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Review

PRNANO

Precis. Nanomed. 2023, 6(4):1134-1156

ANDOVER HOUSE

Recent Advances in Nanocarrier-Based Therapeutic and Diagnostic Approaches in Tuberculosis

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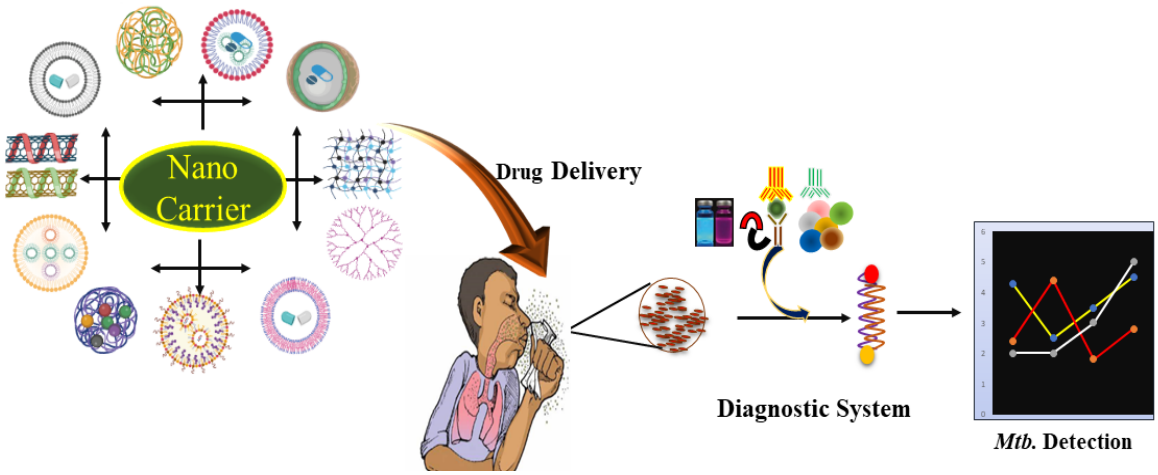
Submitted: August 25, 2023 Accepted: November 16, 2023 Published: November 29, 2023

Graphical Abstract



Abstract:

Tuberculosis is the second most fatal infectious disease; each year, it causes millions of deaths worldwide. Although several anti-TB drugs are currently available, the problem with these drugs is that they require a prolonged duration of treatment, with high drug doses. In addition, patient non-adherence to therapy subsequently leads to the development of multidrug-resistant and extensively drug-resistant tuberculosis. Therefore, we need to develop an effective and robust nano-carrier-based drug delivery system to overcome these issues and improve the therapeutic potential of drug dose/duration and patient compliance. This review article focuses on the currently available various nanotechnology-based therapeutic approaches, including lipid nanoparticles, polymeric particles, carbon nanotubes, glucan, and alginate-based nanocarrier systems for effective anti-TB drug delivery to host macrophage for mycobacterium killing. Finally, we also present some promising recent nanotechnology-based mycobacterial detection systems using silver nanoparticles, silica nanoparticles, magnetic nanoparticles, quantum dots, and magnetic barcode assay that have recently emerged as the latest diagnostic tool. Functionalized nanomaterials for emerging Aggregation-induced photodynamic therapy as next-generation theranostics have also been discussed as a novel option for T.B. diagnosis and therapy.

Keywords: Tuberculosis, Carbon nanotube, Quantum dots, Photodynamic therapy, Magnetic nanoparticles

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Purpose, Rationale, and Limitations

Tuberculosis is a highly contagious disease caused by Mycobacterium tuberculosis. It is responsible for the highest number of deaths and will soon surpass the deaths caused by HIV. The goals of new therapeutic approaches are to ensure a cure without relapse to inhibit deaths, contagions, and the formation of drug-resistant strains. The main directions of antitubercular therapy involve either the development of a new chemical entity with a novel mechanism of action or repurposing of old drugs that show significant activity on drug-resistant strains. Repurposing existing drugs is a promising alternative to the expensive and time-consuming process of drug discovery. Nanotechnology-based anti-TB drug delivery systems offer various advantages over conventional methods. These nanocarriers encapsulate hydrophilic and hydrophobic drugs and enhance their intracellular drug bioavailability by improving the drug release kinetics. By using these nanocarriers, we can deliver the drug in a targeted manner, with minimum drug dose and duration, and minimize the adverse effects of the drug. So, in this review article, our primary focus is to describe the different types of nanotherapeutic approaches: liposomal-mediated drug delivery, solid lipid nanoparticles, polymeric nanoparticles, dendrimers, nanoemulsions, yeast-derived β-glucan, alginate. In the last section, we summarize some of the latest nanotechnology-based diagnostic approaches, such as silver nanoparticles, silica nanoparticles, magnetic nanoparticles, quantum dots, and biosensors. These are the latest diagnostic tools with broad-spectrum biomedical applications in tuberculosis and other diseases. Using these functionalized nanomaterials for emerging aggregation-induced photodynamic therapy (PDT) as next-generation theranostics has also been discussed as a novel option for T.B. diagnosis. This review covers the prospect of using nanotechnology to detect mycobacterial strains and nanotechnology-based anti-TB drug delivery systems potentially capable of effectively eradicating Mycobacterium tuberculosis infections.

Introduction

Tuberculosis (T.B.) is one of the oldest and deadliest infectious diseases caused by *Mycobacterium tuberculosis(M.tb.),* Tuberculosis is highly persistent in developing countries, including China (8.4%), the Philippines (6.0), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%), South Africa (25%), with India (26%) as the uppermost TB-burden countries in the world [1, 2]. *M.tb.* is a non-motile, obligate, acid-fast, intracellular bacteria that causes T.B. by inhalation of aerosolized droplets expelled from individuals with active pulmonary T.B. by coughing or sneezing of bacilli. *M.tb.* mainly infect the lungs and reside within it. Still, it can also affect other parts of the body, including (the kidney, central nervous system (meningitis), lymphatic system, circulatory system (miliary tuberculosis), genitourinary system, and bones [2, 3]. Lungs are rapidly phagocytosed by macrophage cells and targeted to destruction by an innate and acquired immune response. Mycobacteria have evolved numerous intracellular survival strategies to inhibit the intracellular defense mechanisms such as phagolysosomal fusion and autophagy and thus avoid being killed by the macrophage, and instead growing and replicating inside the macrophage [1,3,4]. Based on the pathogenesis of *M.tb*. and the immune response by the host, the infection can progress to active T.B., which is progressively more dangerous, wherein bacteria multiply within macrophages, spread, and cause extensive tissue damage. In latent TB, bacteria persist for a prolonged period in a dormant state and asymptomatic manner without any visible disease symptoms [5-7], and when the body is immunocompromised or co-infected with HIV, these dormant *M.tb.* bacteria become active and cause severe infection in the body. Latent T.B. activation and mycobacteria re-infection pose a greater challenge for conventional anti-TB therapy [6-7].

