The Role of Metallic Nanoparticles to Inhibition of *Mycobacterium Tuberculosis* and Enhances Phagosome Maturation into the Infected Macrophage

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Abstract

This review has focused on the role of Gallium (Ga) nanoparticles (NPs) to enhance phagosome maturation into the *M. tuberculosis* infected macrophage and the role of magnetic iron NPs as nano-carrier of anti-tuberculosis drugs. The literature shows that Ag and ZnO NPs with dimensions less than 10 nm have ability to penetrate directly through macrophage bilayer membrane. Ag NPs increase permeability membrane by motiving the aggregation of proteins in the periplasmic space and forming nano-sized pores. ZnO NPs are able to interact with the membrane of *M. tuberculosis* which led to formation of surface pores and the release of intracellular nucleotides. The colloidal Ag:ZnO mixture NPs with 1:1 ratio not is only able to eliminate *M. tuberculosis*, but showed also lowest cytotoxicity effects on MCF-7 and THP-1 cell lines. Mixture Ag/ZnO nanocrystals are not able to kill *M. tuberculosis,* lonely in *Ex-vivo*. Hence, bimetallic Au/Ag NPs possessed the high efficiency to inhibit *M. tuberculosis* in *Ex-vivo* THP-1 infection model. Co-delivery of mixed MeNPs into a polymeric carrier collaborated to selective uptake by macrophages through passive targeting, initial burst release of ions from the encapsulated MeNPs and eventually reduction of MeNPs

toxicity and plays a pivotal role to increasing of the anti-tubercular activity as compared to use lonely. In addition, Ga NPs have ability to import drugs to the macrophage, inhibit *M. tuberculosis* growth, and reduce the inhibition of phagosome maturation. Magnetic encapsulated NPs exhibited good drug release properties and might be suitable as carriers of anti-tuberculosis drugs.

Keywords: Metal Nanoparticle; *Mycobacterium tuberculosis* $H_{37}Rv$; Phagolysosome; Monocyte Derived Macrophages

Introduction

Tuberculosis (TB) is a major global health problem and *Mycobacterium tuberculosis* (*M. tuberculosis*) has been infected one third of word's population.[1, 2] It was reported that 10.4 million new cases and 1.8 million deaths from TB in 2016.[2] *M. tuberculosis* entered to human respiratory system through the inhalation of aerosols, typically through coughing. Then, *M. tuberculosis* colonized inside the alveolar macrophages and finally granuloma will be formed. After inhalation, the *M. tuberculosis* is ingested by phagocytosis by resident alveolar macrophages and tissue dendritic cells (DC).[3, 4] Finally, the immune cells are contributed and the pathological mark of TB, the granuloma, is formed. In the granuloma, macrophages differentiate into epithelioid cells or foamy macrophages, or fuse to form giant cells, and become surrounded by lymphocytes and a fibroblasts and extracellular matrix proteins. To this condition, the *M. tuberculosis* will be survived until the granuloma fails due to immunosuppression.[5, 6] In fact, *M. tuberculosis* use the granuloma for their benefit upon initial infection, recruiting new macrophages to allow spread between host cells.[7] In active tuberculosis, the caseous granuloma center is containing necrotic macrophages. When the granuloma rupture, spillage of *M. tuberculosis* into the airways achieved and *M. tuberculosis* also allows spread to new individuals.[5, 6]

A main component of TB pathogenesis is that the bacilli survives, grows and replicates of *M. tuberculosis* within the host macrophages. These attributes are partly thanks to the ability of *M. tuberculosis* to prevent maturation of phagosome by blocking phagosome integration with lysosomes to form the phagolysosome.[8] It has been proven that the successful parasitization of macrophages is a clever action by which *M. tuberculosis* keeps away the immune response of the host cells.[9, 10]

M. tuberculosis can also attenuate human immunological function through replicating within macrophages, as target cells. The intracellular replication of *M. tuberculosis* into the macrophages finally led to the death of macrophages and the release of extracellular

pathogens. Support of the *M. tuberculosis* into the macrophages requires that the pathogen be able to continuously set up the infection into the sensitive macrophages.[10]

Current, the treatment of patients with Multidrug-resistant *M. tuberculosis* and extensively drug resistant *M. tuberculosis* strains has been changed to a serious challenge.[11] On the other hand, treatment of patients with tuberculosis requires working with a long time multi-drug regimens that some of which interact with some other antibacterial drugs, increasing possibility for drug toxicity.[12] So that, the investigators indicate that rifampicin is able to induce liver injury in mice[13] and some of anti-tubercular drugs have hepatotoxicity effects.[14] Therefore, there is always a need for simple, long-term, and effective anti-tubercular drug regimens for the treatment of *M. tuberculosis*.

