CHAPTER

9

Tackling the Problem of Tuberculosis by Nanotechnology: Disease Diagnosis and Drug Delivery

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9.1 INTRODUCTION

9.1.1 Disease Severity

Tuberculosis (TB) remains a major public health problem all over the world. India contributes 26% of the global TB burden. Since ancient times, TB has been a leading cause of morbidity and mortality. According to the report of World Health Organization (WHO), approximately 8.6 million people have been infected with TB and 1.3 million died from the disease in 2012 (WHO TB Report, 2013). The earliest references to TB can be found in *Sanskrit* (an Indian language). TB is not new at all; in fact, there is also reference to this disease in Ayurveda. It was also described in Chinese and Arabic literature. The word "tuberculosis" was derived from the Latin word *tubercula* (meaning "small lump") (Rubin, 1995; Sharma and Mohan, 2013), and the causative agent (tubercle bacillus) for the disease was discovered by Robert Koch in 1882 (Frank and Tabrah, 2011). After thousands of years, *Mycobacterium tuberculosis* (MTB) was established as the cause of TB in humans. No other disease in history matches TB in terms of morbidity and mortality in humans. Historically, even though several other diseases like smallpox and plague have killed millions of people, they were relatively short-lived. TB has been ever-present and is becoming difficult to treat because of the emergence of multidrug-resistant (MDR) strains.

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In many developing countries, most notably in Africa where the human immunodeficiency virus (HIV) epidemic is particularly severe, individuals infected with HIV are initiated on antiretroviral therapy (ART) only when their $CD4^+$ T-cell count is less than $200/mm^3$. At this stage, an HIV-infected individual is likely to be coinfected with MTB because of a new MTB infection or latent MTB reactivated attributable to suppression of the immune system. Treatment of HIV-associated TB represents a serious problem (Dean et al., 2002; Kwara et al., 2005; McIlleron et al., 2007; Jiang et al., 2014). However, recent reports suggest new hope for the discovery of new drugs (Thacher et al., 2014).

9.1.2 Distribution of TB

It is reported that the average annual risk of infection (ARI) for TB, as computed from the estimated prevalence, is 1.5%. In India, the ARI showed regional variations; it was higher in the northern (1.9%) and western (1.8%) zones compared with the eastern (1.3%) and southern (1%) zones (Chadha et al., 2005; Sharma and Mohan, 2013). However, in house-based tuberculin surveys conducted among children aged 1 to 9 years in statistically selected clusters during 2000 to 2003 and 2009 to 2010, it was observed that ARI rates decreased by, respectively, 6% and 11.7% per year in the north and west zones; however, no change was evident in the south and east zones. In India, ARI decreased by 4.5% per year between 1998 and 2007 (Chadha et al., 2013).

HIV infection is a potent risk factor for TB. HIV increases the risk of reactivating latent MTB infection and also increases the risk of rapid TB progression soon after infection or reinfection with MTB (Duffin and Tullis, 2002; Garira et al., 2005). In the persons infected with MTB, the lifetime risk of developing TB ranges from 10% and 20% (Kirschner and Webb, 1996, 1997). In the persons coinfected with MTB and HIV, however, the annual risk can exceed 10% (Magombedze et al., 2008). The TB burden in different countries has increased rapidly over the past decade with the generalized HIV epidemic, especially in the severely affected countries of eastern and southern Africa (Raviglione et al., 1997; Wilkinson and Davies, 1997; Corbett et al., 2003). TB is one of the most common causes of morbidity and mortality in HIV-positive adults living in less developed countries (Witten and Perelson, 2004; Garira et al., 2005; Magombedze et al., 2008; De Boer et al., 2010); however, it is a preventable and treatable disease. India is among the 22 countries with a high TB rate and has accounted for an estimated one-quarter (26%) of all TB cases worldwide. Observations from reliable accredited mycobacteriology laboratories in India suggest that the prevalence of MDR-TB is quite low in new TB cases (<3%) compared with previously infected cases (15–30%) (http://www.newtbdrugs.org/pipeline.php, accessed on June 10, 2014).

9.1.3 Aim of the Chapter

Public health experts declared that "virtual elimination of the disease as a public health problem" was in sight (Keshavjee, 2012). In the United States, federal funding for TB provides limited funding for research on TB, thus affecting drug discovery, development of diagnostics, and vaccine research. The first decade of the 21st century has been ravaged by extensively drug-resistant TB (XDR-TB). Recently, concern has been expressed regarding the occurrence of extremely drug-resistant TB (XXDR-TB), super XDR-TB, and totally drug-resistant TB (TDR-TB) in some parts of the world because of current TDR bacteria. The role of nanotechnology for predicting a lasting cure and discovery of newer anti-TB drugs, and development of newer drug delivery and vaccines continue to help to achieve the goal of eliminating TB altogether by 2050.

