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APPLICATION OF METAL NANOPARTICLES IN TREATMENT OF TUBERCULOSIS

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Abstract

Development of drug resistant strains such as multi drug resistant TB (MDR-TB) and extensive drug resistant TB (EXDR-TB) threatens the progress made in TB controls and prompt the need to alternative strategies. The aim of this work was to investigate the anti-mycobacterial activity of two noble metal nanoparticles either in non-conjugated form or conjugated with rifampicin. Gold (spheres and rods) and silver (spheres) nanoparticles were synthesized and characterized using TEM and UV–vis spectroscopy. The anti-mycobacterial activity of silver nanospheres (AgNSs) was studied using the malachite green decolorisation assay (MGDA) and cultivation on Lowenstein Jensen media, and the results indicate that AgNSs have a strong anti-mycobacterial effect with 80 µg/ml MIC. To test the cytotoxic effect of gold and silver nanospheres on RAW264.7 cell line as a macrophage cell model, which is the host of TB, we did a comparative study using XTT assay and microscopic examination. Results indicated that silver nanospheres were severely toxic while the gold nanospheres were safer to murine macrophage RAW264.7 cell. Therefore, silver nanospheres were excluded from this work as its toxicity to host cells. Gold nanoparticles (spheres and rods) were conjugated with rifampicin and the successful conjugation was confirmed with UV–vis spectroscopy. Then, the anti-mycobacterial activity of conjugated and non-conjugated AuNPs with rifampicin against extracellular TB were assayed using the malachite green decolorisation assay (MGDA) and cultivation on Lowenstein Jensen media and Middlebrook agar, the results revealed that the shapes of gold nanoparticles played an important role in the anti-mycobacterial activity, as the rods have a strong anti-mycobacterial activity while the spheres have no anti-mycobacterial effect. The impact of the cell types (RAW264.7 macrophage cell line, HSC-3 human oral squamous cell carcinoma and MCF7 human breast adenocarcinoma cells) on the gold nanoparticle uptake was studied using the dark field scattering microscopy. Results showed that the macrophages have the highest uptake while the other two cancer cell lines showed less uptake. Studying the cell death variation on RAW264.7 murine macrophages and two human cancer cell lines (MCF-7 and HSC-3), after the incubation with functionalized AuNRs with rifampicin (AuNRs@RF), assayed by the cell viability and apoptosis /necrosis assay. The decrease of viability of RAW can be attributed to the phagocytic power of macrophage which is not possessed by other cancerous cell lines. Apoptosis/ necrosis study using the AuNRs either conjugated or not with RF on RAW and two cancer cell lines, showed that AuNRs and AuNR@RF caused apoptotic way of cell death with high apoptotic cells in RAW cell compared with the other two cell lines. The intramacrophage activities of conjugated and nonconjugated AuNPs against intracellular mycobacteria were assayed by the CFU assay. The AuNRs in size 25 length x 5 width represent a promising drug delivery candidate for anti-tuberculosis drug in addition of their anti-mycobacterial activity. However AuNSs and AuNSs @RF had no anti-mycobacterial effect against the extracellular TB, they resulted in significant reduction of the intracellular mycobacterial CFU. Plasmonic photothermal study was conducted on M. tuberculosis, and its host cell (RAW264.7 murine macrophage cell line).The AuNRs (0.5 OD) is promising in killing the TB bacteria by plasmonic photothermal without harming the host cell. We characterized the unique Raman band of RAW cell using the Raman spectroscopy technique. In the future work we are planning to study the Raman vibrational change and it is relation to the cell death. This powerful technique might help in explaining the molecular mechanism of cell death. We concluded that AuNPs especially the conjugated form with rifampicin efficiently internalized and accumulated inside the macrophages. And the AuNRs might be a promising antimycobacterial agent, and or a smart delivery vehicle for anti-tuberculosis drug (Rifampicin), while the AuNSs have no anti-mycobacterial activity, they might be a successful delivery system for anti-tuberculosis drugs able to target safely the M. tuberculosis infected macrophage, the conjugated AuNPs with rifampicin were superior than the free rifampicin in combating the intracellular M. tuberculosis. So the AuNPs as antimycobacterial drug delivery system might be recommended for application in tuberculosis therapy.