Researchers found that the mixed colloidal silver and zinc oxide MeNPs have ability to inhibit *$H_{37}Rv$ M. tuberculosis*, even within the THP-1 cell lines.[15] In addition, mixed AgZnO nanocomposites are able to also eliminate *$H_{37}Rv$ M. tuberculosis* and MDR-TB after phagocytosis by the THP-1 cell lines.[16] Recent studies show that mixed AgZnO nanocomposites and rifampicin have synergism effects against *M. tuberculosis.*[16] In the last years, researchers concentrated on the preparation of co-delivery mixed MeNPs containing anti-tubercular antibiotics encapsulated to non-toxic and biodegradable polymers.[18 ,17] Of course, the toxic effects of MeNPs on THP-1 and normal human lung cells (MCF-7 cell lines) should consider.

Studies show that MeNPs in human tissues and cell-cultures produce several toxins which able to increase the oxidative stress and production of inflammatory cytokines. MeNPs might contribute to the apoptosis of cells. MeNPs can penetrate through bilayers cell membrane, mitochondria, and nucleus, thus; they might lead to destruction of the mitochondria and mutation in DNA. The size, dimensions, chemical composition, shape, surface structure, surface charge, density and solubility of MeNPs are major factors in determination of toxicity.[19] Studies also indicated that initial used concentration of MeNPs plays a key role in their toxicity against human cells, especially in THP-1 cell lines.[16]

The major objective of this work was to introduce of MeNPs as an inhibitor growth of *M. tuberculosis* and inducers to phagosome maturation into the infected macrophages. In view of this goal, we investigated on the survival mechanisms of *M. tuberculosis* into the macrophages, and present the novel chemicals, phytogenic and, encapsulated mono-metallic or bimetallic silver (Ag), zinc oxide (ZnO) and, gold (Au) anti-tubercular MeNPs to inhibition of intracellular *M. tuberculosis* and Gallium (Ga) MeNPs to enhance phagosome

maturation. We also evaluated the role of magnetic iron MeNPs as nano-carrier of anti-tuberculosis drugs.

1. Mycobacterium tuberculosis pathogenesis

TB is known as airborne disease caused by *M. tuberculosis*. *M. tuberculosis* and *M. tuberculosis* complex species including *M. bovis*, *M. africanum*, *M. microti*, *M. caprae*, *M. pinnipedii*, *M. canetti* and *M. mungi* more likely may cause disease in humans[20].

Mostly, TB infection arises from cough, sneeze, shouting, or singing of people suffering from pulmonary or laryngeal TB disease and subsequently when a person inhales droplets including bacilli, it reached through the upper respiratory tract and bronchi to the alveoli of the lungs. The infected droplets are around 1–5 mm.[21] After that, bacilli are phagocytosed to form tubercles in the tissue-resident alveolar macrophages of which invited to the inflamed region. Under the conditions of the week host defense, bacilli may survive for a long time as a source of post primary infection into the alveolar macrophage.[22] In fact, macrophages of which failed to kill the mycobacterial invaders, produced chemo-attractants such as chemokines.[23] Chemokines were produced by resident alveolar macrophage and pneumocytes which they invited the neutrophils, monocyte derived macrophages, NK cells, and T cells, which increase inflammation.[23] Then, the cells formed a hard shell structures that know as granulomas. In this condition, the tubercle bacilli remain in the “dormant state” inside macrophages in the granulomas for years (Figure 1).