9.2 THE PRESENT SCENARIO OF ANTIBIOTICS USED AGAINST TB

TB treatment has been available for the past 50 years. On average, active TB infection occurs in approximately 10 to 15 people every year. If active TB is not treated, it can be transmitted to others. TB is treated with antibiotics; however, antibiotic treatment therapy is lengthy and it takes 6 to 12 months to destroy the MTB bacteria. The treatment duration and the drug type needed are determined according to age, overall health, results of susceptibility tests, and whether the TB infection is active. During the past 10 years, the researchers have made significant progress regarding treatment for MTB. Regimens have been optimized and directly observed therapy short-course (DOTS) initiatives have been implemented (Jalhan et al., 2013). Currently, TB chemotherapy comprises a cocktail of first-line drugs, Isoniazid (INH), Rifampin (RIF), Pyrazinamide (PZA), and Ethambutol (EMB), administered for 6 months. If treatment fails because of drug resistance, then second-line drugs are the alternative. Drugs such as para-aminosalicylate (PAS), kanamycin, fluoroquinolones, capreomycin, ethionamide, and cycloserine can be used, but they have serious side effects (Dheda et al., 2008; Keshavjee and Farmer, 2010a; Udwadia et al., 2012; Mani et al., 2014).

Because of resistance to antibiotics, the incidence of MDR-TB has increased (Han et al., 2005). Isoniazid drug initially kills approximately 95% of organisms during the first 2 days of treatment; some other effective drugs for TB include rifampicin (RIF) and pyrazinamide (Tahaoğlu et al., 2001; Mitchison, 2003; Vasquez-Campos et al., 2004; Kim et al., 2008; Madan et al., 2013; Mani et al., 2014).

9.2.1 The Problem of Drug Resistance in TB Strains

Discovery of streptomycin, para-amino salicylic acid (PAS), and the availability of isoniazid ushered in the modern era of effective treatment of TB in the mid 1940s. With the emergence of short-course in the late 1970s in India, there was optimism in the developed world that TB may cease to be a public health problem. Recognizing the impact of TB globally, WHO declared TB to be a "global emergency" in April 1993. The late 1990s also witnessed the resurgence of drug-resistant TB (DR-TB), with MDR-TB emerging as a major threat (Hwang et al., 2009; Kim et al., 2010; Shubladze et al., 2013). Development of XDR-TB occurred during the first decade of the 21st century, and the report of the occurrence of DR-TB in India has raised concern and consternation (World Health Organization, 2011). TB occurs in the rich and poor alike, with equal disdain.

In recent survey studies, the choice of drugs has been driven by the actual or presumed (in view of past failed treatment) resistance characteristics of the strains of MTB (Tahaoğlu et al., 2001; Vasquez-Campos et al., 2004; Leimane et al., 2005; Keshavjee and Farmer, 2010b). In order of preference, the following drugs can be chosen:

1. First-line drugs: Isoniazid, Rifampin, Pyrazinamide, and Ethambutol.
2. First-line drugs followed by injectable drugs: Streptomycin, Kanamycin, Amikacin, Capreomycin, or Viomycin/tuberactinomycin B, and the related tuberactinomycins A, N, and O.
3. Antibacterial fluoroquinolones, such as Ciprofloxacin, Ofloxacin, Levofloxacin, or the more recent Sparfloxacin, Gatifloxacin, Moxifloxacin, and Sitafloxacin should be included in the regimen. This class of antibiotics has been proven as an indispensable treatment for MDR-TB. Moreover, some of these drugs may lead to shorter anti-TB regimens, although their use in immunotherapy also leads to the occurrence of fluoroquinone-resistant strains of MTB. An actual preference of

fluoroquinolones, especially during the latest generations, for the specific treatment of MDR-TB is still a matter of preclinical and clinical research.

4. Second-line drugs: Ethionamide, Cycloserine, and PAS.
5. Other drugs are also considered. Their use is the subject of debate and only time and proper observations will provide the necessary data. Clofazimine is among these compounds, and it is also used against *Mycobacterium leprae*. The combination of amoxicillin and the penicillinase inhibitor clavulanic acid has an antimycobacterial effect *in vitro*. The same is true for clarithromycin, although its clinical efficacy remains to be established.

9.2.2 New Drugs for MDR-TB

The US FDA approved bedaquiline as a novel diarylquinoline drug for MDR-TB (Chahine et al., 2014). However, anti-TB drugs are generally given in combination, but extensive studies are required to overcome the problem of drug resistance and to develop most efficient drugs. From the literature available, it was reported that no one drug is 100% effective for MDR-TB. However, in some cases where the use of TB drugs is regular, the success rate was reported to be up to 70–80%. It can also be increased in some patients, up to 80–90%, with surgical resection and standard drugs. For the patients with XDR-TB, five drugs are generally used, including Linezolid and Clofazimine. Hence, there is the possibility for the development of new drug molecules with novel mechanisms of action that can provide a maximum success rate (Shim and Jo, 2013).

9.2.3 Side Effects of Chemotherapy

Antimicrobial resistance is one of the most serious health threats. Infections from TB strains resistant to antibiotics are increasing at an alarming rate. Unfortunately, some pathogens have even become resistant to multiple types of antibiotics. The loss of efficacy of antibiotics and the decrease in their ability to fight infectious diseases and manage complications common in vulnerable patients are matters of great concern.