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LIST OF CONTENTS

CONTENTS	Page
1. Introduction	1-7
2. Review of literature	8-32
2.1. Therapy of tuberculosis	8-11
2.2. Preparation, conjugation and characterization of noble metal (silver & gold) nanoparticles	12-13
2.3. Studying the antimicrobial activity of metal nanoparticles	13-21
2.4. Nanoparticles as a drug delivery platform for antituberculosis drugs	21-26
2.5. Studying the cytotoxicity of (silver and gold) nanoparticles	26-30
2.6. Noble nanoparticles and Plasmonic photothermal therapy	31
2.7. Surface Enhanced Raman Spectroscopy (SERS)	32
3.1. Materials	33-38
3.1.1. Chemicals used for preparation and characterization Of metal nanoparticles	33
3.1.2. Media used for culturing the Mycobacterium tuberculosis.	34-35
3.1.3. Materials used for Malachite green decolorisation assay.	35
3.1.4. Material used for uptake study	35-36
3.1.5. Cell viability study	36
3.1.6. Material used for apoptosis /necrosis assay	36-37
3.1.7. Material used for macrophage infection assay	37-38
3.1.8. Material used for studying the cell cycle	38
3.1.9. Material used for Plasmonic photothermal study	38
3.1.10. Surface Enhanced Raman Spectroscopy.	38
3.2. Methods	39-59
3.2.1. Preparation of metal nanoparticles	39-41
3.2.2- In vitro studying the anti-mycobacterial activity of the noble metal nanoparticles	41-43

CONTENTS	Page
3.2.3. In vitro study for comparing the cytotoxicity of silver and gold nanospheres on RAW 264.7 murine macrophage cell line	43-44
3.2.4. Studying the sensitivity of *M. tuberculosis (H37Ra)* to anti-tuberculosis drugs (Rifampicin) called sensitivity test.	45
3.2.5. Conjugation of gold nanoparticles with (Rifampicin).	45-46
3.2.6. In vitro anti-mycobacterial effect of the gold nanoparticles.	46-47
3.2.7. The impact of the cell type on the gold nanoparticles uptake	47-48
3.2.8. Studying the cell death variation after the incubation of functionalizing AuNRs with rifampicin	48-53
3.2.9. Studying the anti-mycobacterial activity of the gold nanoparticles conjugated with rifampicin and the free drug against the intracellular TB	54-55
3.2.10. Photothermal study	56-58
3.2.11. Raman spectroscopy study on RAW264.7 cell	59
4. Results	60-107
5. Discussion	108-128
6. Summary	129-134
7. References	135-145
الملخص العربي	

LIST OF TABLES

No.		Page
1	The cell viability result of exposing RAW 264.7 macrophage cell line.	68
2	Time exposure/ viability of RAW macrophage exposed to fixed concentration (20 µg/ml) of silver or gold Nanospheres.	71
3	Cell viability (XTT assay) of RAW 264.7 murine macrophage, MCF-7 and HSC-3.	81
4	Comparative the effect of gold nanorods (2 OD) either conjugated or not conjugated with rifampicin on apoptosis /necrosis of RAW264.7 cell and two cancer cell lines (MCF-7 & HSC-3).	85
5	Showing the colony forming unit (CFU) of *Mycobacterium tuberculosis*	89
6	The viability of RAW macrophage exposed 2 OD of gold nanorods with and without 2 minutes exposure laser .	91
7	The effect of plasmonic photothermal when 1 OD of gold nanorods have been used , with 4 minutes laser exposure on induction of apoptosis/ necrosis in RAW264.7.	94
8	The effect of plasmonic photothermal when 2 OD of gold nanorods have been used , with 2 minutes laser exposure on induction of apoptosis/ necrosis in RAW264.7.	96
9	The effect of plasmonic photothermal using 1 OD of Au NRs either conjugated or non-conjugated with rifampicin in the cell cycle phases of RAW264.7 cell.	101
10	The effect of plasmonic photothermal using 2 OD of Au NRs either in free or conjugated with rifampicin in RAW cell cycle.	105
11	Surface Enhancement Raman spectroscopy (SERS) Vibrational peaks assignments for the RAW cells in G1 phase.	107

