Journal of Applied Chemical Research, 8, 4, 115-122 (2014)

Journal of Applied Chemical Research
www.jacr.kiau.ac.ir

Antifungal Activity of Silver Nanoparticle in Different Sizes against Some Pathogenic Fungi

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Received 26 Jun 2014; Final version received 20 Sep. 2014

Abstract

Skin infection caused by *Tricophyton rubrum* and some opportunistic fungi such as *Candida. Albicance and Aspergillus*. fumigatus occur in various parts of the body and sometimes are difficult to be treated. Antifungal effects of spherical silver nanoparticles (nano-Ag) were investigated in this study. Although silver nanoparticle has long been used as effective inorganic antifungal agent; the antifungal activity of nano-Ag in different size has not been investigated yet. In this study nano-Ag in diameter size of 10, 20, 40 nm were examined. The minimal inhibitory concentration (completive visual growth inhibition) of these nanoparticles ranged from 4-16 µg/ml for all fungal test strain. Thus, the current study indicates nano-Ag may have considerable antifungal activity, deserving further investigation for clinical applications.

Keywords: *Silver nanoparticle, Different sizes, Antifungal activity, Pathogenic.*

Introduction

In recent years cancer, HIV, diabetes are mostly common in people so patients with these predisposing factors are more susceptible to be affected by fungal infection especially opportunistic fungi [1]. Fungal infections are more frequent in patient who are immunocompromised. It will be worst to considering that the limited number of antifungal drugs available because prophylaxis with antifungals may lead to the emergence of resistant strains [2].

Therefore, there is an inevitable and urgent medical need for antibiotics with novel antimicrobial mechanisms. [1, 3]. Since ancient times and among inorganic antimicrobial

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agents, silver has been employed most widely fight infection. A survey of recent literature showed remarkable findings on the bactericidal activity of silver nanoparticles (nano-Ag) [4,5]. However ,the antifungal effect of silver nanoparticles has received only marginal attention and just a few studies on this topic have been published [3, 5-8].Compared with other metals ,silver exhibits higher toxicity to microorganisms while it exhibits lower toxicity to mammalian cells [1,5].

In this study, silver NP_s with different diameter size (10, 20, 40 nm) was used. The aim of current study is the investigation the effect of NP_S on some pathogenic fungi and also Comparison the effect of NP_s in terms of size.

Experimental

Microorganisms and culture conditions

Tricophyton rubrum [Ptcc 5143 (RI 613)], *Aspergillus fumigatus* (Ptcc 5009) and *Candida. albicance* (Atcc 10231) were cultured in Sabraud dextrose agar (SDA) respectively in 48 hours, 24 hours and one week at incubation 28° *C. Candida. albicanse* in transmition (65-70%) by specterophotometric devices the inoculum size of cells/ml 1-4×10^6 and *Aspergillus fumigatus* (80-87%) Tricophyton rubrum (65-70 %) the inoculum size of cells/ml 1-4×10^4 were obtained.

Characterization of nanoparticles

Nano –Ag colloidal solution was prepared based on the reduction reaction of silver nitrate by sodium borohydride Trinatrium citrate was used as stabilizing agent. Nano-Ag in size of 10nm, 20nm, and 40nm is obtained (Purchesed from Nanozino Co.Ltd. (ZSA, Roshd, Iran).

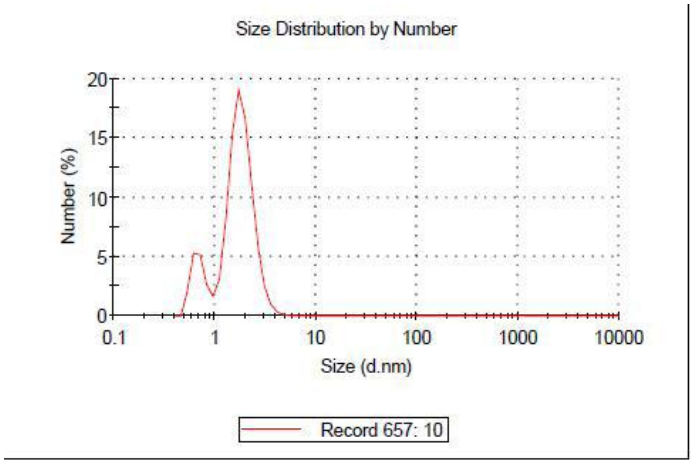

Diagram 1. DLS of Nano-Ag 10.

