Roles of Nanotechnology in Diagnosis and Treatment of Tuberculosis

Attapon Cheepsattayakorn[1,*] and Ruangrong Cheepsattayakorn[2]

[1]*10th Zonal Tuberculosis and Chest Disease Center, Chiang Mai, 10th Office of Disease Prevention and Control, Chiang Mai, Department of Disease Control, Ministry of Public Health, Thailand*

[2]*Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*

Abstract: Nanotechnology has been believed for many years ago to offer new methods for both diagnosis and treatment of tuberculosis which is globally infectious disease burden in many countries, especially in developing and under-developing countries. Nanotechnological diagnosis of tuberculosis is expected to cost cheaper than many currently available diagnostic tools. Many researches are currently ongoing in the field of synthetic carriers for antituberculosis chemotherapy both oral and inhaled routes while the potentially curative drugs are available for over 50 years.

Keywords: Diagnosis, nanotechnology, treatment, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is still one of the main health threats of the world [1]. Delayed diagnosis and misdiagnosis of TB continue to fuel the global epidemic [2]. The diagnostic tools are essentially required to meet the needs of the World Health Organization (WHO)'s expansion of the Directly Observed Treatment, Short-course (DOTS), co-infection with human immunodeficiency virus (HIV) and multidrug-resistant (MDR) TB [3]. Simple and effective point-of-care TB diagnostic tests are not available yet despite considerable improvements in diagnostics for the last few decades [2]. A previous study in Australia was conducted for the application of novel tethered nanoparticles as low-cost, colour-based TB-diagnostic assays as well as another Indian study of an optical biosensor for rapid TB detection with low-cost of less than US$ 1 [3]. Since chemotherapy of TB is complex with requirement of long-period administration of poly-drug regimens, then poor patient treatment adherence is the single most reason for treatment failure [3]. Here we review the current TB diagnostic assays and treatment by nanotechnologies and highlight recent advances in anti-TB drug delivery systems and anti-TB drug encapsulation.

APPLICATIONS OF NANOTECHNOLOGY IN TB DIAGNOSTICS AND THERAPY

1. Diagnostic Applications

Nucleic acid diagnostic tests such as polymerase chain reaction play a crucial role in detection of TB bacilli at an early asymptomatic stage of TB disease progression but nanotechnology is expanding the currently available options which will contribute to better efficiency, especially greater sensitivity. Nanoparticles can be tagged with suitable ligands and can be functionalized with various lectins to make more effective Poly-DL-Lactide-co-glycolide (PLG) nano-particle uptake [4, 5].

1.1. Quantum Dots

Nanotechnology uses semiconductor nanocrystals (or "quantum dots") with no larger than 10 nanometers that can be made to fluoresce in different colours depending on their size to overcome the low specificity of fluorescence or electronic microscope to detect TB bacilli. These minuscule probes can withstand significant more light emissions and more cycles of excitations than typical organic molecules with more readily decomposition [4-6].

1.2. Protein Chips (or Proteomics)

Proteomics is important in diagnosis of the diseases and in drug development. Protein chips can be treated with small modular protein components of TB bacilli that can specifically bind to proteins containing a certain biochemical or structural motif [4].

1.3. Imaging Nanotechnology

Labeling of targeted TB-bacilli molecules with quantum dots or synthetic chomophores such as fluorescent proteins that will facilitate direct investigation of intracellular signaling complex by optical techniques, for examples: confocal fluorescence microscopy or correlation imaging [4].

1.4. Sparse Cell Detection

This method can take advantages of the unique properties of sparse cells manifested in differences in

*Address correspondence to this author at the 10th Zonal Tuberculosis and Chest Disease Center, 143 Sridornchai Road Changklan Muang Chiang Mai, 50100, Thailand; Tel: 66 53 140 767, 66 53 276364; Fax: 66 53 140773, 66 53 273590; E-mail: attaponche@yahoo.com

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deformation of intracellular TB bacilli. Sparse cells are both rare and physiologically distinct from their surrounding cells in normal physiological conditions. It is a challenge to identify and subsequently isolate these sparse cells [4].