Current Drug regimens for T.B.

Due to the emergence of drug resistance in mycobacteria, treatment of T.B. is still quite complex, and the currently available chemotherapeutic drugs having different modes of action are usually given as combination therapy for at least 6 months up to 1 year. Depending upon the bacterial infection and their resistance profiling, the treatment duration may extend up to 2 years for multidrug-resistant (MDR) T.B. [4-6].

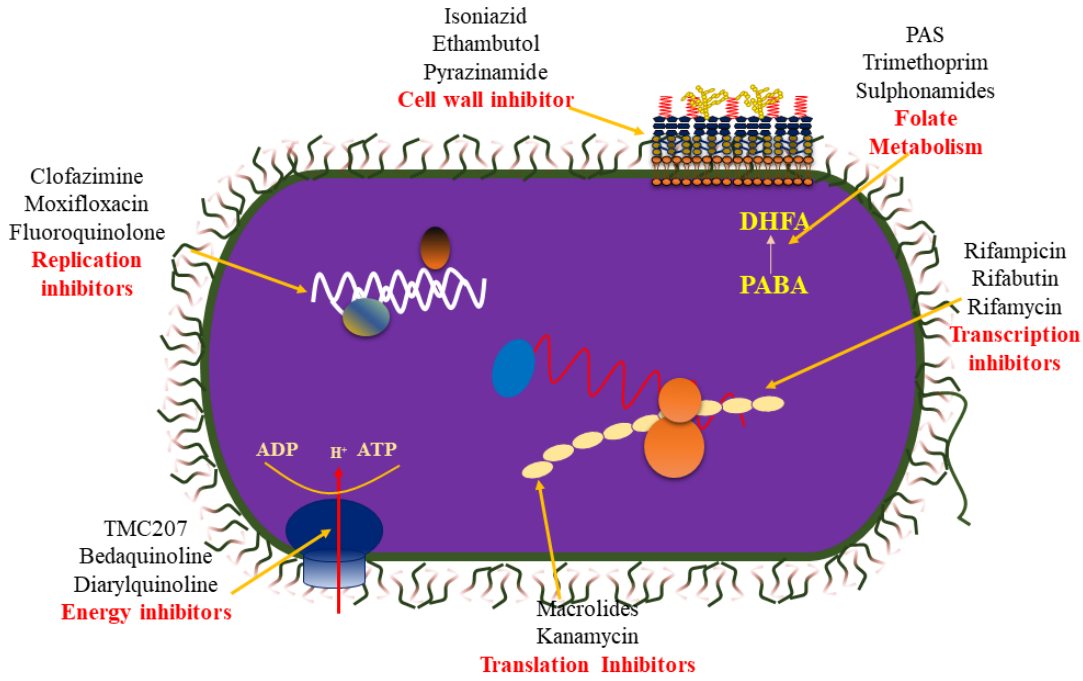

Figure 1: Different types of anti-TB drugs and their mechanism of action.

First-line anti-TB drugs

Usually, four first-line anti-TB drugs, isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) are used for the treatment for more than six months in combination. Initially, INH, RIF, PZA, and EMB are used for two months, followed by INH and RIF for the subsequent four months. The therapeutic efficacy of the first-line anti-TB drugs is high, and it restricts bacterial replication [8, 9].

Second-line anti-TB drugs

Second-line anti-TB drugs have lesser efficacy, are too toxic and costly, and need longer treatment duration as compared to first-line anti-TB drugs **[10].** These are, therefore, given to patients when the bacteria has shown resistance against one of the first-line anti-TB drugs, either RIF or INH, both of which are very potent anti-TB drugs, the situation termed as multidrug resistance (MDR-TB) [9-10].

Third line of anti-TB drugs

The third line of anti-TB drugs is the most advanced form of medication used in T.B. treatment. They are less effective and have a lower safety profile because the adverse effect of these drugs is much higher as compared to first and second-line drugs [8-11]. They are only recommended when the bacteria have acquired resistance against at least isoniazid and rifampin (first-line drugs), a fluoroquinolone, and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin), the situation is termed as XDR-TB 5. The therapeutic efficacy of the drugs is determined by strict treatment adherence and other daily routines. Due to the emergence of drug resistance in mycobacteria, treating T.B. has become quite complicated [9,11,12]. Current conventional therapy relies on the administration of anti-TB drugs by oral or intravenous routes, implying that the drug(s) do not specifically reach the active site in the lungs but are non-specifically distributed in the body through the systemic blood circulation system and, thus, subsequently lead to other complications and toxic effects in the whole body. In addition, the rapidly increasing surge in resistance against existing drugs in recent years has dictated an urgent need to develop novel immunotherapy and potentially more effective medication(s) that would significantly reduce the treatment time [8,11,13].

Nanotechnology-based drug-delivery systems for T.B. treatment

Nanotechnology-based drug-delivery systems have recently emerged as one of the most

promising research areas, with several broad biomedical, clinical, and pharmaceutical applications. Nanotechnology-based drug delivery has various advantages over free drug delivery, as it enhances the bioavailability of drugs in a controlled and targeted manner and thereby minimizes drug-associated patient non-compliance issues [29, 30]. Nano-formulations allow various shape, size, and composition modulations, significantly affecting the drug's intracellular therapeutic efficacy [31]. Due to the high volume-to-surface ratio, these nanoparticles have the potential to reach the deep lung area and release their intracellular drug at the site of infection [29-33]. Using these techniques, we can deliver the drug at the desired site of action, lower the dose and duration, lower the emergence of drug resistance, and minimize the adverse effects of drug toxicity [30]. This review article will discuss the biological features and applications of various nanocarriers, particularly emulsions, micro/nanoparticles, CNT, and their biomedical applications.

