Nanotechnology-Based Approaches for Combating Tuberculosis: A Review

Tamishrana Bagchi* and Sejal Chauhan

School of Nano Sciences, Central University of Gujarat, Sector 30, Gandhinagar, Gujarat, 382030, India

Abstract: Nanotechnology has immensely contributed towards the advancement of almost all the fields where it has been used. The fascinating properties such as small size, high surface area, mechanical strength etc. have been widely exploited for its specific applications. In medicine, several nanotechnology-based devices and formulations have been developed for point of care diagnosis and targeted treatment. The properties of metal nanoparticles such as surface plasmon resonance, conductivity, magnetic field have been used in enhancing the sensitivity of bionanosensors. Thus, the bionanosensor with enhanced sensitivity has been used in diagnosis to detect various analytes up to picomolar concentrations. Polymeric nanoparticles are biocompatible and biodegradable and therefore have been used in drug delivery. Ligand decorated nanocarriers loaded with various drugs have been used for target specificity. Additionally, response to stimuli such as pH, the temperature has allowed the use of nanocarriers for targeted drug delivery and improved the bioavailability of drug at the site. The nanocarriers with a combination of metal and polymers have been used for the dual purpose of diagnosis and treatment. Nanovaccines have also been produced wherein nanoparticles with low immunogenicity and good adjuvant property have enhanced the delivery and immunogenicity of antigen for immunization. Tuberculosis (TB) is among the deadliest communicable diseases caused by Mycobacterium tuberculosis (m.tb). Due to the complicated pathophysiology of m.tb, the diagnosis is extremely difficult during the early stages of infection and therefore the treatment prolongs. Several nanotechnology-based approaches for diagnosis and treatment of TB have been discussed in this review. Also, several approaches in other diseases and its potential application in TB have been discussed.

Keywords: Tuberculosis, nanotechnology, nanoparticles, imaging, targeted drug delivery, nanovaccines.

1. INTRODUCTION

The advent of nanotechnology has brought about a great revolution in the world of science wherein materials have started to be examined at the nanometer (nm) scale. Nanotechnology is the emerging technology in which properties of materials are studied and manipulated at the nm scale for specific applications. Since past few decades, nanotechnology has touched almost all the fields and has made great advancements in energy, engineering, space, agriculture, medicine, food, electricity, communication, material science, manufacturing etc. Properties of nanomaterials such as high surface to volume ratio, strength, electric conductivity, surface plasmon resonance have been employed to develop various nanodevices as per need. For example, solar light harvesting devices with nanomaterials like a solar collector, fuel cell, photocatalysis and solar photovoltaic have been developed with greater efficiency [1]. In the construction industry, nanoengineered concrete has improved the compressive and tensile strength, water resistance, leaching of calcium, self-cleaning and de-polluting properties [2]. Silver nanoparticle-containing paints and coatings show good chemical resistance and thus protect the buildings from corrosion thereby giving better surface appearance [3]. Similarly, in aerospace, high strength, low weight composites, improved electronics and displays with low power consumption and a variety of physical sensors have been proposed using nanomaterials [4]. In the food industry too, nanosensors for bacterial identification, nanocapsules containing bioactive food compounds, nanocomposite coating on food packing materials have improved the production and safety aspects [5]. In this review, several nanotechnology-based approaches in other diseases and its potential application in TB have been discussed. Also, several approaches for diagnosis and treatment of TB have been discussed. In the first part, we have discussed in general, the nanotechnology approaches in diagnosis and therapy of various diseases, including cancer which is one of the major areas of study. Various types of nanoparticles including polymer complexes, metal nanoparticles, dendrimers and aptamers besides others have been highlighted. In the latter half of the review, we have focused on the diagnosis and therapy in TB. It is apparent that the recent advances in nanotechnology could possibly be em-

*Address correspondence to this author at the School of Nano Sciences, Central University of Gujarat, Sector 30, Gandhinagar, Gujarat, 382030, India; Tel: 917923260628; E-mail: tbagchi@cug.ac.in
ployed in the development of methods and materials for combating TB.

2. NANOTECHNOLOGY IN MEDICINE

Nanobiotechnology is the branch of biotechnology in which nanoparticles and nanodevices are constructed for imaging, diagnosis, and treatment. A range of nanoparticles have been synthesized from metals and polymers and have been used for imaging, tracking and drug delivery besides also helping fundamental studies. The various platforms that have been developed include simple metallic nanoparticles made from non-toxic metals such as gold, iron, zinc. These nanoparticles are easy to synthesize and further can be functionalized with different chemical groups like thiol, amine etc. The functionalization helps in the conjugation of these nanoparticles with biomolecules like protein, nucleic acids, polysaccharides and chemical compounds such as drugs and reporter dyes. Thus, nanobiotechnology is widely used for diagnostic imaging and targeted drug delivery as shown in Table 1.

