasma membrane combine to produce covalent compounds.

The physical barrier and thickness of the dynamic PG structure shield microorganisms from potential harm in the microenvironment (Justen AM, 2020). The major part of the cell wall is composed of superficial AG terminals, which are esterified with mycolic acid. The very hydrophobic mycolic acid envelops the bacilli to shield them from complement deposition, oxidative damage, and hydrophilic antibiotics.

The complex structure of the bacteria cell wall is linked to resistance to clearance in infected cells, with the lipid barrier serving as a barrier in hostile conditions. Low permeability is a result of hydrophobicity and the makeup of the cell wall. Additionally, Mtb possesses efflux pumps and the ability to control the components of its cell wall (Boparai JK, 2020).

### 1.3 Time to Search for Novel Antimicrobial Peptides

The search for novel antimicrobial compounds is not new, and peptides have been emphasized in several of these investigations. The three physicochemical characteristics of AMPs—net charge, structure, and solubility—have been used to categorize them. The majority of AMPs are soluble in the majority of organic media because they have both hydrophobic and hydrophilic side chains. AMPs may interact with most microbial membranes due to their positive charge, and their amphipathic characteristics cause them to enter into the membrane, creating pores that result in osmotic shock-induced cell death (Boparai JK, 2020). Cecropin, magainin, LL-37 and its derivatives and AMPs are some of the most significant types of AMPs in these groupings.

### 1.4 Why Nanotechnology

Strategies for delivering AMPs that enhance their stability and toxicity are made possible by nanotechnology. Lipid-based, metal-based and self-assembling nanoparticles are a few types of nanoparticles used to transport AMPs. For example, liposomes with antimicrobial peptides have demonstrated potent antibacterial action against P. aeruginosa and MRSA. Antimicrobial action against bacteria has been demonstrated using silver nanoparticles (AgNPs) (Bruna T, 2021).

Because the extracellular polymeric substances (EPS) that make up biofilm prevent antibiotics from penetrating biofilms, biofilm eradication is difficult. Polysaccharides, proteins, and polynucleic acids make up the EPS, which forms highly structured and diverse networks that are difficult for small molecule medicines to penetrate. Because of this, the current approaches to eliminating biofilms usually include the removal of contaminated tissues together with antibiotic therapy—invasive procedures that are expensive.

Nanomaterials have become a promising platform for medicinal and diagnostic uses. Nanomaterials' size provide them special chemical and physical characteristics, such as multivalent interactions with cellular and biomolecular systems. Because of their inherent properties, nanoparticles can be employed therapeutically as active therapeutic agents or as a means of delivering medications to the site of infection.

To break these challenges, hybrid nanoparticles are therefore preferred. A hybrid nanoparticle consist of a core polymer which engulfs the drug, while its surface is coated with another material to avoid the MPS. Such hybrids through a coating layer, suppress any drug release, allowing for precise targeting.

**1.5 Recent Developments**

In order to penetrate the thick, hydrophobic Extracellular Polymeric Substance (EPS) barrier of mycobacterial biofilms, recent developments concentrate on using stimuli-responsive and lipid-coated hybrid nanoparticles. Modern treatments can actively target the biofilm matrix and deliver medicines directly to the embedded bacteria by altering the hydrophobicity and surface charges of nanoparticles.

**1.5.1 Lipid-Coated Hybrid Nanoparticles (LCHNPs)**

LCHNPs interact electrostatically with the negatively charged elements of the EPS matrix by creating a significantly positive (cationic) surface charge. Hydrophobic antimicrobial medicines, such as anti-TB medications, can dissolve and pass through the lipid-rich layers of mycobacterial biofilms thanks to their highly adjustable vehicles (Buya, A. B., 2021). Drug-loaded nanoparticles have been thoroughly researched and assessed as therapeutic delivery platforms during the past few decades to address the inherent drawbacks of existing antibiotic therapies.

LCHNPs are made up of an organic or inorganic nanoparticulate core covered in one or more layers of lipids, which can be derived lipids (like cholesterol), compound lipids (like phospholipids), or simple lipids (like DOTAP). The LCHNP, which has a core-shell structure, shows promise as a robust and adaptable therapeutic (K. M. Vargas 2019). These characteristics include the ability to encapsulate hydrophilic and lipophilic medicinal substances, with good biocompatibility.