Treatment for HIV has some side effects, but these side effects have been found to increase because of overlapping toxicity profiles and development of drug-resistant strains of both MTB and HIV when drugs for HIV are combined with anti-TB drugs. Drug interaction may lead to diminished therapeutic results, depending on the choice of the drugs. For example, some anti-TB drugs reduce the concentration of certain antiretroviral drugs by as much as 90% (McIlleron et al., 2007). After ART, the recovery of the immune system may result in immune reconstitution inflammatory syndrome (IRIS), which is especially problematic for an individual with TB. Because of these problems, the timing of ART relative to TB treatment for coinfection is an important question that needs to be addressed. Therefore, Abdool-Karim et al. (2011) rightly stated that "the optimal timing for the initiation of ART in relation to TB therapy remains controversial."

ART for TB can be performed in three phases, which may also be called as arms. One is the sequential arm: ART is performed after TB treatment with standard drugs. However, the other two arms are integrated: ART is performed before TB treatment or during TB treatment (Ramkissoon et al., 2012). TB may be caused by drug-susceptible or drug-resistant strains. In such settings many of the social determinants of TB, including extreme poverty, severe malnutrition, and overcrowded living conditions, become the exception rather than the norm. Public health experts declared that "virtual elimination of the disease as a public health problem" was in sight.

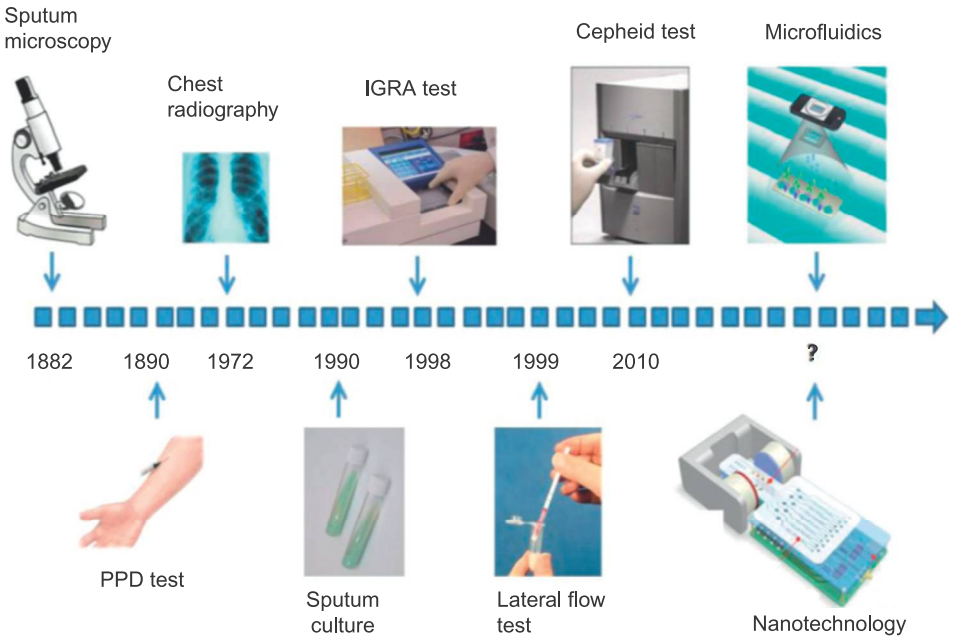

FIGURE 9.1 Different methods generally used for diagnosis of TB. (*Reprinted with permission of publisher, from Wang et al., 2013*).

9.3 NANOTECHNOLOGY AS A NOVEL APPROACH IN DRUG DISCOVERY

The drug resistance in TB presents major problems for the effective control of TB. The TB drugs currently in use were developed 40 years ago, and there is a great need for a new generation of TB drugs. Nanotechnology is a multidisciplinary field that has recently emerged, and it is extremely necessary for TB treatment.

Before the discovery of different nanomaterials (or the science "nanotechnology"), many traditional methods like sputum microscopy, chest radiography, IGRA test, and PPD test were routinely used for the diagnosis of TB. Now, many nanotechnological methods have been developed for the same use. Hence, nanotechnology is important for diagnosis, treatment, and prevention of TB. Figure 9.1 shows the schematic representation of different diagnostic methods used in past and now.

Nanotechnology-based drug delivery will help to deliver even those drug molecules that are poorly soluble in water, and the intracellular specificity with regard to various tissues and cells is an added advantage (Farokhzad and Langer, 2009; Mamo et al., 2010). Nanotechnology-based systemic delivery of anti-TB drugs have many advantages such as controlled release of drugs, which helps to keep the drugs working for longer period of time. It also helps to enhance and modulate the distribution of different drugs such as hydrophobic and hydrophilic into and within different tissues because of their small size (Mamo et al., 2010).