Gold nanoparticle (AuNP) is inert in the biological environment and has a high affinity for biomolecules. Due to this, it is widely conjugated with nucleic acids and proteins (miRNS, siRNA, DNA, peptide, antibody) for its targeted delivery for use in imaging and therapy. A CRISPR-AuNP has been developed for homology-directed repair as an approach in gene therapy. In this, AuNP was conjugated with donor DNA, CRISPR associated protein 9 and the endosomal disruptive polymer. This cargo efficiently corrects the DNA mutation that causes Duchenne muscular dystrophy in mice via local injection, with minimal off-target DNA damage [6]. AuNPs have also been used for plasmic photo-thermal therapy where coating with an antibody specific to epidermal growth factor receptor facilitates the entry of AuNP into tumor cells and following laser irradiation leads to apoptosis of the target (photothermalysis). The X-ray attenuation property of AuNP also enables its use as a contrasting agent for better resolution. Thus, acetylated dendrimer-entrapped AuNPs have shown great potential for in vitro and in vivo CT imaging of cancer cells [7]. AuNPs conjugated with Doxorubicin via a linker peptide which acts as a substrate for matrix metalloproteinase 2 enzyme is used for stimuli-responsive drug delivery. The fluorescence of doxorubicin is initially quenched by AuNP. The abundance of matrix metalloproteinase 2 enzyme at the tumor site cleaves the peptide and releases the doxorubicin and therefore it fluoresces. The imaging of doxorubicin fluorescence fulfills the dual purpose of diagnosis and therapy [8].

Superparamagnetic Iron oxide nanoparticles (SPIONs) are used as MRI contrasting agent as well as for magnetic guidance targeting and hyperthermia. The protein binding capacity enables SPION to be used for targeted delivery as well. The inhalable SPIONs show good transfer and distribution all over the lungs [9] with a less toxic immune response when studied in vitro and in vivo [10]. Keeping these features in mind, folate/NIR 797 conjugated SPIO-albumin nanosphere has been developed for the early diagnosis of cancer along with imaging and monitoring features [11].

Similarly, chitosan and polyethylene glycol (PEG) co-polymer coated SPIONs were labelled with a fluorescent dye followed by conjugation with a monoclonal antibody against the breast cancer proto-oncogene neu receptor. The in vitro and in vivo MRI studies showed specific uptake and enhanced contrast in primary breast tumor and thus establishing the diagnostic potential of these nanoparticles for breast cancer metastases [12]. SPIONs have also been surface-modified by 3-amino propyltriehoxysilane and/or pro- tamine sulfate for magnetic induction hyperthermia induced gene therapy. Using this approach, TNF-α gene was success- fully transfected into the tumor cell for therapeutic use [13].

Other metal nanoparticles in common use are silver nanoparticles which have microbialc activity and have been used to a kill wide range of bacteria [14]. Similarly, titanium dioxide mediated photocatalysis lead to the immediate killing of gram-positive while gradually inactivating gram-negative bacteria and could also disable enveloped and non-enveloped viruses [15]. The effect and mechanism of antimicrobial activity of oxides of zinc, iron, nickel, copper, cad- mium, cobalt, aluminum etc. have also been studied widely [16].

Other than these, carbon nanotubes, graphene and their oxides, zinc oxide-based nanoparticles along with different metal nanoparticles have also been used as bionanosensors for the development of low cost, robust, reliable, easy-to-use, and ultrasensitive diagnostics tools. Early detection of breast cancer by monitoring the plasma levels of overexpressed miRNA-155 has been possible by using bionanosensors rather than by using microarray, northern blot, RTPCR or in situ hybridization methods which are time-consuming. The sensor consisted of a glassy carbon electrode coated first with graphene oxide, followed by a further coating of thio- lated probe functionalized gold nanorods. Upon binding of specific miRNA to the probe, the signals of intercalating oracet blue were measured by differential pulse voltammetry method. This sensor has the ability to detect a minimum of 0.6 fM of miRNA [17]. Similarly, porous magnetic micro- spheres and AuNP based bionanosensor have been able to detect plasma protein APoE and beta-amyloid in Alzheimer's disease [18].

The synthetic polymers such as poly (glycolic acid) (PGA), poly (lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA) and polycaprolactone are biodegradable and biocompatible and have been approved by FDA for drug delivery. These polymers are stable and help in the controlled release of the entrapped compound. This enhances the bioavailability of the compound with maximum retention time. [19]. Doxorubicin-loaded PLGA nanoparticle coated with glutathione-PEG conjugate has been synthesized to treat brain cancer. The in vitro studies demonstrated higher permeability through the blood-brain barrier, of nanoparticles compared to the free drug [20]. A triblock copolymer consisting of PLGA-PEG-PLGA has thermogelling property and in vivo studies showed that doxorubicin loaded triblock inhibits tumor growth with higher efficacy as compared to free doxorubicin at the same dose [21].