LIST OF FIGURES

No.		Page
1	UV- Vis spectra of different samples of gold nanospheres throughout the growth process	60
2	Shows the TEM image of AuNSs width 20 ± 2 nm capped with Sodium Citrate. And the UV spectra showing surface Plasmon resonance band at 520 nm	61
3	Showing the TEM of the prepared AuNRs, Length 25±2nm × 5 ± 0.9nm width and the UV-Vis Spectra showing two surface Plasmon resonance bands	62
4	TEM image of the silver nanospheres showed an average particle size 20 nm and UV-Vis spectra at 420 nm	63
5	Cell viability results for the exposure of RAW264.7 cell line to three different concentration (10, 20 &40 µg/ml) of Ag NSs or AuNSs solutions for 3 hour	68
6	The bright field microscopic images of RAW264.7 cell line treated with 20 µg/ml of AgNSs or AuNSs for different time points	69
7	The cell viability RAW 264.7 cell line treated with 20 µg/ml AuNSs or AgNSs at different time points	70
8	The UV-spectra of AuNPs before and after the conjugation with rifampicin	71
9	Dark field images for exploring the uptake of gold nanospheres by RAW 264.7 cell line, HSC-3 cell and MCF7 cells.	77
10	Dose dependent effect of AuNRs @RF on the viability of RAW264.7 cell	79
11	Cell viability of MCF-7 human breast adenocarcinoma cells upon exposure to different concentrations of Au NRs @RF for 24 hrs	80
12	Viability of HSC-3 human oral squamous cell carcinoma cells upon exposure to different concentration of gold nanorods conjugated with rifampicin	80
13	Apoptosis/ Necrosis data of RAW264.7 cell line upon exposure to 2 O.D of Au NRs conjugated or not Conjugated with rifampicin	82

No.		Page
14	Apoptosis/ Necrosis data of MCF-7 cell line upon exposure to 2 OD of AuNRs conjugated or non Conjugated with rifampicin	83
15	Apoptosis/ Necrosis data of HSC-3 cell line upon exposure to 2 OD of Au NRs conjugated or non-conjugated to RF	84
16	The histogram exploring the mycobacterial colony forming unit versus the different AuNPs and AuNPs @RF, free rifampicin and control	89
17	Photothermal ablation effect using the AuNRs either conjugated or not with rifampicin on RAW 246.7 cell viability	90
18	The plasmonic photothermal effect of 1 OD of AuNRs either conjugated or not conjugated with rifampicin in apoptosis / necrosis of RAW cell.	92-93
19	Apoptosis / necrosis of RAW 264.7 cell line upon exposure to 1 OD of gold nanorods with and without 4 minutes exposure to laser	94
20	Apoptosis/Necrosis of macrophage using Au NRs either in single or conjugated with rifampicin	95
21	The percentages of cells in four populations gated as in figure 20, plotted versus the effect of Au NRs @BSA or Au NRs @RF with and without photothermal ablation	96
22	The cell cycle histograms acquired from flow cytometry. macrophage cells (RAW 264.7 cell line)	97
23	The effect of plasmonic photothermal ablation on RAW cell cycle using 1 OD of AuNRs.	98-100
24	The effect of plasmonic photothermal ablation on RAW cell cycle using 2 OD of AuNRs.	102-104
25	Surface enhanced raman spectra (SERS) of RAW treated with AuNSs @RF	106