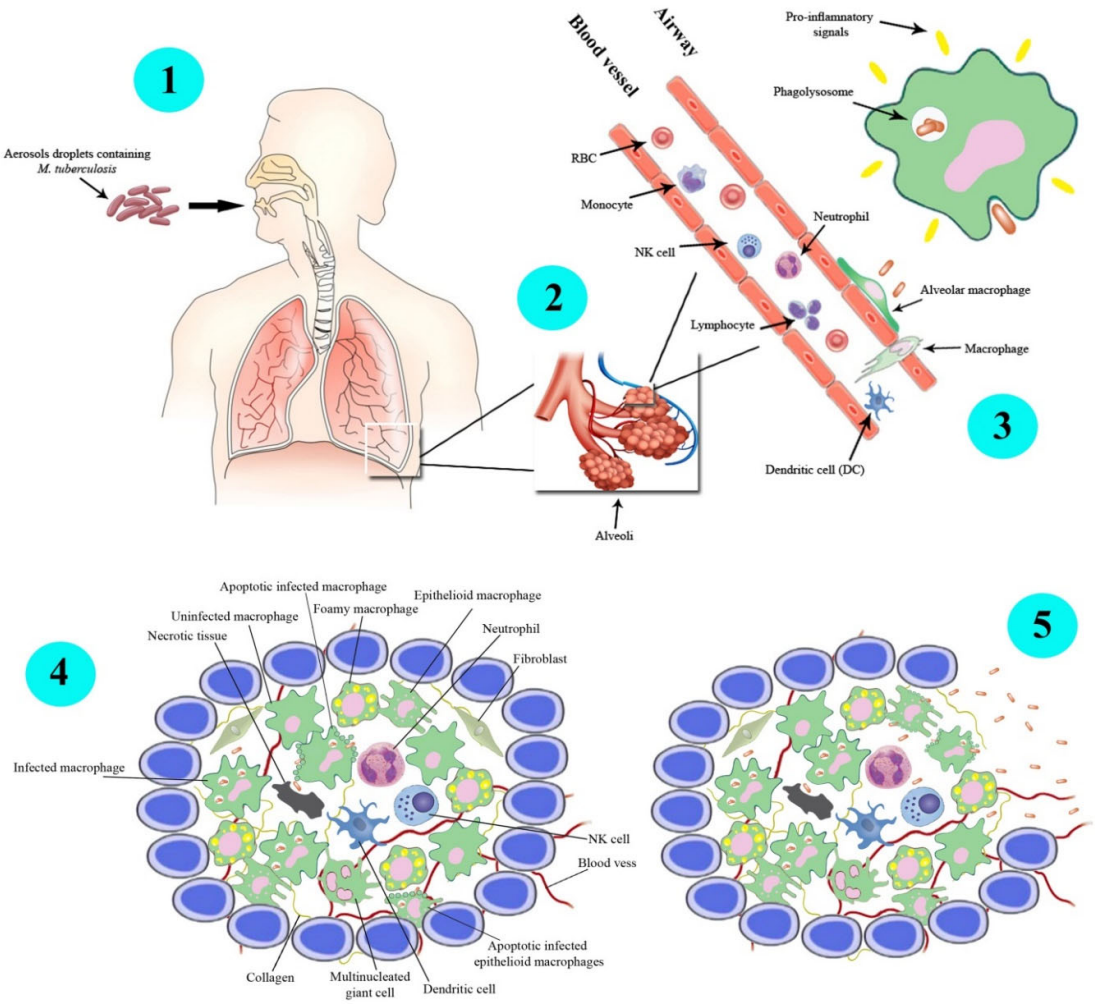

Figure 1: The infection pathways of *M. tuberculosis* in a human macrophage cells conducting to the formation of a granuloma (1 to 4). Resident alveolar macrophages phagocytes inhaled mycobacterium tuberculosis and products the pro-inflammatory response and recruitment of fibroblasts, lymphocytes, neutrophils, NK cells, collagens, necrotic tissues, dendritic cells, foamy macrophages, apoptotic infected macrophage, apoptotic infected epithelial macrophages and epithelial macrophages, and the formation of a granuloma. But if the human immune system for any reason is weakened, *M. tuberculosis* is activated and replicated within the granuloma structure. In this case, the necrotic nucleus of granuloma develops. When the number of *M. tuberculosis* is increased within the granuloma, it is ruptured and the *M. tuberculosis* is spilled into the airways. Each of the bacteria has the potential to infect other alveolar macrophages to the formation of a new granuloma.

In the active form of TB, bacilli are also able to replicate into the host macrophage, broke down cell membrane of macrophage and released to outside of it.[22] The bacilli of which released from demolished macrophage move around through lymph and blood stream to the brain, larynx, lymph nodes, lung, spine, bones, or kidneys which is known as military TB.[21]

2. Antibiotics therapy challenges in treatment of M. tuberculosis into the macrophage

There are several issues in terms of the efficiency of anti-TB antibiotics. First, there is no direct correlation between the plasma concentration of anti-TB antibiotics and its intracellular concentration. Furthermore, the intracellular activity of anti-TB antibiotics is justified by the rate of internalization, excretion rate, cell transformation, and pH.[24] Moreover, anti-TB antibiotics have a flexible internalization and intracellular accumulation path[24] In addition, anti-TB drugs may not penetrate into granulomas of which protect from *M. tuberculosis.*[7] One of other critical challenges is the limitation of the administration routes and the prolongation of treatment of TB.[15] We know the intracellular survival of M. tuberculosis in the host cell is pertain to it adaptation-evading of the immune system, dissemination, and selection of aggressive and MDR-TB.[24] Studies have been shown that the azithromycin could accumulate completely in phagolysosomes; however, it had vastly low antimicrobial efficiency on pathogens. Comparably, moxifloxacin not only was not able to accumulate in phagolysosome but has also high antimicrobial efficiency.[25] Seral et al., concluded that the cellular accumulation of antibiotics and some local environmental conditions (such as pH) are importance parameters for intracellular activity.[25]

3. Survive mechanisms of M. tuberculosis in macrophages

Knowing of the intracellular adaptive mechanisms for survival of the *M. tuberculosis* into the macrophages is essential in formulating convenient therapies. The *M. tuberculosis* has adaptive mechanisms which run away from macrophages and the intracellular location into the macrophages that being discussed below.