1.5. Individual Target Probes

Nano-gold particles studded with short segments of TB-bacilli deoxyribonucleic acid (DNA) form the basis of the easy-to-read test for the presence of TB-bacilli genetic sequences. It binds to complementary DNA tentacles on multiple nanospheres and forms a dense web of visible gold balls then allows the detection of TB bacilli [4].

1.6. Nuclear Magnetic Resonance (NMR) with Microfluidic System

Iron-based magnetic nanoparticles tagged with antibodies are used for binding the *Mycobacterium tuberculosis* bacilli while microfluidic system deliver the *Mycobacterium tuberculosis* and buffer solutions. Concentrating the specimens with the membrane filter can markedly improve the detection sensitivity. Magnetic nanoparticles have high sensitive detection of Bacillus Calmette-Gue′rin.

Integration with quantum dots can detect not only *Mycobacterium tuberculosis* but also *Mycobacterium avium* subspecies " paratuberculosis " [5, 7].

1.7. Noble Metal Nanoparticles

This method demonstrated 94.7% of sensitivity and 99.6% of specificity for detection of *Mycobacterium tuberculosis* while revealed 96.6% of sensitivity and 98.9% of specificity for detection of *Mycobacterium tuberculosis* complex. It can detect *rpoB* mutation associated with drug resistance [5].

1.8. Silica Nanoparticles

This method is luminescence-based nanoparticles combined with immunofluorescence microscopy for detection of *Mycobacterium tuberculosis* with extremely high sensitivity and low false positivity [5].

1.9. Nano-Fabricated Devices

Nano-fabricated devices is ideal for point-of-care application in detection of *Mycobacterium tuberculosis* because of reduced costs of the automated sensitive detection and ability of nanostructured zinc-oxide-films detection of genomic target up to 100 pM in clinical specimens [5].

2. TB Therapeutic Applications

2.1. Drug Delivery

Nanoparticles as therapeutics foe examples: nanoemulsions, nanosuspensions, niosomes, polymeric micelles and other self-assembled structures which are anti-TB drug nanocarriers, and polymeric and nonpolymeric nanoparticles.

Nanoparticles can cross the intestinal permeability barrier directly through the transcellular or paracellular pathways into the circulation [1]. Nanosuspensions have been shown to be potential and promising new anti-TB drug formulations for intravenous route. These nanocarriers demonstrated ability of higher stabilization and drug-carrier capacity, feasibility of incorporation of both hydrophobic and hydrophilic substances and feasibility of routes of administration not only intravenous but also oral and inhalation routes [3].

2.2. Drug Encapsulation

Functional macromolecules " dendrimers " are well-defined, regular hyperbranching with three-dimensional structures and highly adjustable functionality for anti-TB drug encapsulation by the dendrimeric core and complexation and conjugation on their surface [1]. Liposomes are nano- to microsized vesicular carriers comprising a phospholipid bilayer which surrounds an aqueous core whereas the core enables the encapsulation of water-soluble anti-TB drugs. Liposomes help achievement of targeted anti-TB chemotherapy through recognition by the phagocytic cells and are rapidly cleared from the blood circulation. Polyethylene glycolylated (PEGylated) liposomes can extend the circulation times and prevent it elimination. A previous study revealed that liposome-encapsulated rifampicin and isoniazid killed *Mycobacterium tuberculosis* bacilli in lungs at and below therapeutic concentrations which were more effective than free anti-TB drugs. Several recent studies demonstrated that rifabutin and pyrazinamide-containing liposomes had potential and versatility of these nanocarriers. Microencapsualtion by microspheres took the advantages by extension of the time for anti-TB drug release from days to months with the small microspheres and for a year or more with large microspheres. Rifampicin-microsphere formulation demonstrated effective delivery of rifampicin to host macrophages by significant reduction the levels of intracellular replicating *Mycobacterium tuberculosis* bacilli at the delivering effective doses compared to the equivalent doses of free anti-TB drugs when investigated in animal models. Combinations of small-

and large-microsphere formulations would be ideal TB treatment regimens because small microspheres enable targeting the host macrophages while large microspheres enable effectively systemic anti-TB drug delivery [1, 3, 4, 8-10].