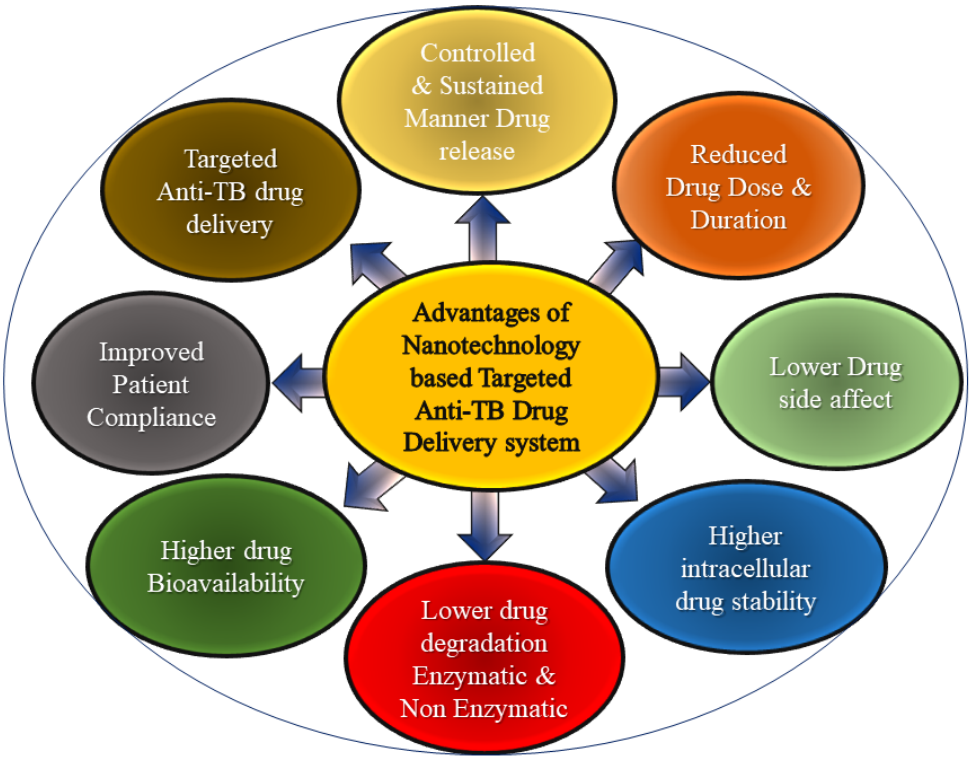

Figure 2: Advantages of Nanocarrier-based Anti-TB drug-delivery system.

Solid lipid nanoparticles (SLNs)

SLNs are one of the phospholipid-derived colloidal systems composed of the phospholipid-derived monolayer with a hydrophobic core that provides space for the entrapment of drug molecules. Due to the presence of the hydrophobic matrix of the SLNs, they can serve as carriers for both the hydrophilic and lipophilic drug molecules. Due to this intrinsic property, SLNs form a promising approach for the combined delivery of various drug molecules and thus find application in drug delivery systems to overcome drug bioavailability and stability issues [35-37]. Several *in vitro* studies have confirmed that SLNs have higher drug retention time in circulation than free drugs. In guinea pigs, first-line drug-encapsulated SLNs have shown significantly higher antimycobacterial efficacy against the bacteria than free drugs [35]. Another *in vitro* study has shown that rifabutin-loaded mannosylated SLNs exhibit higher cellular uptake due to the presence of the mannose coating and also showed higher therapeutic efficacy than free drugs [36]. Other rifampicin-loaded SLNs have higher drug entrapment, 85% efficiency, and higher antimycobacterial activity against the *M.tb.* than free rifampicin [37,38].

Liposomes-based drug-delivery systems

Liposomes, discovered accidentally by Dr. Alec. D. Banghamam, a British hematologist,

in 1961, are spherical microscopic aqueous vesicles surrounded by a lipid membrane(s) composed of natural phospholipids like phosphatidylcholine, serine, ethanolamine, and inositol. Due to their amphipathic nature, they can encapsulate lipophilic and hydrophilic drugs [39-40]. The liposome size is ~0.05 µm to 5 µm and can be synthesized in a desired shape, size, surface composition, and lamellar composition [40]. Liposomes are selectively captured by the phagocytic cells and subsequently undergo lysosomal fusion for enzymatic degradation [39]. Then, they release the encapsulated drug within phagocytic cells [40-41]. There are several benefits of the liposomal system as a drug-delivery system. They are non-toxic, non-immunogenic, biocompatible, and biodegradable, making them suitable carriers for various therapeutic biomolecules such as drugs, siRNA, proteins, enzymes, and vaccines [42]. Liposome-mediated drug delivery enhances therapeutic efficacy by protecting the encapsulated drug from degradation and allowing sustained drug release at the target site. It is successful against intracellular pathogens, which can get deep in the lungs, and is thus suitable for lung-associated diseases such as pulmonary TB [44-46].

Table 1. Liposome-based anti-TB drug-delivery systems

	Liposomes	Encapsulated Anti-TB Drugs	Biological Property	Ref.
1	Di-palmitoyl phosphatidyl choline	Isoniazid, Rifampicin, Pyrazinamide, Ethambutol	Formulation shows higher drug entrapment efficiency by effectively making sustained/prolonged drug release kinetics.	[47]
2	Di-Palmitoyl Phosphatidyl choline	Isoniazid	Study shows controlled and sustained release of entrapped isoniazid from liposomal formulation.	[48]
3	Cholesterol Phosphatidylcholine	Rifampicin Isoniazid	The formulation has shown higher drug entrapment efficiency.	[49]
4	Cholesterol, dicetyl phosphate, O-SAP and monosialogangliosides	Isoniazid,	Formulation shows the high efficiency of drug entrapment.	[44]
5	Cholesterol Phosphatidylcholine	Isoniazid, Rifampicin	Formulation shows a potent antimycobacterial effect and improved drug pharmacokinetics.	[50]
6	Di-palmitoyl, cholesterol	Pyrazinamide	Formulation shows better entrapment efficiency and potent antimycobacterial in mice.	[51]

Niosomes-based drug-delivery systems

Niosome has gained significant attention as a drug-delivery system by the scientific community. A niosome is usually a bilayer structure of 10 nm to 100 nm, formed by self-assembling the non-ionic surfactant and cholesterol molecule in an aqueous system [52]. Thus, they can encapsulate lipophilic and hydrophilic drug molecules [53]. These are non-toxic, biodegradable, biocompatible, thermodynamically stable, and cost-effective, and are, therefore, ideal carrier systems to deliver hormone peptide antigen molecules in a targeted manner [54]. Niosome biosynthesis and stabilization requires non-ionic surfactants (span-60, span-80). Niosome-encapsulated Isoniazid and Pyrazinamide have shown sustained drug release kinetics from the niosomic lipid membrane, enhancing the encapsulated drugs' intracellular bioavailability [36-41].