Nanoparticles were found to be promising for carrying synthetic anti-TB drugs worldwide. Also, natural drugs can be carried and released in infected macrophages for anti-TB chemotherapy. The possible mechanism for

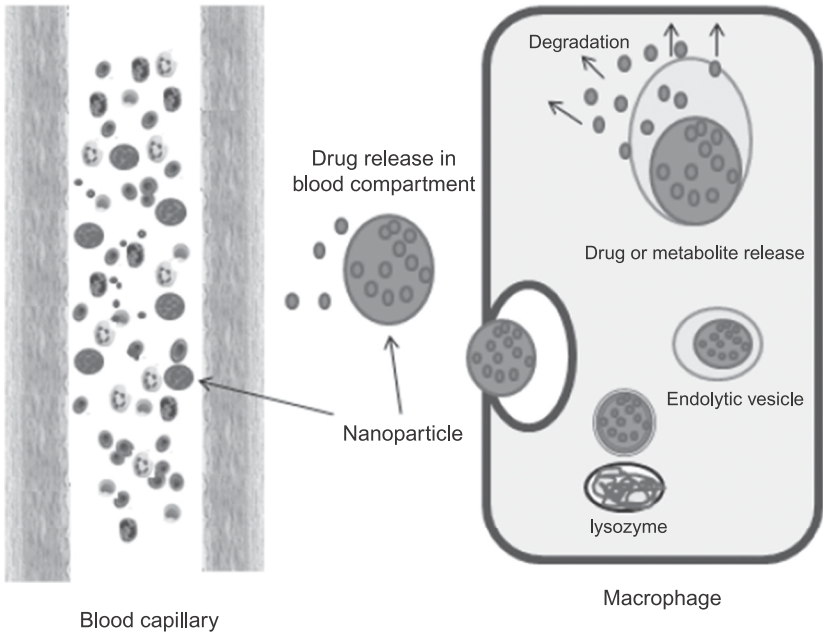

FIGURE 9.2 Possible mechanism for nanoparticles to carry both natural and synthetic drugs and the release of encapsulated drug in infected macrophage for anti-TB chemotherapeutic agents. *(Reprinted with permission of publisher, from Cheepsattayakorn and Cheepsattayakorn, 2013).*

nanoparticle release of encapsulated drugs to the infected macrophages is shown in Figure 9.2. Hence, these nano-based drug delivery systems hold the most promise for their use in clinical treatment and prevention of TB.

9.3.1 Nanotechnology-Based Drug Delivery for TB Treatment

9.3.1.1 Polymeric Nano-Carrier: Dendrimers

Dendrimers are synthetic nanomaterials that are approximately 100 nm in diameter and comprise layers of polymers surrounding a central core. Dendrimers are regularly hyperbranched and three-dimensional macromolecules with low molecular weight and polydispersity; they also have highly adjustable functionality. Drug encapsulation is performed by virtue of the dendrimeric core and complexation and conjugation on the surface (Duncan and Izzo, 2005). Functionality of dendrimers archetypically comprise three different topological components of chemical significance: a poly-functional core, interior layers, and a multivalent surface. The poly-functional focal core can encapsulate various chemical species and exhibits unparalleled properties because of the special nano-environment surrounded by extensive dendritic branching. Because of this structure, they are attractive candidates for the encapsulation and delivery of anti-TB agents for diverse administration routes. RIF-loaded mannosylated fifth-generation polypropyleneimine (PPI) dendrimeric nano-carriers have been developed (Bosman et al., 1999; Kumar et al., 2007). Surface modification with sugar molecules (e.g., mannose) recognizable by lectin receptors located on the surface of phagocytic cells improved the

selective uptake of the drug-loaded nano-carriers by cells of the immune system.

The binding efficacy of RIF with core is approximately 37% and occurs through the hydrophobic interactions and hydrogen bonding. The solubility of RIF within unmodified dendrimers was 52 mg/mL, whereas the superficial mannose molecules sterically hindered the complexation and encapsulation of the drug, and the solubilization of RIF was substantially less efficient at approximately 5 mg/mL (two-fold when compared with the aqueous solubility of RIF). Surface modification with sugar molecules (e.g., mannose) recognizable by lectin receptors located on the surface of phagocytic cells improved the selective uptake of the drug-loaded nano-carriers by cells of the immune system. Increased hemolysis levels shown by amine-terminated dendrimers preclude their clinical application. Mannosylation significantly reduced the hemolytic toxicity of the nano-carrier materials from 15.6% to 2.8%. The use of RIF-containing dendrimers as a carrier was found to be very beneficial and it was reported that the intrinsic hemolytic effect can be reduced from 9.8% to 6.5%. The phagocytic uptake of RIF and RIF-loaded dendrimers was investigated with alveolar macrophages harvested from rat lungs. A clear increase in the intracellular concentration of the antibiotic was apparent. Using a similar approach, more recent work investigated the suitability of RIF-containing fourth-generation and fifth-generation polyethylene glycosylated (PEGylated) PPI dendrimers to sustain the delivery of RIF (Kumar et al., 2007). PEG-grafted dendrimers showed a minimal hemolytic activity (1–3%) as opposed to the NH2-terminated ones (14–20%).