Lipid-coated hybrid nanoparticles (LCHNPs) are composed of a biocompatible lipid shell and a robust polymer core. They significantly improve drug delivery and therapy efficacy against Mycobacterium because they target infected macrophages and penetrate the bacteria's notoriously thick, lipid-rich cell wall.

By promoting fusion between the bacterial membrane and infected host cells, the lipid coating promotes the intracellular accumulation of antitubercular drugs.

Since, Mycobacterium TB hides inside macrophages to hide from the host, LCHNPs can be designed so as to target these macrophages. These remove a major cause of multidrug resistance by harming the mycobacterial biofilms (Jiang, L., 2020).

**1.5.2 Enzyme-Immobilized Delivery Systems**

In the recent years, effective systems which conjugate anti-biofilm enzymes with nanoparticles are being developed. These enzymes not allow easy penetration but also actively dissolves the EPS framework (proteins, polysaccharides). Some of the enzymes used in this regard are proteases, amylases, and dispersin B.

After the scaffold dissolves, the nanoparticles efficiently diffuse into the center of the biofilm. The highly organized, protective 3D matrix (biofilm) that shields dormant Mtb can be effectively broken down by enzymes such as β-glucosidase, glucose oxidase, and ∑-amylase [Varshiny Gopinath, 2024]. By aggressively breaking down the bacterial lipid-rich barrier, immobilized enzyme systems can lower the required inhibitory concentration of antibiotics (minimum inhibitory concentrations have been shown to be 16-fold lower when using LysB composites).

Delivery vehicles are designed to directly enter the host's macrophages, such as liposomes or mesoporous silica nanoparticles. This preserves healthy tissue while delivering the enzyme-drug payload straight to the infection location. Targeted nano-formulations greatly reduce systemic toxicity by raising therapeutic concentrations precisely where the germs are found, such as the lungs.

**1.5.3 Stimuli-Responsive Platforms:**

In response to particular microenvironmental triggers inside the biofilm (e.g., changing pH, specific enzymes, or hypoxia), advanced nanoparticles have been engineered to change their shape or release their payload. Internal and external triggers are the two main categories into which they fall. Endogenous and exogenous.

**Endogenous:** These systems exploit the natural physiological changes that occur inside infected tissues and macrophages:

a. **pH-Responsive Delivery:** Mtb usually lives in necrotic caseum or the acidic environment of macrophage phagosomes (pH = 4.5–5.5). Acid-cleavable polymers or coatings, such as chitosan-coated PLGA, are used in pH-sensitive nanocarriers to dissolve or alter form in acidic pH, resulting in tailored drug release [Elbehiry, A., 2026].

b. **Redox-Responsive Platforms:** Oxidative stress is a characteristic of TB lesions. High levels of intracellular glutathione (GSH) or reactive oxygen species (ROS) can split carriers made with disulfide bonds, releasing antitubercular drugs [Fang, R., 2024].
c. **Enzyme-Responsive Systems:** In order to ensure localized antibiotic release at the infection site, these platforms include substrates that are broken down by enzymes like lipases or gelatinase that are overexpressed during Mtb infection.

**2. Exogenous:** Using external inputs, these techniques enable physicians to manually regulate the dosage and timing of drug release:

a. **Magnetic-Responsive:** Iron oxide-containing nanoparticles can be directed to the lungs by external magnetic fields, and an alternating magnetic field can then cause hyperthermia, or heat, which releases medication locally [M. Colilla, 2020].
b. **Photo-Responsive:** When subjected to Near-Infrared (NIR) light, which may penetrate deep tissues and cause photothermal or photochemical release, nanocarriers can be designed to release their payload [M. Colilla, 2020].
c. **Ultrasound-Responsive:** High-frequency sound waves can be used to destabilize certain liposomes or microcapsules, causing quick drug release exactly where it's needed.