Dendrimers

Historically, Vögtle's principal work on cascade synthesis [59a] opened the door for dendrimers, a term first used by Tomalia [59b]. Dendrimers are highly branched polymeric, nearly monodispersed, symmetric, and spherical structures that allow the covalent or non-covalent binding of drugs [60,61]. The different functional groups on the external surface and/or internal core space may be used to entrap drug(s). Dendrimers can also be chemically modified to improve the biophysical and biochemical properties of the drug molecules [62]. For example, Rifampicin-conjugated G4 PAMAM dendrimers have been shown to exhibit sustained drug release kinetics and enhanced intracellular drug bioavailability [29-62].

Table 2. Niosomes-based anti-TB drug-delivery

SN	Niosomes	Encapsulated Anti-TB Drugs	Biological Property	Ref.
1	Cholesterol, Dicetyl phosphate, stearyl amine	Ethambutol	Enhanced drug-loading efficiency by niosome system and improved drug efficacy.	[55]
2	Dicetyl phosphate, cholesterol, stearyl amine, span 60	Pyrazinamide	This study found the highest percentage of pyrazinamide-loading efficiency using surfactant span-60.	[51]
3	Cholesterol, span-80, span-85, Triton X-100, diethyl ether	Rifampicin	Entrapped drug rifampicin-loaded niosomes are effective targeting *M.tb.*	[56]
4	Triton X 100, PEG 2000, span 80	Rifampicin, Isoniazid	Enhanced entrapment efficacy rifampicin and isoniazid niosomal vesicle.	[57]
5	Dicetyl phosphate diethyl ether, sorbitan, monostearate, cholesterol	Isoniazid	Isoniazid-loaded niosomal vesicles are higher entrapment and potent therapeutic efficacy.	[58]

Micelles:

Micelles are one of the most promising nanocarrier systems prepared by self-assembling phospholipid molecules in an aqueous environment. Micelles are biocompatible, biodegradable, amphiphilic nanostructures that have also been used as drug-delivery systems. Micelles have outer hydrophobic shells and internal hydrophilic cores, allowing efficient delivery and increasing the bioavailability of poorly soluble drugs. Micelles conjugated with anti-TB drugs PZA and INH have shown higher drug bioavailability with improved solubility of the incorporated drug [63-64]. Similarly, nano-engineered INH-RIF-conjugated polymeric micelles have shown significant antimycobacterial activity and were much more effective against the bacteria than free drugs [65]. In another study, Rif-loaded HPMA-PLA nano-polymeric micelles showed potent and improved antimycobacterial activity in resistant and sensitive *M.tb.* Strains [66].

Micro/Nano-emulsions-Based Anti-TB Drug-Delivery Systems:

The concept of a microemulsion-based drug-delivery system was first introduced by Hoar and Schulman in 1943. Microemulsion is a combinational mixture of oil in water, in which surfactant molecules are added to obtain a thermodynamically stable emulsion system. Microemulsions have shown several beneficial effects, including protection of loaded drug(s) from degradation, reduced drug-associated toxicity, and enhanced intracellular drug bioavailability. In recent years, microemulsion-mediated drug-delivery systems have gained much attention due to their safety, easy preparation,

and higher stability of these formulations. Thus, they may play a promising role in effective anti-TB drug delivery [68]. These systems have been found to possess high diffusion and absorption rates and allow the delivery of hydrophilic and lipophilic drugs in a targeted manner [69]. Nanoemulsions are 10-100 nm colloidal particulate systems; they are more recent versions of fine emulsions that have lately emerged as drug-delivery systems. Administration of anti-TB drugs as nanoemulsions via the oral route has been shown to enhance the bioavailability of encapsulated medicines [70]. Recently, a study has shown that rifampicin-loaded nanoemulsions efficiently lower the bacterial burden of ocular T.B. [71]. Emulsions are classified into broad categories as below: oil in water (o/w), water in oil (w/o), oil in water in oil (o/w/o), and water in oil in water (w/o/w) [72].

Table 3. Emulsion-based anti-TB drug-delivery

S.N.	Emulsion	Encapsulated Anti-TB Drugs	Biological Property	Ref.
1	Tween 80, Oleic acid, Ethanol	Pyrazinamide Rifampicin, Isoniazid	Formulation shows better release kinetics with a combination of the multi-anti-TB drug rather than alone, enhancing the encapsulated drug's stability.	[73,74]
2	Sefasol, Tween 80	Rifampicin	The stability of microemulsion is high; microemulsion enhances the drug's strength and improves release kinetics.	[75]
3	Nile red, Brij 96, Ruthenium Dichlorides	Pyrazinamide Rifampicin, Isoniazid	This microemulsion system has shown that rifampicin has a strong interaction dye with pyrazinamide/isoniazid has interaction with ruthenium dichloride.	[60-61]
4	Tween 80, Oleic acid, Ethanol	Rifampicin	An optically clear/ highly stable microemulsion is obtained between the w/o and o/w systems. The microemulsion exhibits controlled/sustained release kinetics.	[68]
5	Tween 80, Oleic acid, Ethanol	Isoniazid	The stability of the microemulsion is high; sustained/controlled release of the drug.	[76]

Polymeric microparticle-based anti-TB drug delivery systems

In the last decade, different polymeric microparticles have gained significant attention because of their non-toxic, biodegradable, biocompatible nature and high stability. These polymeric microparticles can be functionalized by coupling macrophage receptor-interacting ligands such as mannan, galactose, Folate, and N-acetyl glucosamine, enabling efficient particle uptake to macrophage [77]. Various biocompatible and biodegradable polymers have been used to prepare microparticles and nanoparticles, as listed below.

Polylactide (PLA)

Polylactic Acid (PLA) is an eco-friendly, biocompatible, non-toxic biopolymer. This bioactive, recyclable thermoplastic aliphatic polyester is derived from renewable resources, like corn starch, cassava roots, chips or starch, or sugarcane. PLA has also been approved by the U.S. Food and Drug Administration (USFDA) for various medical applications, including the correction of facial fat loss connected with antiretroviral therapy-induced lipoatrophy in HIV patients [78-79] to correct nasolabial fold deficiencies and other facial wrinkles. Due to these properties, PLA finds broad-spectrum applications as an absorbable, injectable implant that progressively restores volume and accelerates collagen formation. PLA also offers promise for delivering various chemotherapeutic drug molecules and tissue engineering. Microparticles incorporating anti-TB drugs INH and RIF have shown higher therapeutic potential as compared to free drugs [66-80] *in vitro* as well as *in vivo* studies [81]. Mouse macrophage *J774* cells efficiently take up these microparticles,

leading to higher intracellular drug concentrations in contrast to equivalent amounts of drugs *in vitro* [82]. Another study has shown that rifampicin and isoniazid-loaded PLA microparticles induce classical activation within mycobacteria-infected mouse macrophages and have a significantly higher antibacterial effect against *M.tb.* as compared to that by an equivalent amount of drugs [80].