Dendrimers are radially symmetric nano-sized molecules with a core, inner and outer shell. They are widely used in medical research due to their polyvalency, electrostatic interaction, stability and low toxicity [22]. Dendrimers are repeti- tively branched thereby providing a wide surface area for
Table 1. Summary of different nanomaterials developed for diagnosis and treatment of various diseases.

<table>
<thead>
<tr>
<th>Type of Nanoparticle</th>
<th>Diseases</th>
<th>Treatment/Diagnosis/Imaging</th>
<th>Target</th>
<th>Therapeutic/Diagnostic/Imaging Agent</th>
<th>Mode of Action</th>
<th>Mode of Detection</th>
<th>'References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNPs</td>
<td>Duchenne Muscular Dystrophy</td>
<td>Treatment</td>
<td>Gene</td>
<td>CRISPR-Cas9</td>
<td>Gene editing &amp; HDR</td>
<td>Fluorescence microscopy</td>
<td>[6]</td>
</tr>
<tr>
<td>- Cancer</td>
<td>Imaging</td>
<td>-</td>
<td>Acetylated dendrimer entrapped AuNP</td>
<td>Contrasting agent</td>
<td>CT</td>
<td>[7]</td>
<td></td>
</tr>
<tr>
<td>- Cancer</td>
<td>Imaging and Treatment</td>
<td>MMP2</td>
<td>Doxorubicin</td>
<td>Chemotherapy</td>
<td>Fluorescence</td>
<td>[8]</td>
<td></td>
</tr>
<tr>
<td>SPION</td>
<td>Cancer</td>
<td>Diagnosis</td>
<td>Folate receptor</td>
<td>NIR dye</td>
<td>Contrasting agent</td>
<td>NIR Fluorescence microscopy</td>
<td>[11]</td>
</tr>
<tr>
<td>- Cancer</td>
<td>Imaging</td>
<td>Neu receptor</td>
<td>SPIO</td>
<td>Contrasting agent</td>
<td>MRI</td>
<td>[12]</td>
<td></td>
</tr>
<tr>
<td>- Cancer</td>
<td>Treatment</td>
<td>TNF-α</td>
<td>SPIO</td>
<td>Magnetic induction hyperthermia and Gene therapy</td>
<td>MRI</td>
<td>[13]</td>
<td></td>
</tr>
<tr>
<td>Graphene oxide and gold nanorods</td>
<td>Breast Cancer</td>
<td>Bionanosensor</td>
<td>miRNA-155</td>
<td>Anthraquinine, Oracet blue</td>
<td>Electrochemical signals</td>
<td>Pulse voltammetry</td>
<td>[17]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Brain Cancer</td>
<td>Treatment</td>
<td>BBB</td>
<td>Doxorubicin</td>
<td>Chemotherapy</td>
<td>Spectrophotometry</td>
<td>[20]</td>
</tr>
<tr>
<td>Dendrimer</td>
<td>Lung Cancer</td>
<td>Treatment</td>
<td>HuR, DNA</td>
<td>Si-RNA &amp; Diamine dichlororhodium</td>
<td>Gene therapy &amp; chemotherapy</td>
<td>Fluorescence microscopy</td>
<td>[24]</td>
</tr>
<tr>
<td>Aptamer</td>
<td>Atherosis</td>
<td>Diagnosis</td>
<td>TBA1 &amp; 2</td>
<td>Rubpy-doped silica nanoparticles</td>
<td>Aptasensor</td>
<td>Fluorescence spectrophotometry</td>
<td>[25]</td>
</tr>
<tr>
<td>- Cancer</td>
<td>Diagnosis</td>
<td>uMUC1</td>
<td>Cobalt ferrite magnetic nanoparticles with silica shell</td>
<td>Aptasensor</td>
<td>Optical, radionuclide and MR imaging</td>
<td>[26]</td>
<td></td>
</tr>
<tr>
<td>- Cancer</td>
<td>Treatment</td>
<td>MUC1, NAS-2A, Cyt C</td>
<td>Graphene oxide</td>
<td>Apoptosis induction</td>
<td>Fluorescence analysis</td>
<td>[27]</td>
<td></td>
</tr>
</tbody>
</table>

* Refer the numbered reference in the text.

drug loading and its subsequent sustainable release. They have been used extensively for both diagnosis and therapy [23]. They can be made from several different types of subunits such as ethylene diamine, phenylacetylene and functionalized with various ligands. Folic acid conjugated polyamidoamine (PAMAM) nanoparticle has been synthesized to treat lung cancer. Human Antigen R (HuR) is an RNA binding protein, which is overexpressed in cancer cells and is responsible for the translation of growth-related mRNAs, and therefore is a potential target for therapy. The nanoparticles loaded with siRNA for HuR and diamine dichlororhodium as anti-cancer drugs were delivered to lung cancer cell line and showed a greater therapeutic effect with less cytotoxicity [24]. (Fig. 1) summarizes the development of different nanomaterial-based formulations for cancer diagnosis and treatment.