3.1. Prevention the fusion of primary phagosomes with lysosomes

Conceptually, *M. tuberculosis* is able to interfere with the transformation of the primary endosomes and phagosome maturation, and subsequently the fusion with lysosomes is delayed or blocked.[26] (Figure 2)

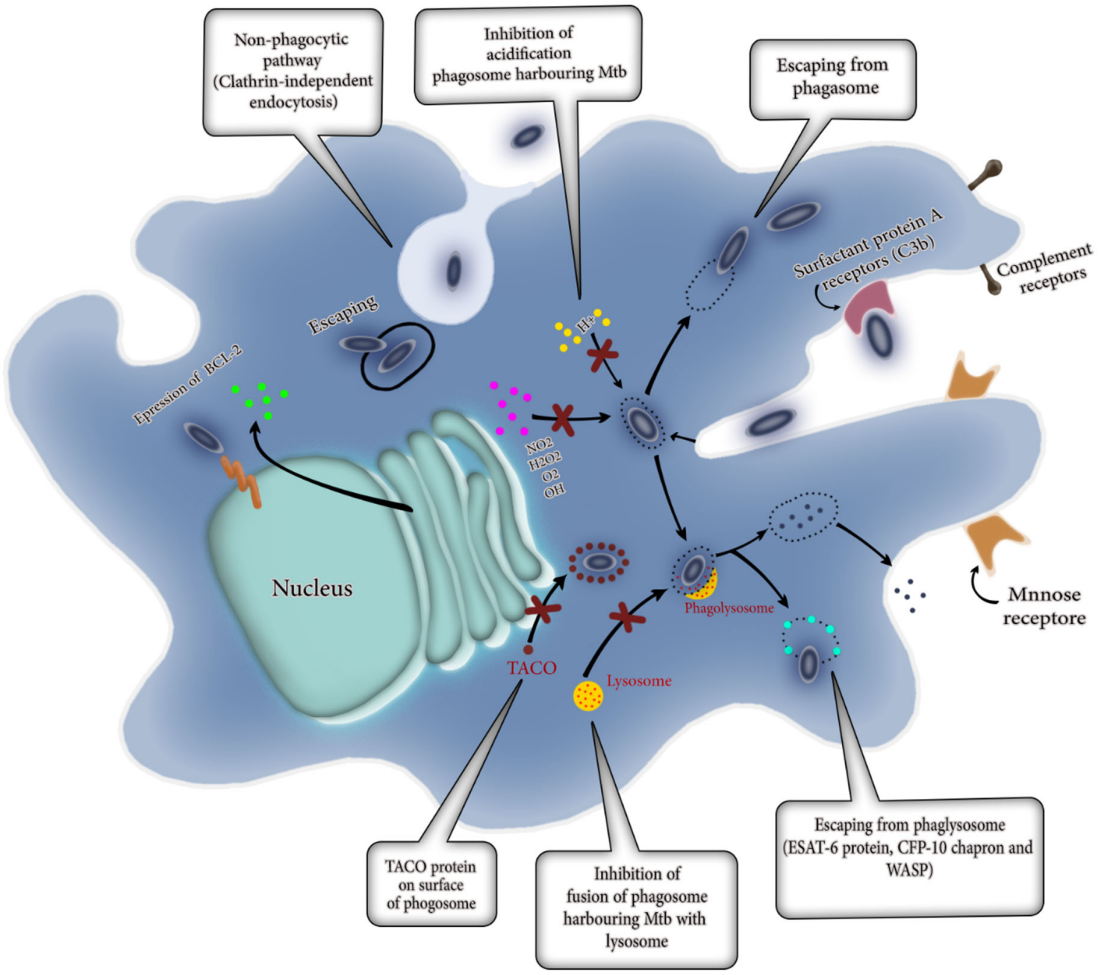

Figure 2: shows manage to survive in macrophages and adaptive mechanisms of *M. tuberculosis*. Some of *M. tuberculosis* is able to enter into the alveolar macrophages through non-phagocytic pathway which called clatrin-independent endocytosis. *M. tuberculosis* then can escape from phagosomes and release into the cytosol of macrophages. *M. tuberculosis* can induce the expression of anti-apoptosis genes (BCL-2) into the macrophages. The absorption of H^+, H_2O_2, O_2, No_2 and OH also are inhibited to control acidification of phagosome harboring *M. tuberculosis*. *M. tuberculosis* is able to escape from phagolysosome by producing ESAT-6 proteins, WASP and CFP-10 chaperone. *M. tuberculosis* prevent from transforming of primary endosomes in phagolysosome via the reducing of levels of proton ATPase inside the endosomes, connecting of the inducible iNOS and elimination of the PI_3P. TACO proteins, represents a component of the phagosome coat that is released earlier than phagosome fusion with or maturation into lysosomes. In macrophages containing TACO, it leads to prevent to forming phagolysosome and following that *M. tuberculosis* can survive within the phagosome.

M. tuberculosis also is able to prevent from transforming of primary endosomes in phagolysosome by the reducing of levels of proton ATPase inside the endosomes, connecting of the inducible nitric oxide synthase (iNOS)[27, 28] and elimination of the phosphatidylinositol 3-phosphate (PI3P)[29], simultaneously (Figure 2).