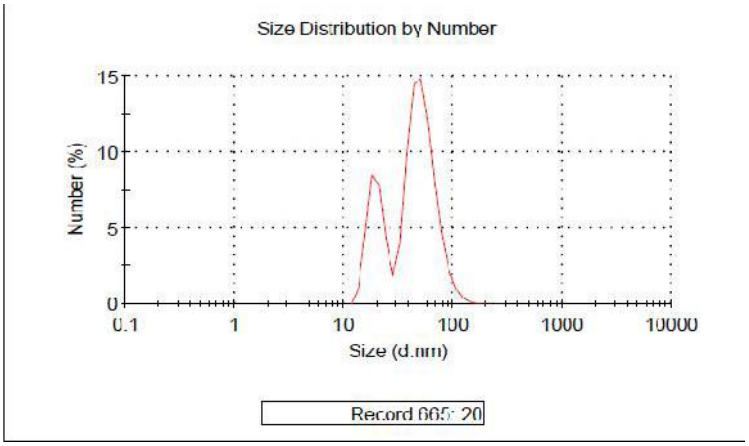

Diagram 2. DLS of Nano-Ag 20.

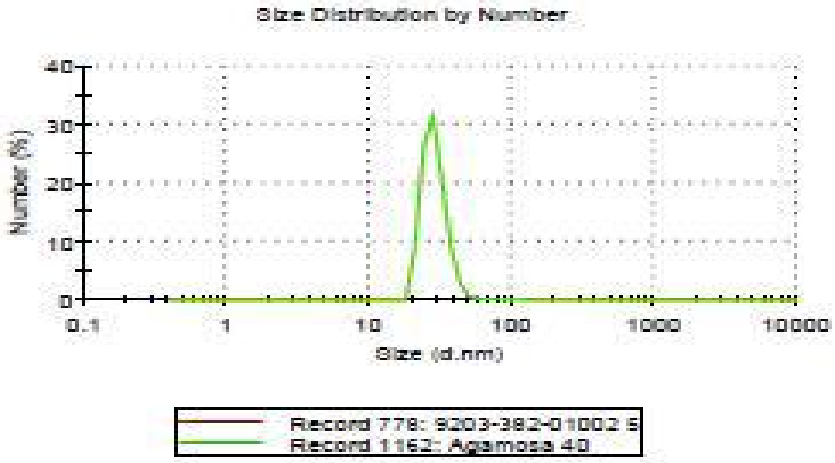

Diagram 3. DLS of Nano-Ag 40.

The morphology of nanoparticles was determined by TEM microscope:

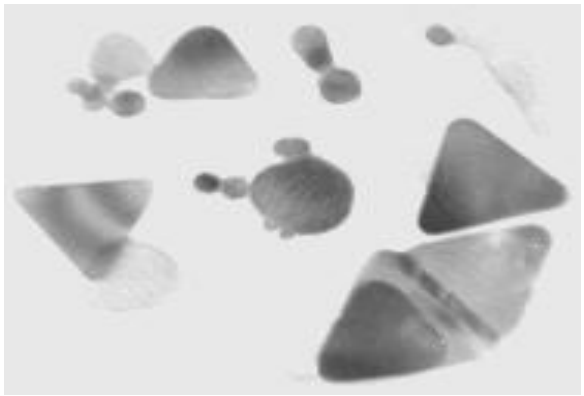

Figure 1. Nano-Ag 10.

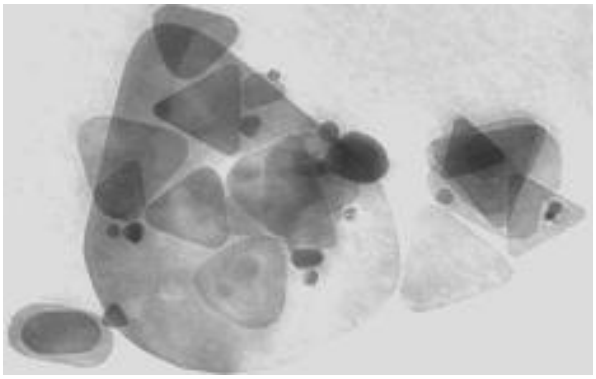

Figure 2. Nano-Ag 20.