Another class of delivery agent, aptamers, are oligonucleotides or peptide molecules that bind to specific target molecules. The nucleic acid aptamers are selected by systematic evolution of ligands by exponential enrichment by a technique known as SELEX. The peptide aptamers are selected in vivo by yeast two-hybrid, phage display, ribosome display technique. Being of high specificity, small size and low immunogenicity make them the best candidates for diagnosis and treatment. Aptasensors are aptamers conjugated with nanoparticles and have been developed for specific, sensitive and simple diagnosis and treatment in various cases. A fluorescent-based aptasensor has been developed for quantitative analysis of thrombin. In this sensor, the TBA1 aptamer for the exosite I of thrombin was coated on RuBpy-doped silica nanoparticle and the TBA2 aptamer for the exosite II of thrombin was coated on magnetic beads. A sandwich complex formed in the presence of thrombin has enabled detection of thrombin up to a concentration of 0.70 nM.
Ag85A, Ag85B, Ag85C from virulence strains have shown a protective immune response in mice and thus are strong vaccine candidates [29]. Majorly 70-80% of infected individuals survive with latent TB because of an immunological equilibrium that exists between the effector and regulatory immune cells. Only 10-20% of the carriers transform into the active TB cases under various immunocompromised conditions e.g. HIV, imbalance between effector and regulatory immune cells, alternate antigen expression etc. Various models have been proposed and studied for understanding the pathogenesis of m.tb and immune responses to the organism [30].

Different dosages of drugs are administered to different patients according to their severity. Under the DOT regimen which is the established WHO approved treatment protocol, due to long periods of treatment and high doses of drug supplements involved, there is a loss of compliance for the treatment. However, this problem may be overcome if the treatment period and dosages can be reduced by resorting to more effective and targeted drug delivery. Therefore, specific drug delivery may assure a short period of treatment with the drug along with low dose and high availability.

The diagnosis of TB also remains one of the major problems due to the slow growth of the organism, m.tb. The visible symptoms occur only after a heavy load of lung-resident bacteria breaches the epithelial lining and enters the mucus [31]. The objective of ending TB globally can be achieved by early diagnosis, low dose effective treatment and an ideal vaccine. As seen from the previous sections, nanobiotechnology has enabled the development of various types of nanomaterials that have been studied for low dose targeted drug delivery and used for the development of point of care (POC) diagnostic tools for other diseases and conditions.

Currently, active TB is diagnosed by sputum smear microscopy, culture-based methods, imaging by chest X-ray or PET-CT and molecular tests like nucleic acid amplification (NAAT), Xpert® MTB/RIF assay, etc. Latent TB diagnosis

3. NANOTECHNOLOGY IN TB

TB has been ranked as the ninth most devastating disease worldwide, which is above AIDS. Millions of deaths and new cases every year have compelled the World Health Organization and United Nations to include this issue in the Sustainable Development Goals, the ultimate being to end the global TB epidemic by 2035 [28].

It is an infectious disease majorly caused by m.tb. The disease is generally spread by sneezing, coughing whereby, the m.tb containing aerosol is expelled into the atmosphere. This aerosol when inhaled by naive individuals, leads to the entry of m.tb into the lungs where professional phagocytic cells engulf them. The survival mechanisms of m.tb evade the phagolysosomal fusion in the phagocytic cells. The m.tb ESX1 type VII secretion system enables it’s spread from cell to cell in the lung tissue. The antigens CFP-10, ESAT-6, Ag85A, Ag85B, Ag85C from virulence strains have shown a...
is done by TST and IGRA test which have their own limitations. These include passive diagnosis which is based on detecting T-cell responses post infection but not memory cell-based detection, and lack of a POC predictive method. The sensitivity and accuracy of these tests are promising only under high microbial load. Consequently, there is no good diagnostic method to detect the early stages of TB infection and its consequent risk of progression [32].

Gold nanoparticles have properties such as chemical stability, high electron density, and affinity for biomolecules, making them drug carriers as well as a tool for diagnosis. Research in this area has led to the development of Au-conjugated PLGA-PEG-SA-PEG-PLGA multiblock copolymer loaded with rifampicin which was demonstrated to exhibit an in vivo sustainable drug release of up to 240 hrs with increased bioavailability [33].