TYPES OF ANTI-TB DRUG CARRIERS

Anti-TB drug carriers are classified: synthetic or natural origin. They allow the flexibility of selecting the route of drug delivery, depending on the drug formulation. Not only the smaller size but also the ability of higher drug encapsulation and enhancement of the orally administered-drug bioavailability is the key difference between the nanoparticles and microparticles. PLG-A nanoparticles are commonly used preparation for emulsification or evaporation.

1. Natural Anti-TB Drug Carriers

Liposomes which consist of a lipid shell [8] or amphiphilic lipid molecules [11] surrounding an aqueous core can be selectively targeted towards the lung tissue by tagging them with o-stearyl amylopectin [8]. Liposome structure was first introduced in 1965 and then proposed as a drug delivery nanoparticle (NP) platform in 1970s [11]. After extensive studies on their lipid-drug and lipid-protein interactions, lipid polymorphism, their fundamental properties, and liposome disposition mechanisms in 1980s, the application of liposome potential was globally recognized and being transferred to medical practice as a drug delivery vehicle [11]. Liposome drug delivery system is able to be made of either natural or synthetic lipids [11]. PEG has been frequently used for conjugation to liposome surface, namely " PEGylated liposomes " [1, 12] to establish a stealth layer which prolongs the liposome-circulation lifetime in the blood circulation [11]. The liposome-based anti-TB drug delivery system have been demonstrated to be suitable for encapsulated anti-TB drug release. Liposome encapsulated rifampicin or isoniazid inhalable administration twice a week for 6 weeks or co-administered rifampicin and isoniazid once a week for 6 weeks in TB-infected mice as well as twice weekly-aerosolized liposomal rifampicin for 6 weeks revealed more effective in TB bacilli clearance than free drugs with no hepatotoxicity. In case of aerosolizing liposome-encapsulated anti-TB drug administration to guinea pigs, it resulted in remaining of the drugs in the circulation and alveolar macrophages from the bronchoalveolar larvage for 24-48 hours and 5 days post-aerosolization [8], supported by the study by Gupta *et al.* that pulmonary delivery of formulations containing anti-TB drugs can be best carried out using handheld devices [13]. Hence, the daily oral dosing could be reduced to once a week [8]. Ligand-appended liposome drug delivery system for pulmonary TB treatment was recently reported by Bhardwaj *et al.* and revealed that it was proved to be more effective for use as a dry powder inhaler [14]. Currently, liposomes are the most globally used antimicrobial drug delivery system [11]. Niosomes, a group of natural anti-TB carriers has similarity to that of liposomes with main composition of non-ionic surfactant and with or without incorporation of lipids [15]. Some previous *in vivo* studies demonstrated that up to 65% of rifampicin can be localized in the lungs and showed higher drug concentration in intrathoracic lymph nodes with intraperitoneal administration [16]. Isoniazid was tried to incorporate in niosomes [17, 18].

In the previous first study, the co-incorporated rifampicin, isoniazid and pyrazinamide-loaded solid lipid nanoparticles were prepared by the emulsion solvent diffusion technique for respiratory administration with 7 weekly doses in TB-infected guinea pigs. The results revealed sustenance of the drugs in plasma and the organs for 5 and 7 days, respectively and completely undetectable TB bacilli in the organs, replacing 46 conventional doses. The similar results were shown in the oral route study which demonstrated better results with 8 day- plasma and 9-10 day-organ maintenance and clearance of TB bacilli with 5 oral doses and 10 day-spacing apart [8]. Alginic acid, a natural co-polymer of guluronic acid and mannuronic acid is ideal nanoparticulate delivery system for encapsulation of rifampicin, isoniazid pyrazinamide [8, 19] and ethambutol [19] microsphere-based drug delivery system for oral administration to guinea pigs [8, 11]. A single oral dose (12 mg/kg for rifampicin plus 10 mg/kg for isoniazid plus 25 mg/kg for pyrazinamide plus 16 mg/kg for ethambutol) administered to *Mycobacterium tuberculosis* H_{37}Rv-infected mice revealed that the administration of three oral doses of anti-TB drug-loaded alginate nanoparticles (1 dose of 4-drug combination and 2 doses of 2-drug combination), and 45 doses of oral free drugs daily administered (15 doses of 4-drug combination followed by 30 doses of 2-drug combination) demonstrated undetectable colony-forming units (cfu) in lungs or spleen compared to about 4 log cfu in untreated control population ($p < 0.001$) [19]. Another study results revealed drug maintenance in plasma and organs for 4-5 days and 7-9 days, respectively and complete mycobacterial clearance after weekly 8-oral doses, same as the daily