9.3.1.2 Cyclodextrins

Cyclodextrins (CD) are a group of structurally related natural products formed during bacterial digestion of cellulose. CD are cyclic oligosaccharides that consist of (α-1,4)-linked α-D-glucopyranose units and contain a somewhat hydrophobic central cavity and a hydrophilic outer surface, and thus are able to host other hydrophobic molecules (Pitha et al., 1986). Several researchers reported on the complexation of RIF by means of different CD molecules, although results regarding the efficiency of this approach are ambiguous. Ferreira et al. (2004) prepared inclusion complexes of RIF with hydroxypropyl-β-cyclodextrin (HPβCD). In this context, poorly water-soluble nitroimidazole P-824, a new anti-TB drug, has shown activity against drug-sensitive and MDR bacilli. *In vivo* experiments in a short-course murine infection model were conducted, a complex with HP-γ-CD was developed, and a CD/lecithin formulation was prepared (Lenaerts et al., 2005). A reduction in the bacterial load in the lungs was observed with 50 and 100 mg/kg doses. CD has been also investigated as a carrier for local delivery to the lung (Evrard et al., 2004).

9.3.1.3 Polymeric Micelles

Polymeric micelles are used for nanotechnology-based drug delivery systems for pulmonary administration and pulmonary targeting. Polymeric micelles have promising applications for drug delivery, cancer targeting, and tumor imaging (Croy and Kwon, 2006). Regarding the use of polymeric micelles as a carrier for drug delivery systems for respiratory and nonrespiratory diseases (pulmonary administration of drugs) (Smola et al., 2008) as well as for systemic targeted treatment of lung diseases (Goel et al., 2013), a novel approach in the field of polymeric drug delivery systems was introduced by the formation of polymeric micelles and subsequently by functionalized polymeric micelles (Jhaveri and Torchilin, 2014). Polymeric micelles are expected to find a wide application in the fields of drug delivery and diagnosis because the of the possibility of coupling to bioactive substances. Nanospheric particles as drug delivery systems are gaining

increasing interest in the biomedical field. Nanospheres and microspheres are also found to be efficient drug delivery systems for intravenous administration because of their comparatively long bloodstream circulation (Hire and Derle, 2014; Wang et al., 2014). It was expected that when the drugs are released after internalization of micelle, by cleavage, they act as pro-drugs and enhance solubility of hydrophobic drugs, reduce drug toxicity, increase bioavailability, specificity, and systematic release of drugs, and prolong the drug action (Moghimi et al., 1993; Croy and Kwon, 2006; Dadwal, 2014). Micelle-forming polymer derivatives of the initial-phase Anti-TB drugs—pyrazinamide, thioridazine, isoniazid, and rifampin—showed potential activity against drug-resistant bacteria (Silva et al., 2007; Amaral and Viveiros, 2012). This potentiates the drug activity when conjugated to the polymer and, hence, is promising with regard to the possible reductions in the dose. The aforementioned derivatives of different drugs were also found to be effective against several virulent strains of mycobacteria such as MTB and *Mycobacterium avium* when the activity was determined by critical micelle concentration (CMC) (Emanuele and Attwood, 2005; Silva et al., 2006; Chen et al., 2007). Similarly, some other drug derivatives like copolymer PEG−PASP, containing pyrazinamide, isoniazid, and rifampin, were successfully synthesized using various processes that can be used as effective drugs for TB (Francis et al., 2004; Hans et al., 2005).

9.3.1.4 Nanosuspensions

Submicron colloidal dispersions of pure drugs stabilized with surfactants are nanosuspensions. Reduction of the average size of solid drug particles to the nanoscale, generally by top milling or grinding, is a useful methodology to improve the solubility of drugs. Dimethyl sulfoxide methanol, ethanol, and ether solvents are used to solubilize the TB drugs for nanonization (Hari et al., 2010). Dimethyl sulfoxide was found to be the most excellent solvent. Spherical particles of various sizes (mean diameters between 400 nm and 3 mm) are needed and sizes are tuned by changing the conditions of the process. This may support TB treatment, especially the local delivery of anti-TB drugs to the lungs. Nanocrystalline suspensions of poorly soluble drugs such as riminophenazines and clofazimine are easy to prepare and to lyophilize for extended storage and represent a promising new drug formulation for intravenous therapy of mycobacterial infections (Peters et al., 2000).

9.3.1.5 Nanoemulsions

Nanoemulsions are thermodynamically stable oil-in-water (o/w) dispersions displaying drop sizes between 10 and 100 nm (Constantinides et al., 2008). Advantages of nanoemulsion are that they are generated spontaneously, can be produced in a large scale without the need of high homogenization energy, and can be sterilized by filtration. The enhanced uptake of nanoemulsions (lipid emulsion) by cells of the phagocytic system reported and they have a potential role as a novel antimicrobial agent (Seki et al., 2004).

9.3.1.6 Niosomes

Niosomes are biocompatible, nonimmunogenic, and biodegradable in nature and exhibit flexibility in their structured characterization (Junyaprasert et al., 2008). Stable liposome-like vesicles produced by different methods like hydration of cholesterol, charge-inducing components like charged phospholipids, and nonionic surfactants have advantages such as higher stability, entrapment of more substances, and no need for handling or storing in special conditions. Niosomes have the ability to hold hydrophilic drugs within the core and to hold lipophilic drugs by entrapment in hydrophobic domains.