Similarly, a dual channel lateral flow assay has been designed for the detection of antibody specific for m.tb 38 kDa antigen in patients serum. The minimum analyte detectable was 5 ng/ml, therefore, this has great potential to be used as a POC tool [34]. Further, the surface plasmon resonance property of AuNP has been exploited to detect m.tb DNA. When the AuNP linked single strand DNA binds to target TB DNA, AuNPs aggregate and thus lead to a change in the surface charge density and therefore color change. This paper-based device showed the visible color change that can be read out with a smartphone up to a minimum of 0.0195 ng/ml of ds TB DNA [35].

M.tb is becoming tolerant to classical antibiotics and therefore resulting in the emergence of MDR and XDR species of the bacteria. While looking for different strategies for new drug discovery to combat the emergence of MDR and XDR species of the bacteria, the Small Molecule Variable Ligand Display (SMVLD) approach may prove helpful. In the SMVLD method, combinations of small organothiol ligands are attached to AuNPs to create a library of mixed ligand-modified nanoparticle conjugates that are subsequently screened for bacterial growth inhibition activity. The mixed ligand monolayer-AuNP conjugates have inhibited the growth of M. smegmatis at 8 uM concentration and might have the potential to serve as the next generation TB antibiotic [36].

Studies on in vivo instillation of AuNPs have shown that maximum fraction was phagocytosed by macrophages within one hr while only a small fraction entered in to the systemic circulation [37]. In vitro studies have shown that AuNPs have the ability to get translocated and distributed across epithelial tissues [38]. In a similar fashion, folic acid modified dendrimer-entrapped AuNPs, also have a great potential to be used as imaging probes, for targeted CT imaging of human lung adenocarcinoma [39]. The inhalable AuNPs with anti TB drug/ fluorescent dye targeted to the lung granuloma may provide a promising approach for treatment/diagnosis.

Magnetic nanoparticles functionalized with specific antibodies have been used in magnetoresistive biosensors for m.tb detection. Streptavidin-coated magnetic nanoparticles with anti-m.tb biotinylated antibodies were developed to capture BCG. When BCG is captured on the biochip surface, they are detected by an array of spin-valve sensors, which are sensitive to a small change in the magnetic field. This is an efficient POC method to detect bacteria in sputum sample as compared to the Ziehl-Nielsen sputum smear microscopy test [40]. Similarly, early detection of CFP10 has been possible in the culture magnetophoretic immunoassay in which magnetic nanoparticles were decorated with AuNPs and further coated with anti CFP10 antibody allowing a detection limit of 0.3 pm [41, 42]. The simultaneous detection of latent TB markers IFN-γ, TNF-a and IL-2 has been made possible by an electrochemiluminescence-based immunosensor. In this, luminol and carbon quantum dots and CdS, quantum dots were integrated onto the AuNP separately followed by its immobilization onto magnetic beads. These beads were then decorated with secondary antibodies for each marker. The primary antibody for each marker was immobilized onto the indium tin oxide electrode to capture the respective analytes from the serum. When magnetic beads bind to immobilized analyte, it generates electrochemiluminescence signals with intensities corresponding to the analyte concentration. The IFN-γ, TNF-a and IL-2 levels can be detected as low as 1.6 - 200 pg/ml [43]. SPIONs conjugated m.tb specific antibody in combination with MRI have the potential to diagnose extrapulmonary TB, thus providing a non-invasive diagnosis of TB [44]. Also, iron oxide nanoparticles coated with isoniazid entrapped crosslinked starch have been synthesized which shows good mucoidhesiveness and drug release property [45]. This formulation can be used to treat pulmonary TB by pulmonary delivery. (Fig. 2) summarizes the different approaches used in the diagnosis and treatment of TB.

Silica nanoparticles have a promising role in emerging nanomedicine because of their low cytotoxicity and efficient drug delivery potential. Silica nanoparticles conjugated with beta-glucan have been formulated to deliver isoniazid molecules. Cargo includes the anti TB drug, isoniazid, which is encapsulated in beta (β)-glucan-conjugated silica nanoparticles, showing a good host immune response [46]. Similarly, mesoporous silica nanoparticles (MSNPs) coated with polyethyleneimine showed greater loading capacity as compared to the uncoated preparation. Isoniazid loaded MSNPs were further capped with pH-responsive cycloextrin and were successfully internalized by macrophages endosomes. The acidic pH of endosomes helps to remove the cycloextrin cap and thereby releases the drug which kills the endocytosed m.tb [47].

Exhaled volatile organic compounds (VOCs) have been studied as biomarkers for various diseases like asthma, lung cancer, chronic obstructive pulmonary diseases (COPD), cystic fibrosis and other pulmonary infections. These VOCs are by-products of either host or bacterium or metabolites produced by their interactions which are then exhaled by the lungs. The VOCs such as naphthalene, 1-methyl- and cyclohexane, 1,4-dimethyl- have been identified by GC/MS as biomarkers for active TB with 100% specificity and sensitivity [48]. The VOCs of TB patients and healthy control have been identified by GC/MS followed by simulation analysis with predictive algorithms. Using this approach, the results identified active pulmonary TB with 80% accuracy, 71.2% sensitivity and specificity of 72% [49]. The detection of VOCs with nanosensors is easy and is real time in contrast to...
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Fig. (2). Different approaches used in diagnosis and treatment of TB.