3.2. Demolition of endosomes or early phagosomes walls and escape into the cytosol

M. tuberculosis has ability to elude from the degradation in phagolysosomes by demolishing the endocytic vesicle wall and entering in the cytosol. In fact, escaping the endocytic vesicle is an essential step in the intra-macrophage survival for *M. tuberculosis* in the cytosol.[30] (Figure 2) In 2003, Jafari et al., recorded images of which showed some of *$H_{37}Rv$* strain of *M. tuberculosis* escaped from the phagolysosomes and arrive in the cytosol.[31] (Figure 2)

On the other hands, Brown et al., discovered that *M. marinum* also is able to escape from its phagolysosome and move around by the motive force of actin through Arp2/3 complex-mediated actin reorganization dependent on activation of "Wiskott-Aldrich syndrome protein" (WASP). [32, 33] Besides this, proteins secreted by *M. tuberculosis*, such as ESAT-6 play important roles in virulence. In fact, escaping from the phagosome depends to ESAT-6 alone or in complex with its chaperone CFP-10 (ESAT-6: CFP-10).[34] (Figure 2)

These proteins led to increased replication rates in the cell cytoplasm.[35] In addition, more likely to the mycobacterial cell envelop can be mistaken for a phagosome membrane due to its lipid-rich structures.[36] One explanation for the presence of cytosolic *M. tuberculosis* in some preparations is that exists of host triacylglycerol in both prevention of phagosome maturation and persistence in granulomatous lesions.[6]

4.3. Internalization in macrophages by non-phagocytic pathways which are not likely coupling with lysosomes

Internalization of *M. tuberculosis* in macrophages by non-phagocytic pathways involves interactions between *M. tuberculosis* and the membrane of the macrophages cause of the formation of vesicles. [37] In the other hands, internalization in macrophage has achieved by coupling of *M. tuberculosis with* lipid rafts [37] and receptors of which mediate a non-phagocytic endocytosis.[37] Studies indicated that the synthetic antimicrobial polymers have ability to induce membrane lysis and to bind to the genomic material of mycobacteria, thereby inducing mycobacterial cell death and was also able to kill the intracellular mycobacteria effectively without inducing any toxicity to mammalian cells.[38] Yavvari et al., showed that synthetic antimicrobial polymers has clathrin independent penetration and escape from hydrolytic lysosomal degradation and effectively kill the intracellular bacteria. [38] *M.*

tuberculosis also is able to interact with the macrophage apoptotic mechanisms to increasing of expression of anti-apoptotic molecules such as Bcl_2. [39] (Figure 2)

4. The entrance pathway of particles into the macrophage

Macrophages have ability to engulfing of opsonized NPs, modified mannose, IgG, and many complements larger than 500 nm including *M. tuberculosis* [40] from phagocytic pathways. [41] Macro-pinocytosis is an actin dependent pinocytic pathway by which macrophages can engulf droplets of extracellular fluid within large vacuoles formed by fusion of a plasma membrane extension with non-extended plasma membrane as indicated in Figure 3. In fact, the macrophage is able to using from macro-pinocytosis pathways for engulfing of agglomerated particles, functionalized NPs (such as ligand-modified NPs and viral NPs) and non-functionalized NPs such as the polyethylene glycol (PEG) NPs. It may more likely, macro-pinocytosis pathways occurred for swallowing of agglomerated particles.[42] Studies indicated that, ligand-modified NPs and some viral NPs have ability to entrance into the macrophage by specific receptors such as clathrin-mediated, caveolin-mediated or clathrin/caveolin-mediated pinocytosis. [42]