The prepared microsized (8–15 mm) RMP-loaded niosomes contain Span 85 as the surfactant. *In vivo* studies have revealed that by adjusting the size of the carrier, up to 65% of the drug can be localized in the lungs (Junyaprasert et al., 2008). Niosomes have been used for improving the stability of entrapped drug RMP.

9.3.1.7 Polymeric and Nonpolymeric Nanoparticles

Polymeric nanoparticles (PNPs) have been extensively used for the drug solubilization, stabilization, and targeting (Delie and Blanco-Prieto, 2005). High stability, given the high loading capacity for drugs and feasibility of administration by different routes, has made PNPs one of the most popular approaches for drug encapsulation (Brannon-Peppas, 1995). Depending on application, two types of system can be developed, namely, nanocapsules and nanospheres. The drug solubilized in aqueous or oily solvents is surrounded by a polymeric membrane. There are several biomaterials available for the production of PNP. PNPs are removed from the body by opsonization and phagocytosis (Owens and Peppas, 2005).

To prevent recognition by the host immune system and to prolong circulation time in the blood, the modification of the surface with highly hydrophilic chains (e.g., PEG) has been performed (Hari et al., 2010). This approach was one of the most extensively investigated with respect to anti-TB drug delivery systems (Kataoka et al., 2001). Du Toit et al. (2008) developed INH-loaded polymer-based nanosystems by means of a salting-out approach (nanoprecipitation). Hari et al. (2010) reported that encapsulation of different TB drugs within poly(*n*butylcyanoacrylate) (PBCA) and poly (isobutylcyanoacrylate) (PIBCA) nanoparticles increase in the concentration of such drugs when tested for the accumulation in human blood monocytes *in vitro*.

9.3.1.8 Liposomes

Liposomes are nano-sized to microsized vesicles comprising a phospholipid bilayer that surrounds an aqueous core (Cheepsattayakorn and Cheepsattayakorn, 2013). In liposomes, the core encapsulates the water-soluble drugs and the hydrophobic domain is responsible for entrapping insoluble agents. After administration, liposomes are usually recognized by phagocytic cells and are expelled from the blood rapidly. To prevent better efficacy of liposomes, they are usually PEGylated. In more recent investigations, pyrazinamide and rifabutin-containing liposomes were also produced. Reports of INH and rifampin encapsulated in lung-specific stealth liposomes against MTB infection revealed that liposome-encapsulated drugs at and below therapeutic concentrations were more effective than free drugs against TB (Pandey et al., 2004).

9.4 NANO-BASED DNA VACCINES FOR TB

Bacillus Calmette–Guerin (BCG) is the only vaccine that has been discovered for TB. Unfortunately, its efficacy varies from 0% to 80% (Fine, 1995; Lin et al., 2007). Clinical trials of BCG have shown total lack of protection in regions of the world where the disease is common, and thus BCG vaccination is considered ineffective. In addition, BCG is a live vaccine and can cause disseminated disease in immunocompromised individuals. Therefore, these disadvantages indicate the urgent need to develop more effective vaccines against TB (Dhanasooraj et al., 2013). BCG is used in many countries with a high prevalence of TB to prevent childhood TB meningitis and other diseases like miliary disease. However, BCG vaccine is not generally recommended in countries with a low risk of infection with MTB, like the United States (www.vaccines.gov/

diseases/tb/ accessed on June 15, 2014). BCG also showed variable effectiveness against adult pulmonary TB and its potential interference with tuberculin skin test reactivity. According to TB experts, the BCG vaccine can be used for very selective individuals who meet specific criteria (www.vaccines.gov/diseases/tb/ accessed on June 15, 2014). Development of a novel and effective vaccine against MTB for preventing TB infection is a challenge (Feng et al., 2013). Therefore, the concept of nano-based vaccines like chitosan-based DNA vaccine came into existence. A few studies have been performed concerning the use of nanomaterials (mostly chitosan nanoparticles) for the development of nano-based DNA vaccines, which are reviewed in this chapter.

Bivas-Benita et al. (2004) contributed extensively to the use of nanomaterials in different forms for the control of TB infections. The authors formulated the conjugate of DNA plasmid from MTB encoding for different restricted T-cell epitopes with chitosan nanoparticles. Later, they investigated the effects of these conjugates on pulmonary delivery and reported that chitosan–DNA conjugates induce the maturation of dendritic cells, which cannot be achieved by chitosan nanoparticles alone. This indicates that release of DNA from nanoparticles stimulates the dendritic cells and increases levels of interferon-gamma secretion compared with delivery of plasmid in intramuscular immunization routes. Hence, these findings proved that use of DNA vaccines (DNA encapsulated in chitosan nanoparticles) against TB would be more efficient than intramuscular immunization because it increases immunogenicity. In another study, they reported that pulmonary delivery of DNA encoding MTB antigen Rv1733c conjugated with poly (D,L-lactide-co-glycolide) (PLGA)-polyethyleneimine (PEI) nanoparticles (NP) (PLGA-PEI) augmented the T-cell responses in a DNA prime/protein that boosts the vaccination regimen in mice (Bivas-Benita et al., 2009). From both studies it can be concluded that conjugates of DNA encoding for different antigens from MTB with various nanoparticles would be helpful to boost the immune response against TB.