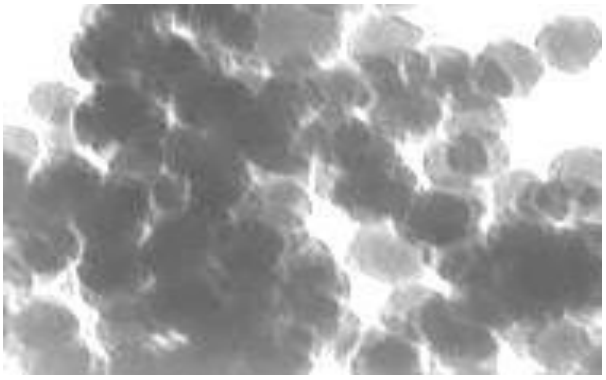

Figure 3. Nano-Ag40.

Measurement of Minimum Inhibitory Concentration (MIC)&Minimum Inhibitory Fungicidal

The MICS for *C. albicance, T. rubrum* and *Asp. fumigatus* were determined by a broth dilution method based on the National Committee for Clinical and Laboratory Standards (NCCLS; now named as Clinical and Laboratory Standards Institute, CLSI ,2000). Different concentration of Nano-Ag(64,32,16,….0/06) µg/ml was added in broth medium. Each fungal culture was incubated at 35ºc.To establish the antimicrobial activity of Nano-Ags, the C. albicance, T. rubrum and Asp. fumigatus were determined by turbidity of the fungal culture solution containing different concentration of each Nano-Ag after 24-72 h. The minimum fungicidal concentration (MFC) was determined by inoculating the contents of all of the testing earlens onto a new plate with SDA without silver NP_s . Flowing 72-h incubation, the MFC was recorded as the lowest concentration of the tested agent inhibiting the visible growth of microorganisms.

Results and discussions

The fungistatic activity of Nano-Ags with an average diameter 10-40 nm against *C.albicance ,T.rubrum* and *Asp. fumigatus*

as models for fungi was investigated by the standard dilution method , Nano-Ags exhibited a potent antifungal activity against fungal tested. The obtained results showed that the very low concentrations and the inhibition was dependent on the size of Nano-Ags (Table 1). The growth of C. albicance , T.rubrum and Asp. fumigatus was inhibited totally at MIC_s equal to 4-8-16 µg/ml for Nano- Ag ^{10} ,8-8-16 for Nano- Ag ^{20} and 16-4-16 for Nano- Ag ^{40} respectively.

The obtained MFCs are considerably similar in comparison to MICs. Silver nanoparticles killed *C. albicance, T. rubrum* and *Asp. fumigatus* at the concentration of 4-8-16, respectively.

Table 1. Antifungal activity of nano-Ag.

Fungi	Nano-Ag^{10}	Nano-Ag^{20}	Nano-Ag^{40}
C.albicance	4-4-8	8-8-4	8-16-16
Asp.fumigatus	4-4-8	8	4
T.rubrum	16-8-8	8-8-16	4-8-16

The obtained results of the antifungal activity clearly reveal that the growth of funguls was inhibited at low concentration. Nano- Ag^{10} has more efficiency on *C.albicance* in comparable with another fungal tasted (Diagram 4). There is no significant between fungi for Nano-Ag^{20} (Diagram 5). Nano- Ag^{40} has significant impact at first on *C. albicance* and secondly on *Asp.fumigatus* (Diagram 6).

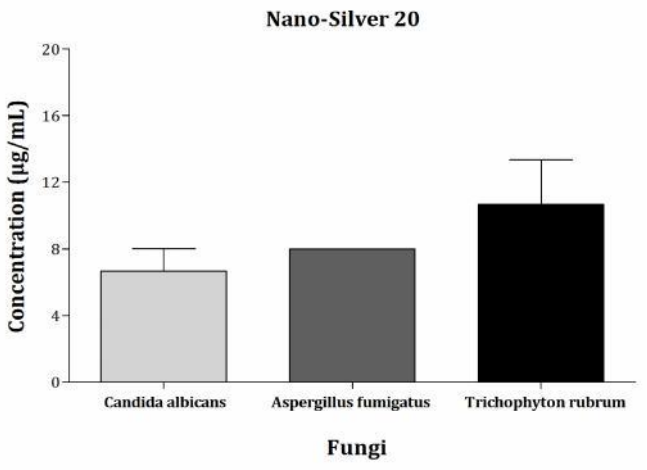

Diagram 4. The untifungal effect of Nano-Ag^{10}.