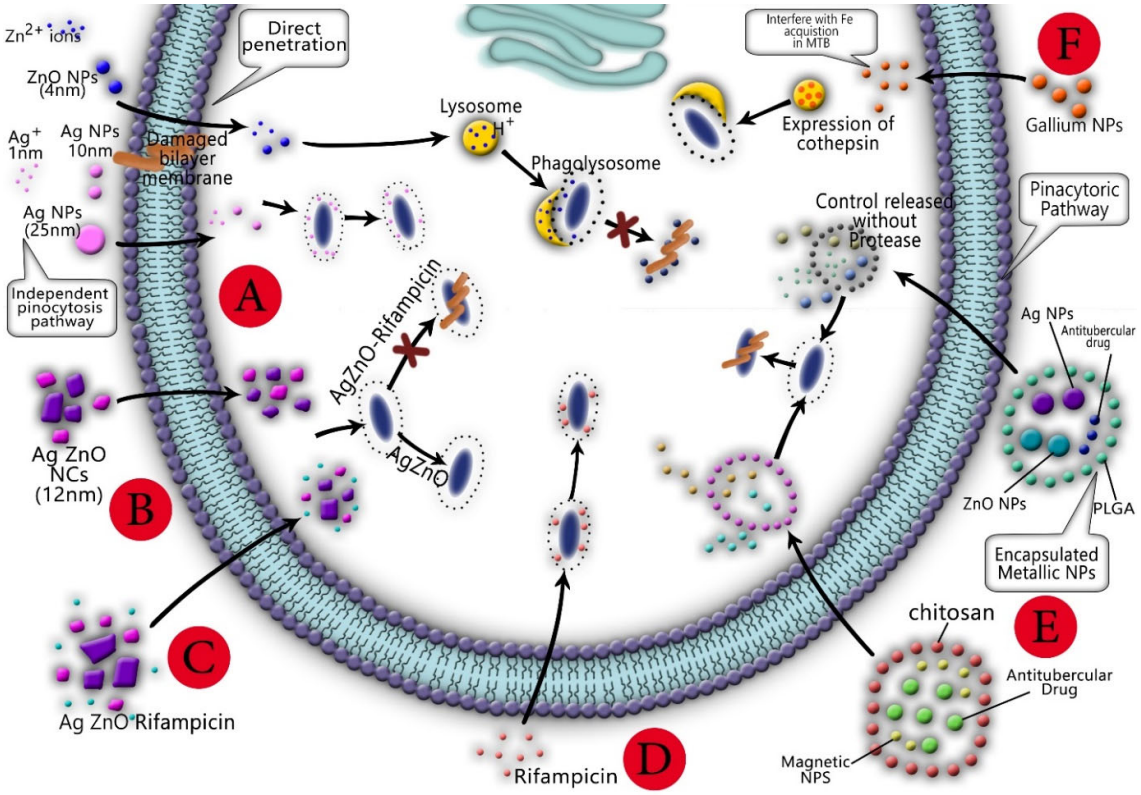

Figure 3: Colloidal ZnO NPs able to penetrate thought the bilayer membrane directly and accumulate into the lysosomes. **(A)** Then, lysosomes containing NPs of ZnO NPs are

integrated to infected phagosomes and eliminate *M tuberculosis*; Colloidal Ag NPs alone has not ability to kill *M. tuberculosis.* **(B)** Opposed to mixture Ag/ZnO nanocrystals, Ag/ZnO-Rifampicin able to eliminate *M. tuberculosis* into the phagosome. **(C)** Encapsulated magnetic NPs and antibiotics loaded polymers import to macrophage by endocytosis and subsequently release NPs and antibiotics in cytosol. Mixed magnetic NPs and anti-tubercular NPs have able to kill *M. tuberculosis* into the macrophage. **(D)** The macrophage of which infected with *M. tuberculosis* presented an expression of cathepsin D which plays an important role in macrophage activation.

Interestingly, MeNPs and dendrimers with dimensions between 4 and 10 nm are capable of direct penetrating into the macrophage (Figure 3). Based on the TEM images of previous study, THP-1 cells of which had been exposed to silver and zinc oxide NPs was able to penetrate through cell bilayer membrane and entered into the cell (Figure 3).

5. The role of phagosomes maturation in anti-tubercular properties

The phagocytosis of *M. tuberculosis* thanks to role of receptor-mediated phagocytosis of macrophage. Then, the phagosome was formed and subsequently a series of fusion events are achieved into endocytic pathways called "Phagosomal maturation". The phagosome of which interact with endosomes and then lysosomes obtain a wide range of antimicrobial properties designed to destroy completely of *M. tuberculosis*. The phagosomal maturation stages in macrophages can be identified based on the proteins present on the phagosomal membrane. In fact, the proteins correspond to the endosome type with which the phagosome has interacted.[24] To connection between the phagosome and the endosomal network and lysosomes have two hypotheses; the kiss and run-hypothesis and the fusion hypothesis.[24] In addition, phagosomes can obtain hydrolases from the 6-thioguanine nucleotide (6-TGN). A microtubule-based transport system also is more likely to be responsible for providing the scaffold for endosome movement. In addition, membrane rafts and glycol lipoprotein micro domains play a key role in phagosomal maturation. As well, the fusion and fission events eventually is done in a oxidative, acidic, and degradative phagolysosome environment which designed to very effectively eliminate invading microbes.[24] In fact, the main antimicrobial mechanisms of the mature macrophage phagosome are as a result of acidification, activation of the NADPH oxidase NOX_2, activation of iNOS, antimicrobial peptides and protein degradation.[43] (Figure 3)

6. *The intercellular anti-tubercular impact of mono-metallic/bimetallic nanoparticles and nano metallic carriers*

The MeNPs are accepted as anti-tubercular agents which destroy the integrity of bacterial membranes.[44] The metallic nanoparticles are potentially capable to attach and stick to cell wall of *M. tuberculosis* and subsequently destroy it , mechanically.[44] In general, toxicity of metallic NPs is thanks to oxidative stress and free radicals, called reactive oxygen species (ROS).[45] Conceptually, anti-tubercular mechanisms of metallic NPs arrived from the interaction between the *M. tuberculosis* and NPs, as well the bio-reactive properties of the dissolved ionic fraction (Figure 3).[46-49]