LIST OF PHOTOS

No.		Page
1	Malachite green decolorisation assay.	64
2	Lowenstein Jensen media inoculated with mycobacteria suspension treated with AgNSs.	65
3	Testing the antimycobacterial activiy of AgNSs using malchite green decolorisation assay and lowenstein jensen media.	66
4	Bright field images of RAW 264.7 cells treated with 40 µg/ml of Au NSs or Ag NSs for 3 hrs.	67
5	The bright field images were taken for Raw264.7 after 3 hrs. incubation with 10 µg/ml Au NSs or Ag NSs.	67
6	Lowenstein Jensen media showing that H37Ra is sensitive to rifampicin with 2 µg/ml MIC.	72
7	Testing the antimycobacterial effect of two concentration (1&2OD) Of AuNPs either conjugated or not conjugated with rifampicin using the MGDA.	73
8	Lowenstein Jensen media inoculated with 100 µl of H37Ra suspension treated for 7 days with Au NPs or Au NPs @RF or rifampicin inoculated on Lowenstein Jensen media.	74
9	Assaying the antimycobacterial effect of AuNPs (2OD concentration) by MGDA.	75
10	Middle brook agar plates inoculated with 100 µl of mycobacterial suspension treated with 2 OD of Au NPs either conjugated or not with rifampicin and free rifampicin.	76
11	Dark field image of RAW264.7 cell line treated with AuNSs @ RF (0.5 OD) for 3 hrs.	78
12	Microscopic image of RAW264.7 monolayer before and after infection with. H37Ra.	86
13	RAW264.7 monolayer infected with H37Ra and treated with AuNPs for 3 days.	87

INTRODUCTION

Tuberculosis (TB) is one of the most leading cause of morbidity and mortality and remains a major public health concern worldwide especially in developing countries. The World Health Organization (WHO) estimates about one third of the the world populations is infected with *Mycobacterium tuberculosis*, and more than 8 million new cases of active TB occurs annually **(WHO, 1999).**

However the availability of effective treatment for almost half century. Chemotherapy of tuberculosis and other mycobacterial diseases remains a complex task due to the requirements of multidrug regimes that need to be administrated over long periods. Thus the poor patients' compliance is the most common reason for chemotherapy failure in TB **(Parbakaran *et al.*, 2004).** Also the drugs reach mycobacteria infected macrophages in low concentrations and/or do not persist long enough to develop the desired anti-mycobacterial effect; and the current antimycobacterial agents are associated with severe side effects. As these side effects are due to the action of the drug on hepatocytes and neuronal cells rather than on macrophages, selective delivery of these antibiotics into macrophages has the potential to increase greatly their therapeutic index by achieving higher drug concentrations locally where the *M. tuberculosis* replicates while limiting systemic toxicities. Moreover, because drug resistance develops when bacteria are treated with sub therapeutic doses of antibiotics, a system that

delivers high concentrations of antibiotic to the site where bacteria divide would facilitate sterilization of sites of infection and minimize emergence of drug resistance.

Nanoparticles is now recommended as anti-mycobacterial, and nanoparticles (NPs) have anti-mycobacterial effects including membrane damage and toxicity **(Hsiao *et al.*, 2006).** The mechanism of NPs inhibiting bacterial growth remains less understood. It has been reported that size and surface modifications of NPs could affect their antibacterial effect. Nanotherapeutics have a potential advantage in treatment of tuberculosis over the free drug. As they can selectively deliver the antimycobacterial drugs in high concentrations to the infected macrophages, the main host cell of TB, while avoiding off target effects, which limit the doses of many current TB drugs. Because one of the main routes by which TB spread in infected individual with tuberculosis is the infected macrophages and the therapeutic drug feebly enter inside macrophages, Because *M. tuberculosis* resides and multiplies within host mononuclear phagocytes and because mononuclear phagocytes showed high capacity to internalize particles more efficiently than other host cells, encapsulation of anti-tuberculosis drugs within nanoparticles offers a mechanism for specific targeting of *M. tuberculosis*-infected cells. Indeed, because nanoparticles have been shown to be taken up by macrophages of the reticuloendothelial system and to accumulate in the liver, spleen, and lung **(Lee *et al.* 2011 and Zhang *et al.* 2010).** They are ideally suited to treat *M.*

tuberculosis, which infects macrophages in these organs. An additional advantage of nanoparticle delivery of anti-tuberculosis drugs over free drug is that it protects the drug from degradation or modification prior to delivery of the drug to infected tissues.