Additionally, the intratracheal administration of these rifampicin and isoniazid-loaded PLA microparticles to rats leads to the development of much higher intracellular drug concentrations within host macrophage than that by oral route of administration. All these studies have exhibited that phagocytic uptake of these particulate polymeric drug carriers delivers a bolus of encapsulated drugs to mycobacterium-infected macrophages much more effectively than that by the diffusive uptake of drugs dissolved in body fluids, as in the case of conventional oral administration [83]. Another study has also confirmed that the therapeutic efficacy of rifabutin against mycobacteria is significantly enhanced by loading within inhalable polymeric PLA-based microparticles [80-84].

Poly (lactide-co-glycolide) (PLGA)

Polylactic co-glycolate acid (PLGA) polymeric microparticles are also used for targeted delivery of anti-TB drugs to macrophages. The chemical properties of PLGA polymer, including the degree of branching and chain length of monomers, greatly influence encapsulated drugs' pharmacokinetics [85-88]. Recently rifampicin-loaded PLGA (R-PLGA) polymeric microparticles were prepared and their therapeutic effect against mycobacteria was evaluated, where it was found that R-PLGA microparticles phagocytosed by macrophage cells and exert more potent bactericidal influence on *Mycobacterium bovis Bacillus Calmette-Guérin* infected cells than soluble rifampicin [85]. The administration of R-PLGA microparticles to Wistar rats led to a 10 times higher amount of rifampicin in alveolar macrophages than free drug [87]. Also, the administration of R-PLGA microparticles to *M.tb.* infected Sprague-Dawley rats led to efficient bacterial killing and prevented granuloma formation within their lungs [88]. The therapeutic efficacy of these particles has shown a significant antimycobacterial effect and lowered the intracellular bacterial burden within the lungs.

Polycaprolactone (PCL)

Polycaprolactone (PCL) is a non-toxic, biodegradable semicrystalline polymer used as a carrier for various drugs in various diseases, including cancer, diabetes, TB, etc. The polycaprolactone-based drug-delivery system efficiently delivers therapeutic drug molecules without causing any adverse effects [89]. Recent *in vitro* and *in vivo* studies have shown that the intracellular antimycobacterial product of isoniazid-loaded PCL (I-PCL) microparticles induces the generation of intracellular nitric oxide (NO). Administration of I-PCL microparticles within the rats minimized the severity of the drug-associated toxic effects and is thus expected to improve patient compliance [90].

Yeast-derived β-Glucan Particles-Based Anti-TB Drug-Delivery Systems

β-Glucans are natural carbohydrate polymers, mainly found in the cell walls of microbes such as bacteria, fungi, mushrooms, and yeast, and also in cereals such as barley and oats. β-Glucans are structurally different from species to species in their degree of branching and chain composed of glucose residues with a backbone of β (1→3)-linked β-D-glucopyranosyl units with β(1→ 4) - or β(1→6)-related side chains [91]. β-glucans have been ingested for possibly thousands of years, especially in China and Japan, and have long been considered to improve general health [92]. β-Glucans have been granted the GRAS (Generally Regarded As Safe) status by the US FDA, and the European Food Safety Authority also recommends it for proper body functioning. Recently, yeast-derived particulate β-Glucans have been developed as hollow porous 2 to 4 μm particles, and the hollow cavity of these particles has been used to encapsulate various therapeutic molecules such as drugs, DNA, siRNA, and proteins [93-94]. β-Glucan particles (G.P.s) have been used for therapeutic and prophylactic applications against various diseases, including cancer, diabetes, T.B., etc [95]-[96]. In our lab, we used glucan particles as a carrier for pulmonary drug delivery. Rif has been encapsulated (precipitated) inside the G.P.s using a hydrogel matrix composed of calcium alginate or chitosan that seals the G.P. pores, slowing down drug kinetics [97]. Such particles contain precipitated nanoparticles of Rif caged within hollow beta glucan microparticles. β-Glucans also behave as "natural polysaccharide immunomodulators"

and are known for their ability to stimulate the immune system. β-glucan acts as a pathogen-associated molecular pattern, identified by various pattern recognition receptors on macrophages, such as toll-like receptors, Dectin-1, lactosyl ceramide receptor, and scavenger receptors. These things make G.P. an ideal drug-delivery vehicle to target phagocytic cells in the immune system. GP-based Nano-in-Micro (NIM) delivery systems containing anti-TB drug rifabutin (R.B.) have been prepared to provide sustained delivery of the drug [98]. *In vitro,* preliminary efficacy testing of these NIM glucan particles in mycobacteria-infected macrophage cell lines exhibited that the GP-RB formulations (containing ~4 µg/ml R.B.) were effective at reducing colony-forming units of *M. tuberculosis* strain *H37Ra* by 98.7% within 72 h. In contrast, administering 2.5× higher soluble R.B. (10 µg /ml) resulted in ~90% reduction in CFU [97]. This demonstrates that GP-targeted RB delivery to macrophages significantly enhances antimicrobial effects.