GC/MS technique [50]. A chemiresistive sensor made up of magnetic nanoparticle linked with poly(3,4-ethylenedioxythiophene) as a conducting polymer has been developed. The lung cancer biomarkers methanol, ethanol, acetone, benzene, and toluene were detected at concentrations as low as 1 ppm [51]. Use of such sensors in TB diagnosis can help the POC diagnosis with associated cost benefits.

PLG encapsulated anti TB drug (ATD) nanoparticles have been prepared for oral/aerosol delivery in tuberculosis. This delivery approach enhances the bioavailability of the drug for 60 days. The controlled release from these formulations for a period of 6-10 days, clears the bacterial load with only 2-5 doses, as compared to 45 doses in the case of the DOT’s regimen [52-54]. PLG encapsulated ATD nanoparticles were coated with WGA for targeted delivery to epithelial cells of dunkin hartley guinea pig where an enhanced drug retention of 15 days was seen as compared to 9 days in the case of uncoated nanoparticles [55]. PLGA encapsulated ethionamide nanoparticles when given orally to mice for controlling MDR TB, have shown better pharmacokinetic parameters as compared to the free drug [56]. PLGA encapsulated rifampicin which was spray dried on porous nanoparticles aggregate particles (PANPs) for its aerosol delivery to guinea pig, showed initial burst following by a sustained release of up to 8 hrs [57]. Similarly, alginate with ATD clears the m.tb with only 3 doses of 15 days interval in infected mice. Alginate-chitosan microspheres ATD gives better bioavailability in guinea pig compared to conventional treatment [58]. A rifampicin loaded poly(ethylene oxide) monomethyl ether block-poly(e-caprolactone) polymer based nanoparticle has been synthesized. These nanoparticles are engulfed by macrophages wherein they enter the lysosomal compartment and release the drug [59]. (Table 2) summarizes the different approaches used for the diagnosis and treatment of TB.

Dendrimers are symmetrically branched structure with the functional group at its terminal which can be modified or functionalized for targeting and drug delivery. Mannosylated dendrimers loaded with rifampicin when targeted to alveolar macrophages, showed good biocompatibility and sustained release into macrophages [60]. The association of rifampicin with a 4th-generation PAMAM dendrimer was investigated by means of molecular dynamics simulations. It was seen that the rifampicin-PAMAM complex was highly stable under physiological pH and resulted in a rapid release of rifampicin molecules under acidic medium. Thus, providing an interesting switch for drug targeting since the bacteria reside within acidic domains of the macrophase [61]. Furthermore, researchers have developed inhalable microspheres of third generation PAMAM dendrimers loaded with rifampicin. The in vitro pharmacokinetic studies and in vivo studies using them have shown sustainable release of the drug and possibilities to attain the minimum inhibition concentration of the drug in the plasma [62].

Chitosan is an N-deacetylated derivative of chitin and widely used in drug delivery system due to its nontoxic, bioabsorbable and slow drug release properties. Rifampicin loaded chitosan dry powder nanoparticles were synthesized and delivered in vitro. The nanoparticles showed sustained drug release up to 24 hrs and slow clearance from the lungs which promise its therapeutic application [63]. Likewise, chitosan-PEG nanocarriers have also been studied for rifampicin encapsulate efficiency and their sustained release [64]. The microparticles developed from low molecular weight chitosan (50-190 kDa) have 89% isoniazid entrapment capacity. These microparticles are mucoadhesive which increases its retention time in the alveolar environment and can activate alveolar macrophages upon drug release [65].

Many of the aptamers developed for diagnosis and treatment are under clinical trials and a few of them have been approved by the US FDA for commercialization [66]. Aptamer-based sensors have been extensively studied also for TB diagnoses. The aptamer Apt22 which was isolated by the protein SELEX method using magnetic beads shows very high affinity to the target m.tb secretory protein Ag85A (FbpA). Further Apt22 was attached on graphene oxide for a fluorescent-based assay which could detect up to 1.5 nM
Table 2. Summary of nanotechnology-based approaches for diagnosis and treatment of TB.