In the present time drug resistance TB, especially multi-drug resistant TB (MDR-TB) which is defined by resistance to the two most important anti-tubercular drugs isoniazid and rifampicin respectively or extensive drug resistance XDR-TB (resistance to all first-line drugs and to fluoroquinolones and at least one of the three second line antituberculosis injectable drugs *i.e.* (apreomycin, kanamycin, and amikacin), has been recognized as a potentially catastrophic challenge to global public health. The development of better and more rapid diagnostic assays and new classes of anti TB drugs are urgent priorities for containment of MDR-TB and XDR-TB.

Nanotechnology has provided a huge improvement to pharmacology through the designing of drug delivery system able to target phagocytic cells infected by intracellular pathogens as mycobacteria. The increased therapeutic index of antimycobacterial drugs, the reduction of dosing frequency, and the improvement of solubility of hydrophobic agents, allowing the administration of higher doses, have been demonstrated in experimental infection. These advantages may lead to new therapeutic protocols that will improve patient compliance and consequently lead to more

successful control of mycobacterial infections. Many researchers studied the possibility of using nanoparticles for delivery of therapeutic drug inside macrophages.

Not less numbers of authors tested the biodegradable and liposomal nanoparticles either in vitro or in vivo as a drug delivery platform for antituberculosis drugs. Some of studies use the poly (lactic-co-glycolide) polymer as a vehicle for delivering rifampicin **(Khalluru et al 2013, Hirota et al 2010), Yoshida and co-workers (2006),** incorporated rifampicin into PLGA microspheres, and they found that RF-PLGA microspheres were superior to free rifampicin in clearing the intracellular tubercle bacilli. **Also Makino and his co-worker (2004)** found that loading rifampicin into PLGA microspheres result in accumulation of rifampicin into alveolar macrophages 19 time than free rifampicin. Few studies used other polymers, **Kisich and his group (2007)** encapsulated the moxifloxacin within poly (butylcyanoacrylate) nanoparticles and they observed that the nanoparticles were distributed throughout the macrophages cytoplasm. **Anisimova and her team (2000)** evaluated two structures of nanoparticles (poly-n-butylcyanoacrylate and poly-isobutyl cyanoacrylate) as a delivery system of isoniazid, streptomycin and rifampicin. Also many studies administrated the liposomal nanoparticles delivery system for TB drugs **(Pandy and Khuller. 2004, Justo and Maroes 2003, Pandey et al 2004, and Vyas et al, 2003)**. Beside the potential advantage of biodegradable and liposomal nanoparticles delivery system for antimycobacterial

drugs, some disadvantages may be recorded, **Lam and his team, (1993)** observed that the phagocytosis of the biodegradable PLGA associated with cell damage, cell lysis and cell death. Also **Marques *et al.*, (2004)** reported over production of proinflammatory cytokines from mononuclear cells cultured in vitro with poly-L-lactide polymer. **Foradada and co-workers (2000)** demonstrated the chemical degradation and hydrolysis of the liposomes with the serum realizing some metabolites which may be explain the intrinsic toxicity of cells. While huge number of authors studied the use of liposomal and biodegradable nanoparticles and microspheres for delivery the anti-TB drugs, only **Clemens *et al.* (2012)** used functionalized Mesosporous Silica Nanoparticle for delivery and targeting anti-tuberculosis drugs into Mycobacterium tuberculosis-infected macrophages

Metal nanoparticles have been introduced in multiple biomedical applications, diagnostics and therapeutics, and mostly in cancer therapy, as Plasmonic photothermal ablation agent and a platform for targeted delivery of anti-cancer drugs **(Powell *et al.*, 2010 and Dreaden *et al.*, 2012).** However the gold and silver nanoparticles have shown antimicrobial activities **(Song *et al.*, 2006),** they revealed antimycobacterial activities against extracellular TB **(Zhou *et al.*, 2012).** Consequently testing silver and gold nanoparticles as antimycobacterial or as a drug delivery vehicle and loading them with anti-TB drugs in an attempt to target the