oral administration of the free drugs [8] whereas alginate-chitosan system documented better therapeutic results with only half of weekly therapeutic dose-oral administration [8]. A single oral administration of the above formulation in alginate-chitosan microspheres could sustained therapeutic drug concentrations in the plasma or organs of the mice or guinea pigs for 2 weeks. The similar results were also demonstrated in guinea pigs with aerosolized administration whereas a total clearance of TB bacilli was achieved after 6 weeks of 3 doses of the above formulation [8]. Another similar study on chemotherapeutic potential of alginate-chitosan microspheres as oral anti-TB drug carriers for rifampicin, isoniazid and pyrazinamide administered to *Mycobacterium tuberculosis* H_{37}Rv-infected guinea pigs showed that treatment with either a therapeutic dose of anti-TB drug-loaded microspheres (5 doses), a half-therapeutic dose (7 doses) or parent drugs (46 doses) all resulted in undetectable cfu in lungs or spleen (< 1.0 cfu based on the lowest dilution tested) and alginate-chitosan microspheres lies not only in reducing the dose frequency, but also the dose itself such as the half-therapeutic dose of formulation also resulted in bacterial clearance whereas untreated population demonstrated comparable bacterial load ($p > 0.05$) to animals receiving empty microspheres [20]. Sabitha *et al.* reported a recent study on chitosan-calcium alginate microcapsulation of rifampicin-isoniazid-pyrazinamide *in-vitro* release for oral use which revealed that the release rate was 95.46% (3 hours) for rifampicin, 98.99% (30 minutes) for isoniazid and 96.44% (30 minutes) for pyrazinamide [21]. This indicated the promise as a potential natural polymer-based oral anti-TB drug carriers for better TB treatment [21]. A designed-rifampicin carriers, chitosan and polyethylene glycol 600 (PEG) nanoparticles were recently proposed and expected to be a promising system for rifampicin delivery in TB treatment [22]. Isoniazid-PEG-poly(aspartic acid) conjugated with micelles, the submicroscopic aggregates (20-80 nm) of surfactant molecules resulting in liquid colloid were studied and demonstrated 5.6-fold increase in anti-TB activity against *Mycobacterium tuberculosis* compared to free drug [23]. Incorporation of rifampicin and pyrazinamide in micelles (< 100 nm) was tried to minimize renal filtration and prolonging mean residence times in the blood circulation with improvement of antimycobacterial activity [24, 25]. Dendrimers are well definded, highly branched macromolecules and represent a novel class of structurally controlled three dimensional macromolecules that radiate from a central core and are mainly derived from a branches-upon-branches structural design [15]. The phagocytic uptake of rifampicin and rifampicin-loaded dendrimers in alveolar macrophages harvested from rat' s lungs demonstrated a clear increase in the intracellular concentration of the antimicrobial agent [26]. Recently, researchers from Monash Institute of Pharmaceutical Science (Melbourne, Australia) developed PEGylated Polylysine dendrimers in collaboration with Starpharma Holdings Ltd for TB treatment including treatment of HIV, cancer and lymphatic diseases [15].

2. Synthetic Anti-TB Drug Carriers

PLG is a co-polymer of glycolic acid and lactic acid with its completely biocompatibility and biodegradability and human non-immunogenicity which allowed repeated administration in human and development of different PLG formulations such as encapsulating rifampicin such as hardened, porous and non-porous [8]. Hardened PLG microparticles (PLG-MP) for rifampicin demonstrated best sustained drug release for 42 days and 12-14% encapsulation while replacement with isoniazid revealed 49 days of drug release. In mice model, PLG-MP encapsulated rifampicin-isoniazid combination by implantation demonstrated drug release for 6 weeks but it has possible risk of N-methyl pyrrolidone which used in the preparation process. Hence, parenteral route application is preferred. A single subcutaneous injection of anti-TB drug with loading of PLG-nanoparticles showed sustenance of drug in the plasma and organs of TB-infected mice for 32 and 36 days, respectively with complete mycobacterial clearance from the organs of the mice. Once-month release injectable microspheres of leuprolide acetate, a superactive agonist of LH-RH had been investigated to support the application of PLG-based nanotechnology for mycobacterial infections and is currently available in the third market. PLG-MP encapsulated rifampicin-isoniazid-pyrazinamide combination *via* oral administration with a once-weekly regimen for 6 weeks was evaluated in mice model and showed an impressive reduction of *Mycobacterium tuberculosis* bacilli counts as well as other similar study results. A study of co-encapsulated PLG nanoparticles (186-290 nm in size) of rifampicin, isoniazid and pyrazinamide with oral administration in mice was evaluated and demonstrated that the plasma drug levels were sustained above the minimum inhibitory concentration (MIC_{90}) for 6-9 days in the plasma, lungs, spleen and liver whereas free drugs were cleared from organs and plasma with 12-24 hours. Similar results were shown in the higher animal models [8]. PLG-nanoparticle-based