The silver and zinc oxide NPs are considered as antibacterial agent against pathogenic bacteria.[50] It was reported that silver NPs increase bacterial cell permeability by motiving the aggregation of proteins in the periplasmic space, forming nano-sized pores in the bacterial membrane. Increasing of bacterial membrane permeability may facilitate increased penetration of an intracellular antibiotic.[51] In other hands, zinc is a natural metal of which used by alveolar macrophages to increased bactericidal pressure against internalized *M. tuberculosis* within the endosome. For this reason, ZnO NPs are able to interact with the membrane of bacteria which led to formation of surface pores and the release of intracellular nucleotides.[51] Up to now, many researchers have been synthesized the colloidal,[19] nano-crystals,[16] and encapsulated[51, 52] anti-tubercular silver and zinc oxide NPs by chemical and biological methods.[53] (Figure 3)

6.1. Chemical mono/bimetallic silver and zinc oxide anti-tubercular nanoparticles

In 2017, Jafari et al., synthesized colloidal silver and zinc oxide NPs by chemical reduction and deposition methods. The average size of colloidal silver and zinc oxide NPs have been estimated about 13±3.14 nm and 4±0.88 nm.[54] They reported that the colloidal silver NPs, however was not able to eliminate intracellular *M. tuberculosis* into the macrophages, completely (MIC≥25 ppm).[19] Whereas, the colloidal zinc oxide NPs demonstrated anti-tubercular behavior into the macrophage against $H_{37}Rv$ *M. tuberculosis*[19] but it was toxic against human normal lung cells (MCF-7 cell lines) *Ex-vivo*. Based on this assumption, 0.663 ppm of 5_{Ag}:5_{ZnO} not only was able to kill *$H_{37}Rv$ M. tuberculosis*, but showed also lowest cytotoxicity effects on MCF-7 and THP-1 cell lines.[19] They also had been synthesized mixture silver/zinc oxide nanocrystals by oxalic reduction method and the average size of 12 nm. The results showed mixture nanocrystals were not able to eliminate *$H_{37}Rv$* strain of *M. tuberculosis,* lonely *in Ex-vivo.*[46] It seems more likely that ability to penetration of metallic

colloidal NPs into the macrophages be more than agglomerated metallic nanocrystals. Furthermore, the anti-tuberculosis properties of metallic NPs are likely arising from the formation of metal ions in the aqueous medium, and subsequently due to penetration into the macrophage.[55] (Figure 3)

6.2. Encapsulated of silver and zinc anti-tubercular nanoparticles

Studies shows that encapsulating of silver and zinc oxide NPs into the poly (D,L-lactide-*co*glycolide) (PLGA), a biodegradable and biocompatible polymer, a multi-metallic micro particle vehicle was formulated to transport relevant antibiotics via aerosol into the *M. tuberculosis* infected endosomal network of alveolar macrophages. In fact, co-delivery of silver and zinc oxide NPs into a larger micron-sized carrier collaborated to selective metallic/metal oxide NPs uptake by macrophages through passive targeting, initial burst release of ions from the encapsulated silver and zinc oxide NPs and eventually reduction of metallic/metal oxide NPs toxicity (Figure 3).[51, 52] Pati et al., in 2016, synthesized zinc and rifampicin, and encapsulated it into transferrin-conjugated silver quantum-dots to improve delivery in macrophages.[56] They found that encapsulated zinc and rifampicin into the transferrin-conjugated silver quantum-dots NPs play a pivotal role to increasing of the anti-tubercular activity as compared to Zn-RIF, RIF and Zn (Figure 3). [56]

6.3. *Phytogenic mono-metallic/bimetallic silver, zinc and gold anti-tubercular nanoparticles*

Biosynthesis of anti-tubercular metallic NPs should be taken into consideration due to its economic feasibility, low toxicity, and simplicity of the procedure.[57] Punjabi and et al., in 2018, has synthesized extracellular silver and zinc NPs with average size of 40 and 60 nm by *Pseudomonas hibiscicola.*[57] The MICs value in both of silver NPs and zinc NPs against *$H_{37}Rv$* strain and MDR strain of *M. tuberculosis* were reported 1.25 mg/ml.[57] Banu and Rathod et al., reported anti-tuberculosis activity of biosynthesized silver NPs against *M. tuberculosis* and clinical isolates of multi-drug resistant *M. tuberculosis* (MDR-TB).[58] A report stating biosynthesizing of silver nanoparticle by utilizing the alcoholic extract of *Plumbago auriculata* and investigating anti-tubercular effects against *M. tuberculosis.*[59] The silver NPs with average size 15 to 45 nm showed anti-tubercular activity with MIC value of 1.6 μg/ml.[59] In 2016, Singh et al., synthesized the silver, gold and bimetallic silver/gold NPs using *B. prionitis* leaf extract, *P. zeylanica* root extract, and *S. cumini* bark extract.[60] The silver NPs of which synthesized by leaf extract of *Psidium guajava* demonstrated inhibitory

effect on *M. tuberculosis.*[60] Bimetallic gold/silver NPs were able to inhibit 90% of mycobacterial growth at 3 μg/mL and it presented great mycobactericidal potency in contrast with gold NPs alone.[60] In common the fact that, bimetallic gold/silver NPs possessed the high efficiency to inhibit *M. tuberculosis in Ex-vivo* THP-1 infection model (Figure 3). [60, 61]