Heuking et al. (2013) investigated the role of TLR-1/TLR-2 agonist–functionalized pDNA nanoparticles on human bronchial epithelium. They reported that chitosan-based DNA delivery permits the uptake into monocyte-derived dendritic cells, which are the most important cells of human immune systems. For example, in the human lung it induces antigen-specific immunity. From these findings they proposed that such a DNA delivery approach was attractive for potential DNA vaccination against intracellular pathogens in the lung (e.g., MTB or influenza virus). Another attempt has been made regarding vaccine delivery system for TB using nano-sized hepatitis B virus core protein particles. According to Dhanasooraj et al. (2013), nano-sized hepatitis B virus core protein particles (HBc-VLP) were suitable and can be easily taken up by antigen-presenting cells.

It was well-known that the antigen culture filtrate protein 10 (CFP-10) is an important vaccine candidate against TB. However, it was reported that without any adjuvant, these antigens showed very low immune response and, hence, has low protective efficacy. However, when these proteins (CFP-10) were used in combination with HBc nanoparticles, it provided higher protection compared with the native antigen alone.

Feng et al. (2013) developed a novel nanoparticle-based recombinant DNA vaccine. It was a complex of Esat-6 three T-cell epitopes (Esat-6/3e) and fms-like tyrosine kinase 3 ligand (FL) genes (Esat-6/3e-FL) enveloped with chitosan (CS) nanoparticles (nano-chitosan). This complex is termed nano-Esat-6/3e-FL). Further, they demonstrated the immunologic and protective efficacy of these nano-chitosan-based DNA vaccines (nano-Esat-6/3e-FL) in C57BL/6 mice after intramuscular prime vaccination with the plasmid DNA and nasal boost

with the Esat-6/3e peptides. The findings showed that the immunized mice had significantly enhanced T-cell responses and protection against MTB. These findings indicate that the nano-chitosan can significantly elevate the immunologic and protective effects of the DNA vaccine and would be useful vaccine against TB.

All these reports collectively proposed the efficient use of nano-based DNA vaccines for the control of TB infections in mice. Further extensive studies are required for the development of novel and 100% efficient nano-based vaccines against TB infections in humans.

9.5 ROLE OF NANOBIOSENSORS IN DIAGNOSTICS OF TB

The tubercle bacterium is sluggish in growth, taking 1–2 months for *in vitro* growth (Tortoli et al., 1997; Davies et al., 1999). Therefore, it is difficult to find the presence of infection at its early stage. Ziehl-Neelsen staining is the conventional method for its identification. This staining is needed for preliminary identification of the causative organism, but it lacks the sensitivity (Moore and Curry, 1998; Mahaisavariya et al., 2005). The conventional methods of cultivation of mycobacteria are time-consuming and need several weeks. Polymerase chain reaction (PCR) is a sensitive method for early detection of mycobacterium, but the amplification process requires ample processing time, chemicals, and reagents, which contribute to the high cost of the assay. Additionally, it is labor-intensive and expensive (Tombelli et al., 2000; Minnuni et al., 2005). Therefore, there is an urgent requirement for the development of a rapid, low-cost, and convenient diagnostic method for detection of TB. In this respect, biosensors appear to be a good option. There is increasing demand for biosensor technology for fast and precise detection of TB with high affinity and specificity. Biosensor technology has the potential to provide a qualitative and quantitative analysis and is free from radioactive or fluorescent tags (Tombelli et al., 2000, Zhou et al., 2001; Yao et al., 2008).

In recent years, in view of the benefits of various remarkable studies performed in nanotechnology, important efforts have been made to combine it with highly sensitive and accurate biosensor technology to develop nanobiosensors. Nanobiosensors systems are efficiently used in diagnosis of diseases, environmental monitoring, food quality control, and defense as a smart approach (Zhou et al., 2011; Rai et al., 2012; Singh et al., 2014). For instance, by using gold-coated nanobiosensors, Duman et al. (2009) demonstrated detection of target molecules (synthetic and PCR products) very effectively at nanomolar levels. A single-stranded oligodeoxynucleotide carrying a thiol group at the end and complementary of the target characteristic sequence of the MTB complex was used as the probe immobilized on the gold-coated surface of the surface plasmon resonance slides. It is interesting to note that the sensor platform is reusable and has long shelf life. A quartz crystal microbalance (QCM) biosensor in combination with AuNPs has been developed for the detection of MTB (Kaewphinit et al., 2012). According to the study, AuNPs improved the sensitivity of immobilized gold electrode of quartz crystal using the specific thiol-modified oligonucleotide probe. The QCM has been shown to detect up to 5 pg of MTB genomic DNA without showing any cross-hybridization with other mycobacteria.