formulation of rifampicin-isoniazid-pyrazinamide was orally [27] and subcutaneously [28] administered in mice for 5 doses every 10 days and single injection, respectively and aerosolizingly [29] and orally [29] administered in guinea pigs both for 5 doses every days demonstrated the promised anti-TB drug delivery system. Co-encapsulated PLG nanoparticle of 4 drugs (rifampicin, isoniazid pyrazinamide and ethambutol) *via* oral administration with a single therapeutic dose was also investigated in mice and showed sustainability of in the plasma for 3, 6 and 8 days for ethambutol, rifampicin and isoniazid or pyrazinamide, respectively whereas in the tissues, rifampicin, isoniazid and pyrazinamide were maintained up to 9 days except ethambutol which was sustained up to 7 days. The inhalable microsphere-based drug delivery of anti-TB drugs *via* nebulization or insufflation to guinea pigs, 24 hours prior to aerosol TB infection was studied and revealed significant reduction of the cfu counts of the *Mycobacterium tuberculosis*, strain " $H_{37}Rv$ " after 28 days post-infection compared with free drugs. A second dose of microspheres was administered to the half of studying guinea pigs at 10 days post-infection and demonstrated significant decrease of cfu only in lungs in cases receiving single dose of the same formulation while administration of two doses resulted in significant reduction of cfu both in lungs and spleen [8]. In a previous study, a single aerosolization of PLG nanoparticles co-encapsulating rifampicin, isoniazid and pyrazinamide with 1.88 μm of the mass median aerodynamic diameter suitable for deep lung delivery was administrated to guinea pigs and demonstrated maintenance of therapeutic drug concentration in the lungs for 9-11 days whereas it was 6-9 days plasma.

Comparison with free anti-TB drugs, there was a significant improvement in the half-life, relative/absolute bioavailability and mean residence time of encapsulated drugs. Five aerosolized doses of PLG nanoparticles co-encapsulating rifampicin, isoniazid and pyrazinamide with 10 days spacing apart administered to *Mycobacterium tuberculosis* $H_{37}Rv$ infected-guinea pigs revealed undetectable cfu in the lungs replacing 46 conventional doses. This was the first study of inhalable PLG nanoparticles for anti-TB drug carriers [8].

Comparison with microparticles, firstly, the decrease of lung cfu was better, and secondly, co-administration of three anti-TB drug encapsulation was possible in nanoparticles delivery system. In case of aerosolization of lectin-functionalized PLG nanoparticles to TB-infected guinea pigs, the therapeutic drug concentrations were sustained in the plasma and in the organs for 6-10 and 15 days, respectively whereas administration of every fortnightly, only 3 doses of the formulation was able to demonstrate undetectable cfu