6.4. Gallium Nanoparticles

The antimicrobial activity of Gallium (GIII) nanoparticle has been investigated against *M* tuberculosis[10, 62] and nontuberculous mycobacterial (NTM) pathogens, *M. avium* complex (MAC) and *M. abscessus.*[63] Gallium and iron have similar chemical properties. In other hands, Gallium has ability to interfere with iron acquisition by microorganisms.[10] Recently, the blocking iron acquisition by Gallium compound in *M. tuberculosis* is confirmed which due to reduce the growth of intracellular *M. tuberculosis* into the macrophage.[10] A report stating that the Gallium NPs also is able to interfere with iron acquisition in *M. tuberculosis* and inhibit intracellular growth within macrophage.[10] Choi et al., in 2017, reported that Gallium NPs shows significant growth inhibition of *M. tuberculosis* in THP-1 macrophages on days 3 and 6 after infection.[10] In common the fact that the macrophage of which infected with *H_{37}Ra M. tuberculosis* exhibited a significant higher expression of cathepsin D - an aspartic endo-protease that is distributed in lysosomes- compare to galactin 3 - a member of the beta-galactoside-binding protein family that plays an important role in macrophage activation- as well.[10] Choi et al., found that uptake of Gallium nanoparticles by macrophage led to promoting of phagosome maturation and subsequently let to increasing of anti-tuberculosis effects of macrophage.[10] To sum up, the Gallium nanoparticles have ability to deliver drug to the macrophage, inhibit *M tuberculosis* and nontuberculous mycobacterial[64] growth and reduce the inhibition of phagosome maturation (Figure 3). [10]

6.5. Iron nanoparticles as a nano-carrier

Iron, an essential nutrient for nearly all living cells, plays a critical role in many important enzymatic reactions as a cofactor. Its ability to redox cycle between Fe(II)/Fe(III) enhances electron transfer.[10] In humans, iron is tightly bound to transferrin, lactoferrin, ferritin and heme. Pathogenic bacteria must acquire iron mainly from these iron complexing proteins for growth and metabolism. Many pathogens possess highly efficient iron uptake mechanisms. These bacteria release iron solubilizing (chelating) compounds, siderophores, to obtain Fe^{3+} from host iron-binding molecules for growth. Carboxymycobactin and mycobactin are

mycobacterial siderophores that chelate Fe^{3+} extracellularly and intracellularly, and are critical to the virulence of these organisms in vitro and in vivo.[10]

Olakanmi et al., reported that *M. tuberculosis* within the phagosomes of macrophage can acquire Fe from extracellular transferrin and sources within the macrophage.[65] Lu et al., in 2018, synthesized encapsulated Fe_3O_4/hyperbranched polyester-(2-dodecen-1-yl) succinic anhydride2-Dodecen-1-/isoniazid magnetic NPs with controlled drug release characteristics. They suggested that this encapsulated microparticle containing iron exhibited good drug release properties and might be suitable for the treatment of tuberculosis.[66] In 2017, a report stating from Saifullah et al., as regards designing a novel anti-tubercular multifunctional formulation containing fabricating graphene oxide with iron oxide magnetite NPs, as a nano-carrier of ethambutol.[67] They found this formulation had prolonged sustained release of ethambutol and had ability to reduce the dosing frequency for treatment of *M. smegmatis* compare to ethambutol alone.[67] El Zowalaty et al., prepared streptomycin-loaded, chitosan-coated magnetic NPs. Remarkably, the MIC of this formulation against *M. tuberculosis* obtained about 732 µg/mL (Figure 3).[68]

7. Conclusion and Future Perspectives

A real therapeutic advance in terms of elimination of intracellular *M. tuberculosis* can be done merely by fulfill careful studies in the field of nano-therapy. Using metal NPs in therapies of respiratory infection may contribute to achieving an effective tuberculosis treatment. The key role of MeNPs in medicine are diagnosis and target therapy, however, it seems that the time has come to use of anti-tubercular MeNPs in the treatment of intercellular *M. tuberculosis*. MeNPs and metal ions can penetrate throughout bilayer infected macrophage membrane due to the small size and relative mobility; can also freely attach to *M. tuberculosis* of which immerse in cytosol and even may penetrate to inside of phagolysosome. Mixing of colloidal MeNPs can also be a good way to increase antibacterial properties and, in turn, reduce the toxicity of MeNPs to human cells. In addition, mixture MeNPs can be considered suitable for co-delivery along with anti-tuberculosis drugs to eliminate of intracellular *M. tuberculosis* into the macrophages and even granuloma. Further advances are needed in terms of change the concept of nanotechnology into a realistic medical application as the next generation of intracellular anti-tuberculosis drugs. Perhaps it is exaggerated, but we believe that the anti-tuberculosis agents of whom we need to treat *tuberculosis;* they have been created in nature.

Conflicts of interest

The authors have no conflicts of interests.

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