### 1.5.4 Physical Synergy:

Combining nanoparticles with physical stimulation, like Low-Frequency Ultrasound (LFUS), has significantly increased their penetration efficiency into dense biofilm matrices. Low-Frequency Ultrasound (LFUS) in the range of 20-100kHz are used in treating tuberculosis. This LFUS have improved the culture decontamination and also helped in targeted drug delivery [Xie, S., 2020]. Shock waves and microstreaming are produced when LFUS causes acoustic cavitation, which is the quick formation and collapse of microbubbles. Mycobacterium tuberculosis (MTB) biofilms' protective extracellular matrix may be damaged by these forces.

According to research, LFUS makes mycobacterial cell walls and biofilms more permeable, which makes anti-tuberculosis medications (such levofloxacin and ciprofloxacin) significantly more efficient at penetrating and killing the germs than medications by themselves. Compared to conventional chemical decontamination techniques (such as NaOH-NALC), LFUS may efficiently remove rapidly developing contaminants without damaging MTB, leading to higher isolation and recovery rates. [P. Tian, 2024].

### 1.5.5 Mesoporous Silica Nanoparticles (MSNs):

These work as biomimetic carriers to greatly increase the intracellular absorption of medications into infected cells because they are coated in mycobacterial lipids [C. W. Ang, 2022]. Mesoporous Silica Nanoparticles (MSNs) are sophisticated, focused nanocarriers that combat Mycobacterium tuberculosis (Mtb). Their large surface area, adjustable porosity, and functionalizable surfaces allow treatments to be delivered intracellularly to infected macrophages and increase the bioavailability of poorly soluble medications. MSNs can have pH-sensitive valves or be coated with polymers (like polyethyleneimine). This allows them to target acidified endosomes inside macrophages and release drugs (such rifampin or isoniazid) exactly where Mtb hides.

### 1.5.6 Carbohydrate-Functionalized Carriers:

Lipid nanocarriers targeted with fucosyl residues send the nanoparticles straight into the endosomal compartments where Mycobacterium thrives by engaging with macrophage mannose receptors. These make up an advanced targeted delivery system for the treatment of Mycobacterium tuberculosis (Mtb). By coating nanocarriers (such liposomes or polymeric nanoparticles) with specific sugars, researchers can target immune cells and exploit the bacteria's natural invasion mechanisms.

Mycobacterium mostly survives and replicates in alveolar macrophages. Carriers functionalized with sugars like fucose or mannose immediately bind to the Macrophage Mannose Receptor (MMR). Consequently, the immune cells aggressively absorb the nanomedicine by receptor-mediated endocytosis.

Nanomaterials exhibit significant potential as both delivery systems and standalone antimicrobials, particularly in targeting planktonic bacteria due to their multivalency and tunability. They are also considered promising treatments for difficult illnesses as they can effectively break down biofilms. Nevertheless, to fully harness the antimicrobial capabilities of nanoparticle-bacterial systems, extensive research is required to better understand their fundamental biological and pharmacological properties.

## CONCLUSION

New drug delivery methods must be developed due to the rising incidence of infectious diseases and the microorganisms' developing resistance to existing treatments. By improving bioavailability and precise targeted delivery,

the special physicochemical characteristics of nanoparticles offer a workable solution for common drug formulation limitations. This surely enhances the therapeutic efficacy while also diminishing the drug resistance.

However, there are several challenges in creating effective nanodrug delivery systems, from the complexities of microbial resistance to the challenges posed by intracellular and biofilm-related disorders. However, the manufacturing procedure and drug loading of the nanoscale devices still require refinement. Combination therapy, better diagnostics, ethical antibiotic use, and the creation of novel antibiotics are strategies that make the use of nano drug systems easier. The primary cause of Mycobacterium TB's persistent threat to global health is its formidable, thick, lipid-rich cell wall, which provides innate resistance to conventional antimicrobials. In addition to restricting drug access, this impermeable barrier encourages lengthy treatment plans and the development of multidrug resistance. This area is a sophisticated development in this area.

Carbohydrate-based carriers mimic host glycoconjugates to increase absorption and extend delivery, whereas lipid-coated carriers promote solubility and membrane fusion. For targeted drug release, enzyme-based nano delivery devices take advantage of pathogen-specific enzymatic activity. Their integration into clinical practice may reduce the length of treatment, address drug resistance, and fundamentally alter how drug-resistant tuberculosis is managed.

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