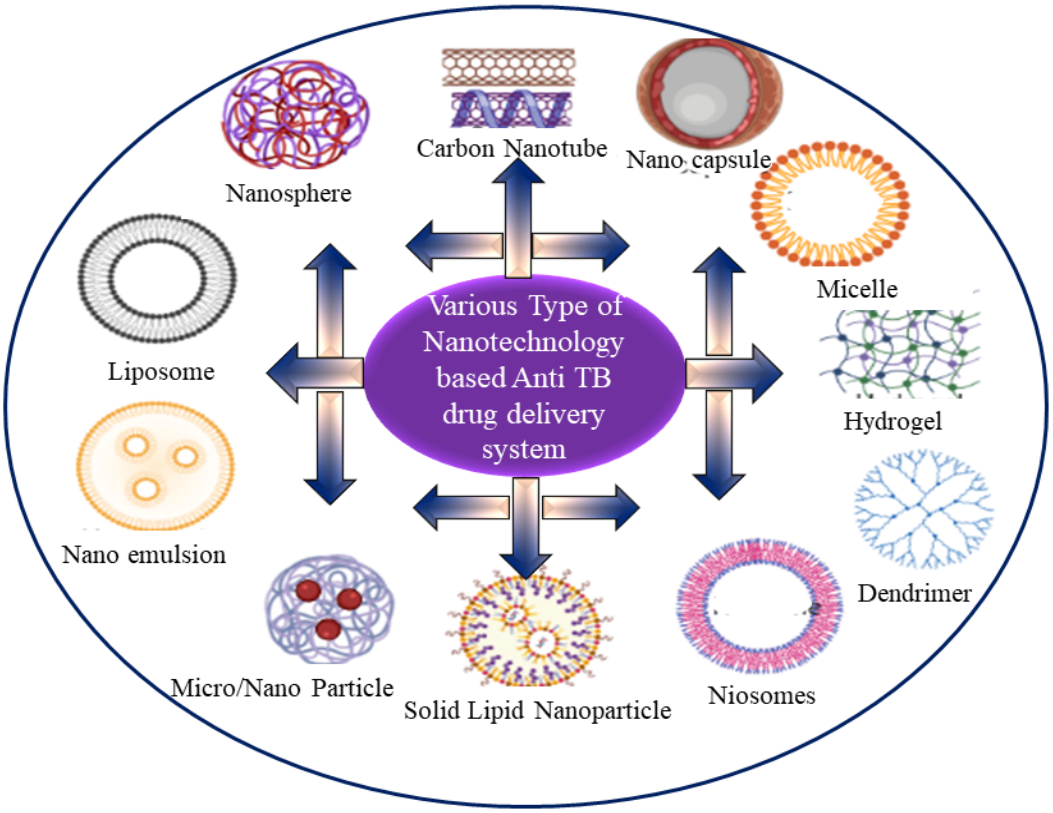

Figure 3: Different types of nanocarrier-based anti-TB drug-delivery systems

Alginate-Based Systems

Alginate is a natural polymer with broad applications in the food industry, cosmetics, and pharmaceuticals as gelling/solidifying agents. Alginate is a non-toxic, biodegradable, biocompatible polymer approved by USFDA [99]. Alginate has various intrinsic biological properties such as epithelium adhesiveness, high porosity, control, and sustained release kinetics, enabling its use as a drug-delivery system in therapy against cancer and T.B. [100]. The use of the ionotropic gelation method for encapsulation of anti-TB drugs within an alginate matrix has shown significant enhancement in its drug-loading efficiency, *viz.*, 80–90% for rifampicin, 70-90% for isoniazid and pyrazinamide and 88 to 95% for ethambutol, [101]. Alginate-based nanoparticles have been standardized as carriers for pulmonary drug delivery systems [99-103]. Inhalable alginate-based nanoparticle formulations were seen to impart significant enhancement in drug bioavailability and antimycobacterial effect as compared to that by free drug(s) [103]. The alginate particles incorporated anti-TB drugs at 70-90% encapsulation efficiency and sterilized the lungs in three doses administered at 15-day intervals. Another

experimental study has shown that the combined use of alginate with chitosan significantly improves the drug encapsulation efficiency (>90%) and bioavailability and enhances the drug release kinetics for the nano-formulation [104].

Carbon nanotubes

Carbon-based nanomaterials have recently gained significant attention from the scientific community [105]. They are found in various allotropic forms with different biochemical properties for various therapeutic applications [106]. Carbon nanotube-based drug-delivery systems result from advancements in nanobiotechnology and material science. The last decade has witnessed a surge in the exploration of carbon nanotubes for applications such as drug and gene delivery against T.B., cancer, tissue engineering, and diagnostics [106,107]. The dimensions of a carbon nanotube are a few micrometers in average length and 1-100 nm in diameter. These diverse molecules differ in shape, size, density, and geometry [105]. Depending on the number of outer layers, they can be single, double, or multi-walled. Carbon nanotubes are organized into different forms by coupling the functional groups [108]. The attachment of various functional groups on the surface improves the biopharmaceutical utility of carbon nanotubes by connecting with peptides, nucleic acids, and drug molecules that can be easily delivered to the target site [109]. Due to the presence of a high surface-to-volume ratio, CNTs can penetrate cells and cause potent intracellular reactive oxygen species (ROS) generation and membrane damage, subsequently leading to microbial killing [110]. Due to the antibacterial effects, they have opened many new possibilities in anti-TB drug-delivery systems. Several studies have reported that isoniazid-loaded, chitosan-embedded carbon nanotubes have shown significant inhibition and decline in mycobacterial colonies [111,112].

Table 4. Carbon nanotube-based anti-TB drug-delivery

S.N.	Type of carbon nanotube Functionalization	Mechanism of action	Reference
1.	Hyperbranched Poly (Ester) Amide (HBPEA)–Microwave functionalized Multi-walled carbon Nanotube	Significant activity against *M.smegmatis*	[111]
2.	Polyethylene Multi-wall carbon Nanotube (PE-MWCNTs) G.O.	Significant activity against *M. smegmatis*	[113]
3.	Tri ethylene glycol monomethyl ether chains	Significant activity against *M. avium*	[114]
4.	Isoniazid conjugated Multi-walled carbon nanotube	Significant antimycobacterial effect against *M.tb. H37Rv*	[115]

Recent nanodiagnostics approaches to detect TB

The conventional diagnosis of *Mycobacterium tuberculosis* is done by Ziehl-Neelsen staining of clinical samples, culture, immunological, and molecular tests. The limitations of these methods are in terms of sensitivity, specificity, bacterial growth, and the requirement for specialized, sophisticated instrumental facilities to carry out all these tests [116,117].

In recent years, nanotechnology-based diagnostic approaches have offered rapid, cost-effective detection systems for detecting clinical conditions with high sensitivity and specificity [118]. Various studies have shown that nanoparticles such as carbon, gold, silver, silica, magnetic nanoparticles, nanotubes, nanoshells, nanopores, and quantum dots (Q.D.s) have significant potential for nano diagnostic application in various diseases, including T.B. [38-117].

Gold nanoparticle-based nanodiagnostics

Gold nanoparticles (AuNP) are generally inert and non-toxic and have excellent optical properties that enable their use in different clinical fields, including the detection of pathogens [120-121].