During the past few years, many techniques have exploited the materials at the nano-scale level for designing biosensors with high specificity and efficacy. Among all nanomaterials, metal oxides are of particular interest because of their unique physical, chemical, and catalytic properties (Shi et al., 2014). Das et al. (2010) have made an important attempt to diversify the application of such metal oxides in the generation of nanobiosensors for

detection of mycobacteria. They deposited nanoscale zinc oxide on the indium-tin-oxide (ITO)-coated glass plate. The presence of nanostructured ZnO films allowed an increase in the electro-active surface area for DNA molecule loading and for detecting genomic target DNA up to 100 pM, which enables the direct detection of pathogens in clinical samples at point of care. The main characteristics of the technique are: (i) the covalent immobilization of the sensor without using any cross-linker that might limit its sensitivity; (ii) detection limit of 0.065 ng/μL; (iii) detection process requires only 60 s; (iv) can be reused up to 10 times; and (v) stable up to 4 months at 4°C. Therefore, it is an efficient nanobiosensor for rapid and accurate diagnosis of mycobacteria (Das et al., 2010). Zirconium oxide (ZrO_2) is an important oxide of metal with greater stability and inertness. Moreover, it has affinity toward groups containing oxygen. Das et al. (2011) developed zirconium oxide and carbon nanotube ($NanoZrO_2$-CNT) nanocomposite-based nucleic acid nanobiosensors deposited on ITO. The group utilized this electrode ($NanoZrO_2$-CNT/ITO) for immobilization of single-stranded probe DNA (ssDNA) specific for MTB to reveal its application to biosensor for nuclei acid detection.

Thiruppathiraja et al. (2011) fabricated and evaluated the DNA electrochemical biosensor for genomic DNA of *Mycobacterium* sp. using a signal amplifier as dual-labeled gold nanoparticles (AuNPs). The method involves the sandwich detection strategy comprising two types of DNA probes: the probes of enzyme ALP and the detector probe conjugated on AuNPs. Both of these probes were specific for *Mycobacterium* sp. genomic DNA. The study claimed that under optimized conditions, the detection limit of the method was 1.25 ng/mL genomic DNA. The said nanobiosensors were also promising and evaluation of the clinical sputum samples showed the higher sensitivity and specificity. Another study (Torres-Chavolla and Alocilja, 2011) with different approaches also fabricated the DNA-based biosensor encompassing AuNPs and amine-terminated magnetic particles (MPs) to detect the mycobacteria. The study made use of thermophilic helicase-dependent isothermal amplification (tHDA) and dextrin-coated AuNPs as electrochemical reporters. The AuNPs and MPs were functionalized independently with different DNA probes that specifically hybridize with a fragment within a gene of mycobacteria. Later, that group separated the MP-target–AuNPs complex magnetically from the solution and detected AuNPs electrochemically. Torres-Chavolla and Alocilja (2011) claimed the sensitivity of this method was 0.01 ng/μL of isothermally amplified target of 105 bp. Such a sensor thus can be used to regularly analyze the clinical samples suspected to have mycobacteria.

In addition to metal oxide nanoparticles, porous silicon is also getting more attention regarding biosensor applications, mostly in label-free applications. Wu et al. (2012) made use of a nanoscale porous silicon micro-cavity biosensor for fast sero-diagnosis of MTB. Through a series of experiments, the study testified the feasibility of this biosensor for the detection of interaction between 16 kDa antigen and 16 kDa antibody. The detection of MDR-TB is of utmost importance. In a recent study, Li et al. (2014) reported development of a DNA sensor for the specific detection of the rpoB gene of MDR-TB by using ruthenium (II) complex–functionalized grapheme oxide (Ru-GO) as a suspension-sensing interface and ferrocene-labeled single-stranded DNA (FC-ssDNA) as an electrochemiluminescence (ECL) intensity controller. The assay relies on the principle that when mutant ssDNA target hybridizes with FC-ssDNA, it is released from the Ru-Go surface, leading to recovery of ECL. The assay is reported to have a detection range from 0.1 to 100 nM and 0.04 nM sensitivity.

More studies are needed that mainly focus on the fabrication of various nanobiosensors for diagnosis of such a dreadful disease. Because this technique is highly sensitive, it requires little sample preparation and is fast, specific, cheap, and easy to use; it has great potential for the clinical diagnosis of TB.

9.6 CONCLUSION AND FUTURE PERSPECTIVES

It is evident that TB is still a major public health problem because of the emergence of MDR strains of *Mycobacterium*. The emergence of MDR strains of TB in HIV patients in developing countries like Africa has made the problem more complicated and, thus, it is a matter of great concern. In fact, the disease should be eliminated as a major public health problem. WHO emphasizes that there is a "global emergency" to eradicate drug-resistant strains of TB. Taking these facts into consideration, there is a pressing need to develop newer anti-TB drugs, drug delivery systems, and development of vaccines to combat the grave problem of MDR mycobacterium by 2050. In this context, nanotechnology may play a vital role in fighting the MDR strains of TB. Development of nanobiosensors for early diagnosis and use of nano-based drugs in combination with the existing antibiotics and delivery system may provide new ways to combat the MDR problem.

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