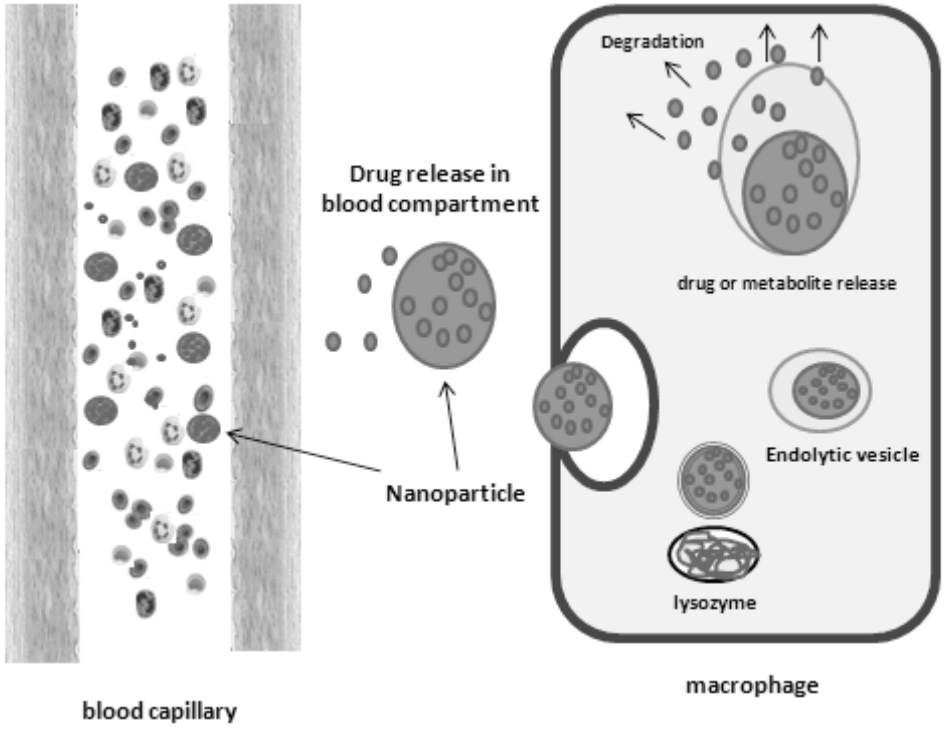

Figure 1: Proposed mechanism of both natural and synthetic drug carriers by which nanoparticle encapsulated drug can be released in infected macrophage for anti-TB chemotherapeutic agents.

in the lungs and spleen [8]. A similar study on lectin-functionalized PLG nanoparticle-based formulations of rifampicin-isoniazid-pyrazinamide in TB-infected guinea pigs orally [29] and aerosolizingly [29] with 3 doses fortnightly both revealed promisingly [29] as well as a previous study results on solid lipid nanoparticle-based formulations of rifampicin-isoniazid-pyrazinamide with 7 doses weekly in guinea pigs [30]. Dry power inhalation with encapsulation of rifampicin and isoniazid in poly-lactide microparticles to rats was investigated and showed higher drug concentrations in the alveolar macrophages compared with vascular delivery of free drugs. Inhalable microparticulate delivery system for para-aminosalicylic acid in dipalmitoylglycero-3-phosphocholeline was also studied in order to reduce the dosages [8]. Human intestinal and respiratory epithelial cells contain receptors for wheat germ agglutinin which is a commonly occurring plant lectin having low immunogenicity. Thus, it suitably used for oral and inhalable drug delivery with detection of the lectin-coated anti-TB drug in tissues up to 15 days compared with 11 days in case of lectin-uncoated formulation from a previous study results [8]. Polyalkylcyanoacrylates [31, 32] and functionalized mesoporous silica [33] nanoparticles are other promisingly synthetic anti-TB drug carriers which increasingly mentioned worldwide. Proposed mechanism of both natural and synthetic drug carriers by which nanoparticle encapsulated drug can be released in infected macrophage for anti-TB chemotherapy is shown in Figure **1**.

FUTURE PERSPECTIVES AND CONCLUSIONS

Development and performance with tremendous advancement of novel nanotechnological approaches for rapid detection of *Mycobacterium tuberculosis* and drug-resistance prediction have been invested and investigated in the last few years. Nanodiagnostics for TB within hours is an obvious advantage. However, only a very small volumes of them have been translated to the clinical TB molecular diagnostics and very few techniques are available for direct application in respiratory samples. Practical points indicated that oral-route anti-TB drug delivery with PLG nanoparticles could be the preferred one. Large-scale development of anti-TB drug PLG-nanoparticles, especially in combination with alginate-based nanoparticles should be emphasized on the future investigations. Nanodiagnostics' future trends will provide ability of non-specialized health personnel to use them through miniaturization of biochip technology to the nanoscale range for point-of-care diagnostics with a specimen-in-answer-out approach.

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