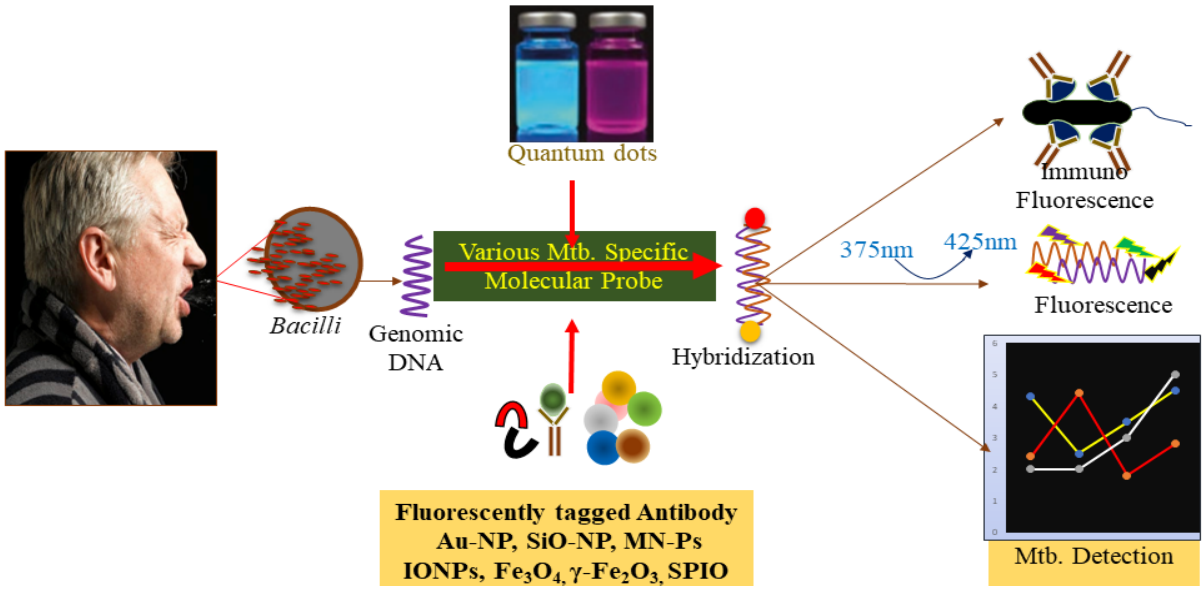

Figure 4: Nanotechnology-based mycobacterial detection system.

AuNPs are used in the calorimetric detection of mycobacteria. AuNPs are coupled with a DNA probe using an oligonucleotide sequence derived from the *M. tuberculosis* RNA polymerase subunit to detect the mycobacteria in the sample.

Exposing the sample to a 526 nm light, the nanoprobe solution turns pink if the sample contains complementary sequences to the DNA probe. If not, then it turns purple [122]. This calorimetric approach provides more sensitivity and accuracy for detecting mycobacteria than other methods [123]. Other AuNP-based *M.tb*. detection methods are slight modifications of the approach mentioned above by using AuNP functionalized indium tin oxide electrode, which can detect mycobacterial genomic DNA (gDNA) [124]. This method uses a capture and gold nano-detection probe linked with the enzyme alkaline phosphatase. The capture probe is then immersed within genomic DNA containing buffer for hybridization to sequence. After that, the electrode allows for generating the signals and detecting mycobacteria. Another AuNP nano diagnostic approach, AuNP-Mediated Dipstick assay, is used for mycobacterial detection in which colloidal AuNP is coated with *M.tb*. Antigenes that are used as a detector of *M.tb.* Antibodies in a serum sample. When *M.tb*. Antigen-coated AuNP reacts with a serum sample, allowing rapid binding with antibodies and, thereby, rapid detection via the development of red color [125].

Silica nanoparticles-based T.B. diagnostics

Silica-based nanoparticles have been used for numerous applications in various biomedical fields, including drug delivery, bioimaging, and biosensing. Mesoporous silica-encapsulated particles have been used to deliver chemotherapeutics [126]. Fluorescence-labeled silica-based nanoparticles have been used in the detection of mycobacteria. The fluorescence intensity of this assay is much higher than that of the conventional fluorescein isothiocyanate-based detection method. Silica-based nanoparticles provide rapid and accurate detection of the mycobacteria within 2 hours in sputum samples [127].

Magnetic nanoparticle-based diagnostics

Magnetic nanoparticles are one of the most promising inventions of nanobiotechnology. These nanoparticles have potential applications in various biomedical fields, such as intracellular bioimaging and the detection of targets. These can be further functionalized by conjugating antibodies, antibiotics, and polymer coating for microbial detection. Different types of magnetic nanoparticles have been synthesized, such as superparamagnetic iron oxide nanoparticles (Iron oxide nanoparticles IONPs composed of magnetite (Fe3O4) or maghemite (γ-Fe_2O_3 nanoparticles) that are widely used in drug delivery and for intracellular localization in MRI [128-129]. Magnetic nanoparticles are also used in Diagnostics Magnetic Resonance

to detect mycobacterial DNA [130,131]. Another use of magnetic nanobeads includes detecting gene mutation in T.B. In this, streptavidin-tagged and biotin-labeled nanobeads very specifically detect the rpoB gene mutation [132]. Magnetic nanoparticles serve as an excellent tracker for the detection of T.B. Superparamagnetic Iron Oxide (SPIO) is used in the MRI imaging of T.B., wherein SPIO is activated and conjugated with anti-*M.tb*. surface antibody and subsequently allowed for incubation with mycobacterium and MRI images indicate the mycobacterial intracellular localization. This method is significant as it exhibits improved sensitivity and specificity for MRI-mediated *M.tb.* detection [128-133].

Magnetic Barcode assay-based nanodiagnostics for tuberculosis

The Magnetic Barcode assay-based mycobacterial detection system is an example of the recent advancement of nanobiotechnology. This assay requires a cDNA sequence of the mycobacteria as a molecular probe to detect [118-134]. The DNA of the bacteria captured by the molecular probe is further conjugated by the complementary magnetic nanoparticle probes and viewed under NMR [135]. This nano technique allows efficient detection of mycobacteria from the clinical sample without DNA extraction and polymerase chain reaction amplification.