<table>
<thead>
<tr>
<th>Type of Nanoparticle</th>
<th>Drug Delivery/Diagnosis</th>
<th>Target</th>
<th>Therapeutic Agent/Diagnostic Agent</th>
<th>Mode of Action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au, PLGA-PEG-SA-PEG-PLGA</td>
<td>Drug delivery</td>
<td>m.tb cells</td>
<td>Rifampicin</td>
<td>Chemotherapy</td>
<td>[33]</td>
</tr>
<tr>
<td>AuNP</td>
<td>Diagnosis</td>
<td>m.tb 38 antigen</td>
<td>Antibody</td>
<td>Surface Plasma Resonance</td>
<td>[34]</td>
</tr>
<tr>
<td>AuNP</td>
<td>Diagnosis</td>
<td>m.tb DNA</td>
<td>DNA</td>
<td>Surface Plasma Resonance</td>
<td>[35]</td>
</tr>
<tr>
<td>AuNP-SMVLD</td>
<td>Treatment</td>
<td>m.tb cells</td>
<td>SMVLD</td>
<td>Bacteriostatic</td>
<td>[36]</td>
</tr>
<tr>
<td>SPIO</td>
<td>Diagnosis</td>
<td>m.tb cells</td>
<td>Anti-m.tb antibody</td>
<td>Magnetoresistive biosensor</td>
<td>[40]</td>
</tr>
<tr>
<td>SPIO, AuNP</td>
<td>Diagnosis</td>
<td>CFP10</td>
<td>Antibody</td>
<td>Immunoassay</td>
<td>[41, 42]</td>
</tr>
<tr>
<td>SPIO, AuNP, CdS &amp; Carbon quantum dots</td>
<td>Diagnosis</td>
<td>IFN-γ, TNF-α IL-12</td>
<td>Antibody</td>
<td>Immunosensors</td>
<td>[43]</td>
</tr>
<tr>
<td>SPIO</td>
<td>Diagnosis</td>
<td>m.tb cells</td>
<td>Antibody</td>
<td>Contrasting agent</td>
<td>[44]</td>
</tr>
<tr>
<td>SPIO</td>
<td>Drug delivery</td>
<td>m.tb cells</td>
<td>Isoniazid</td>
<td>Chemotherapy</td>
<td>[45]</td>
</tr>
<tr>
<td>Silica NP, beta-glucan</td>
<td>Drug delivery</td>
<td>m.tb cells</td>
<td>Isoniazid</td>
<td>Chemotherapy</td>
<td>[46]</td>
</tr>
<tr>
<td>MSNP</td>
<td>Drug delivery</td>
<td>m.tb cells</td>
<td>Isoniazid</td>
<td>Chemotherapy</td>
<td>[47]</td>
</tr>
<tr>
<td>PLG</td>
<td>Drug delivery</td>
<td>m.tb cells</td>
<td>Isoniazid, rifampicin, pyrazinamide</td>
<td>Chemotherapy</td>
<td>[52-55]</td>
</tr>
<tr>
<td>Chitosan</td>
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<td>m.tb cells</td>
<td>Rifampicin</td>
<td>Chemotherapy</td>
<td>[60-62]</td>
</tr>
<tr>
<td>Dendrimer</td>
<td>Drug delivery</td>
<td>m.tb cells</td>
<td>Rifampicin</td>
<td>Chemotherapy</td>
<td>[63-65]</td>
</tr>
<tr>
<td>-</td>
<td>Drug delivery</td>
<td>m.tb cells</td>
<td>Rifampicin</td>
<td>Chemotherapy</td>
<td>[66]</td>
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<tr>
<td>-</td>
<td>Diagnosis</td>
<td>Ag85A</td>
<td>Apt22</td>
<td>Flow cytometry</td>
<td>[67]</td>
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<tr>
<td>-</td>
<td>Diagnosis</td>
<td>H37Rv</td>
<td>Thioaptamer</td>
<td>MSPQC sensor</td>
<td>[69]</td>
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<tr>
<td>-</td>
<td>-</td>
<td>CD44</td>
<td>Thioaptamer</td>
<td>Fluorescence microscopy</td>
<td>[70]</td>
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b Refer the numbered reference in the text.

FbpA protein in the serum [67]. Direct and indirect dot blot assays using enzyme-linked aptamers have shown high specificity with a detection limit as low as 10⁴ cells/ml when compared to the routine smear test in LTBI infection. Also, smartphone-based detection of the results gives a point of need diagnosis [68]. An aptamer specific for H37Rv was selected by the whole-cell systematic evolution of ligands by exponential enrichment technique. The aptamer was immobilized by thiol bond on the gold interdigital electrode of multichannel series piezoelectric quartz crystal. Further, following conjugation of single-walled carbon nanotubes on the aptamer, it was used for the detection of m.tb. Due to high affinity of the H37Rv for the aptamers, the single-walled carbon nanotubes were replaced by H37R, and thus a change in electrical signal was measurable. The sensor could detect 100 cells/ml within 70 min, thus offering a robust diagnostic tool for detection [69]. Thioaptamers with CD44 targeting moiety were conjugated to discoidal silicon mesoporous microparticles to enhance accumulation of these agents/carriers in the infected macrophages in the lungs. Thioaptamers targeted carriers significantly diminished bacterial load in the lungs and caused recruitment of T lymphocytes for better immunity [70].