Quantum dots-based diagnostics

Q.D.s are an emerging technology that also finds wide applications in the biomedical field for diagnostic purposes. Q.D.s are semiconductor-based nanocrystals, and their size ranges from 1 nm to 10 nm. They have been found to possess some unique physiochemical optical properties, including broad absorption spectra, narrow emission spectra, and slow decay rate, making them suitable for pathogen detection [136]. Q.D.s also possess excellent intrinsic fluorescence that can be used for diagnostic purposes [137]. Therefore, using Q.D.s is a more efficient method than other available fluorescence-based approaches. Coupling with various other fluorochromes can be used to identify various targets simultaneously in a single clinical sample. Combining Q.D.s and magnetic nanobeads for mycobacterial detection has proven to be a versatile approach allowing mycobacterial detection in clinical samples. In this method, M.tb. A specific molecular probe binds the 23S rRNA gene sequence of the mycobacteria, and another probe binds the IS900 highly conserved sequences in mycobacteria. It is treated with sulfurous acid chromium Q.D.s and hybridized with the gene sequence of the mycobacterium DNA. After that, hybridized conjugates, consisting of mycobacterial DNA from T.B. patient samples on quantum dot-magnetic bead conjugates, when viewed under ultraviolet (UV) light, emit a red fluorescence that is easily visualized [137]. Another study has shown that $CdSeO_3$ QDs tagged with streptavidin can precisely detect mycobacteria's species-specific surface marker antigen. Usually, this method requires two biotinylated molecular probes that specifically detect the mycobacterial genomic DNA, with a detection limit of 104 cells/ml of sample [138]-[139].

Photodynamic therapy:

Photodynamic therapy (PDT) is an emerging technology extensively used for therapeutic purposes in various biomedical fields like cancer, TB, etc. This therapy employs photosensitizers and laser light that induces ROS within target cells and selectively damages them [140]. Recently, photodynamic therapy was modified by coupling specific aggregation-induced emission fluorophores encapsulated within functionalized nanomaterials for microbial killing within specifically targeted cells. This safe and minimally invasive therapy has shown promise as an alternative option for the treatment of *MDR-TB* and *XDR-TB* [141-142]. Recently, researchers prepared aggregation-induced functionalized transient triplet differential nanoparticles by using (1,2-distearoylsn-glycero-3-phospho-ethanolamine-N-(methoxy polyethylene glycol) that have the potential to detect and target T.B. granuloma lesions and thus act as novel theranostics for T.B. [143-144]. These particles have better tissue penetration, cause granuloma destruction by generating ROS and bright fluorescence, and cause destruction of inhabiting mycobacterium within infected macrophage [144].

Additionally, non-toxic upconversion nanoparticles (UCNP@pyrolipid) with oleic acid (O.A.)-capped upconverting nanoparticles (UCNPs) loaded with rifampin (RFP), an anti-TB drug in their core and self-assembled asymmetric photosensitizer pyrolipid in their shell to achieve dual anti-TB activities. The near-infrared light irradiation of these UCNPs triggers

photosensitizer activation that generates ROS, killing surrounding mycobacteria. In addition, the RFP carried to lesions by UCNPs kills the remaining mycobacteria after the PDT.

Future Prospectives in T.B.

The recent updates in the management of T.B. include three drugs, namely Bedaquiline, Delamanid, and Pretomanid, with a novel mechanism of action that has received approval for treating drug-resistant TB. WHO reported newer antitubercular drugs in clinical trial phases: Contezoid, Delpazolid, Macozinone, Sutezolid, OPC-167832, Telacebec (Q203), SQ109, TBA-7371, and TBI-166 [145] The Nix-TB is the first T.B. clinical trial to test a new drug combination; pretomanid, bedaquiline, and linezolid. It is also collectively known as the BPal regimen, predicated to cure XDR-TB in six to 9 months [146]. Multiple active targeting of nanocarriers to the macrophages using various ligands is one of the research strategies recently explored by different researchers [147]. Further, drug-loading, characterization, and scalability advances are needed to develop optimized and scalable formulations for tuberculosis treatment. A multidisciplinary approach is required for effective and focused tuberculosis treatment.

Recently, GSK has reported that (the M72/AS01$_E$) tuberculosis vaccine in a phase IIb trial has the potential to reduce active pulmonary TB by half in adults with latent T.B. infection [148]-[149]. Recently, a new Mtb detection method (MTB-MCDA-CRISPR) was developed by combining the MCDA with the CRISPR-Cas12a system. *(MTB-MCDA-CRISPR) the* assay can detect *M.tb.* gDNA at as low as 40 fg per reaction in 1 hr, with high sensitivity and specificity in clinical samples. Thus, the newly developed *(MTB-MCDA-CRISPR)* assay is expected to become an important method for *M.tb.* Infection diagnosis [150]. Hopefully, it would be a better therapeutic alternative for the management of T.B.

Discussion

Nanotechnology-based drug delivery has various advantages over conventional drug delivery, as it enhances drug bioavailability in a controlled and targeted manner, thereby minimizing drug-associated patient non-compliance issues and targeted delivery. By using this technique, we can prepare different types of carrier-based nano/micro-formulations that allow various modulations in terms of shape, size, and composition, significantly affecting the drug's intracellular therapeutic efficacy. Due to their targeting potential and high volume-to-surface ratio, nanoparticles have the potential to release their intracellular drug at the site of infection. Using these techniques, we can deliver the drug at the desired site of action, lower the dose and duration, lower the emergence of drug resistance, and minimize the adverse effects of drug toxicity. The above factors imply that nanocarriers have tremendous potential for treating T.B. Their foremost advantages, such as long shelf life, reduced dosing frequency, and enhanced drug bioavailability, make this technology convenient and reasonable.

Conclusion

The success of this technology will depend on the toxicological profiles linked with understanding the fate of polymeric components of nanocarriers in the body. Based on this opinion, drug carriers made from natural polymers like glucan, alginate, and chitosan suggest a smart outlook, but they still require more exploration. Appropriate clinical studies should be done to find out whether or not nanoparticle-based drug delivery systems might be a much-anticipated solution for improving patient compliance with T.B. chemotherapy.

Conflict of interest:

All authors declared no conflict of interest. For a signed statement, please contact the journal office at editor@precisionnanomedicine.com.

Acknowledgments:

The authors acknowledge DST for providing financial support (DST-SERB/CRG/2019/007035/BHS) and infrastructural support under the DST-FIST program. We thank the Dean's Office, R&D, and Integral University for providing the manuscript communication number (IU/R&D/2022-MCN0001634).

Credit of Authorship:

The conceptualization of the rough draft, editing, artwork, and discussion revisions was done by Firoz Ahmad, with proofreading by Niharika Pandey and Kritika Singh. Figures: Shad Ahmad. Rolee Sharma and Mohd Khubaib did the final editing suggestions and corrections.

Quote this article as Ahmad F, Pandey N, Singh K, Ahmad S, Khubaib M, Sharma R, Recent advances in NNanocarrierbased therapeutic and diagnostic approaches in mycobacterium Tuberculosis, Precis. Nanomed. 2023, 6(4):1134-1156, https://doi.org/10.33218/001c.98699

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