Perfluorocarbons (PFCs) are hydrophobic inert liquids, composed of C-F bonds; they are non-metabolizing, biocompatible compounds with a high gas carrying capacity. They are non-toxic, when injected intravenously, pass through reticuloendothelial system and are eliminated by the lungs. PFCs have contrasting agent property for an ultrasound, MRI and CT [71]. Transplanted pancreatic islets are critical for maintaining glucose homeostasis in diabetic patients. In vivo imaging for monitoring islet survival is currently only possible by 1H MRI through SPIO labelling of the cells. PFC which contains bromine i.e. PFOB (perfluoroctylbromide) is a contrasting agent for CT and ultrasound, MRI. When these nanoparticles were targeted to in vivo transplanted pancreatic islets, they could be detected by ultrasound and CT. Also, 19F MRI detection could be possible when labelled with fluorine [72]. This non-toxic and non-invasive method of in vivo imaging helps to monitor the graft physiology in real time and further such modality can be useful for diagnosis of pulmonary and extrapulmonary TB.
Nanovaccines are nanoparticles onto which the immunogen is immobilized. The major properties of nanovaccines are site-specific delivery and bioavailability. To be a good candidate for nanovaccines, the nanoparticles should be non-antigenic, have good adjuvant property, without producing any inflammatory response and show good cellular uptake. These aspects of nanomaterials have been employed to generate nanovaccines against various pathogens [73]. An intranasal nanovaccine for influenza has been produced where three sequential repeats of the ectodomain of the matrix 2 protein are presented on the self-assembled recombinant human heavy chain of ferritin to form a nanoparticle. The intranasal delivery of this induces a high titre of IgG and IgA, with T-cell response in mice and also shows complete protection against homo-subtype H1N1 and hetero-subtype H9N2 virus [74]. Similarly, immunization through antigen expressing mRNA delivery into the host has been made possible. In vitro and in vivo studies have shown that dendrimer nanoparticles loaded with mRNA replicon for H1N1 influenza, Toxoplasma gondii, and Ebola virus antigens elicit both T-cell and antibody responses. This platform can be used for immunization against TB as well [75]. So far, nanotechnology has opened up several avenues for improved diagnosis and treatment in the control of TB. AuNP and SPIO which are well known for photothermal therapy may also be used as a carrier of ATD entrapped in different polymers; for chemotherapy, photothermal therapy, as well as for imaging purpose. Inhalable nanoparticles can also be formulated using biocompatible polymers such as PLG, PLGA, peptides, polysaccharides etc. along with ATD drug entrapment for low dose therapy. Similarly, pH-responsive nanoparticles can be formulated with ATD to target low pH physiology of granuloma. The CRISPR-Cas9 based therapeutic nanoparticles can be developed for targeting essential genes of MDR and XDR m.tb strains resident in the granuloma. This may help to reduce the bacterial load and thus prevent disease development.

CONCLUSION

The major preventive measures for TB are effective vaccines, early diagnosis and treatment with less frequency of reduced drug dose. Nanomaterials have demonstrated their potential to improve the classical diagnosis and treatment strategies in several diseases and therefore in TB as well. The bionanosensors have enabled detection of the whole m.tb cell in the sputum besides TB DNA, specific antibodies to m.tb antigens and VOCs at very low concentrations. These bionanosensors have increased the sensitivity and specificity of the diagnosis of TB. Nanoparticles act as contrasting agents for MRI, CT or ultrasound which can be used for targeted non-invasive in vivo diagnosis of TB. Thus, early diagnosis of infection can be made possible through nanotechnology. Existing nanotechnology strategies for targeted drug delivery have proven the potential for low dose treatment at site of infections. Yet there are more possible targets such as granuloma cells yet to be evaluated for pulmonary tuberculosis. Potential vaccine candidates such as CFP-10, ESA1-6, Ag85A, Ag85B, Ag85C can be evaluated for nanovaccines for immunization. Thus, nanotechnology approaches for TB may reduce the epidemic and mortality and improve the quality of life and help the WHO objective of eliminating the disease by 2035.

LIST OF ABBREVIATIONS

ATD = Anti TB Drug
AuNP = Gold Nanoparticle
PAMAM = Polyamidoamine
PEG = Polyethylene Glycol
PFC = Perfluorocarbon
PGA = Poly(glycolic acid)
PLA = Poly(lactic acid)
PLGA = Poly(lactic-co-glycolic acid)
POC = Point of Care
SMVLD = Small Molecule Variable Ligand Display
SPION = Super Paramagnetic Iron Oxide Nanoparticle
VOC = Volatile Organic Compound

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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