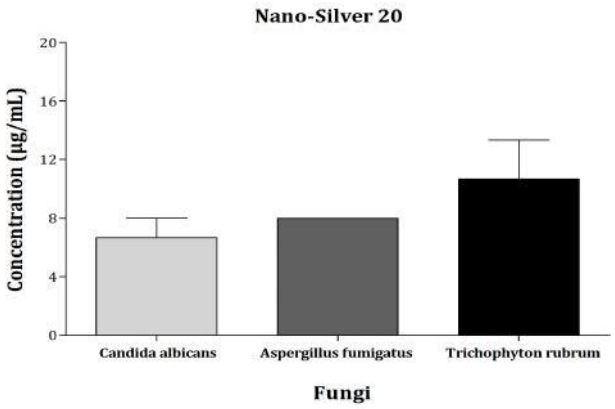

Diagram 5. The untifungal effect of Nano-Ag[20].

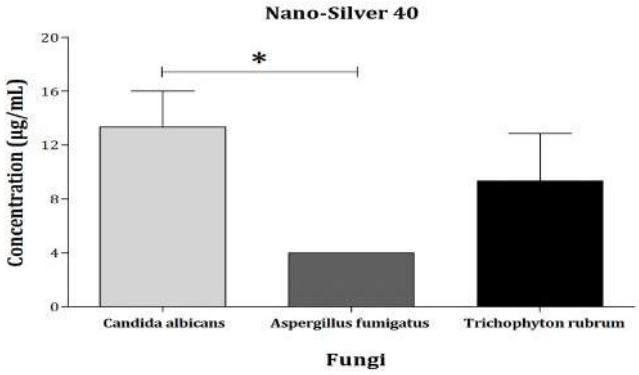

Diagram 6. The untifungal effect of Nano-Ag[40].

The effect of different size of Nano-Silver on each fungi was illustrated. Nano- Ag[10] has more effective on *C. albicance* and *Asp. fumigatus* in compared with *T. rubrum* ,but Nano- Ag[20] has approximately same effect on all fungi tested. The effect of Nano-Ag[40] on fungi has shown, higher concentration for all fungi in compared Nano- Ag[10,20] (Diagram 7-9).

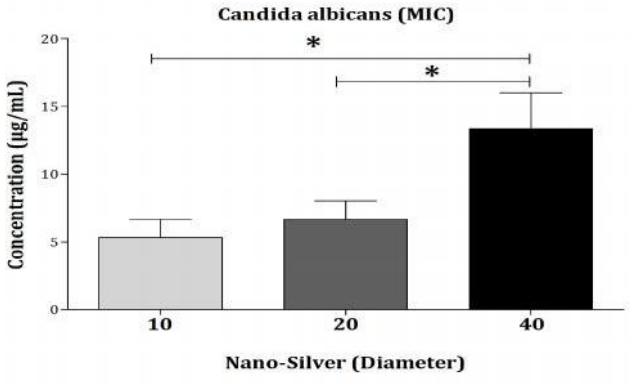

Diagram 7. The effect of different size of Nano –Ag on *Candida .albicance.*

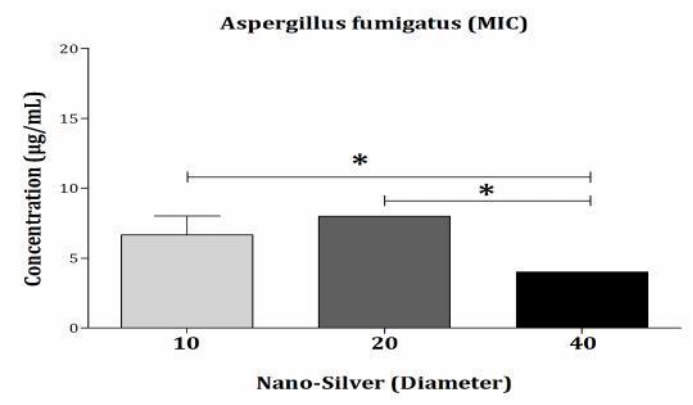

Diagram 8. The effect of different size of Nano –Ag on *Aspergillus fumigates.*

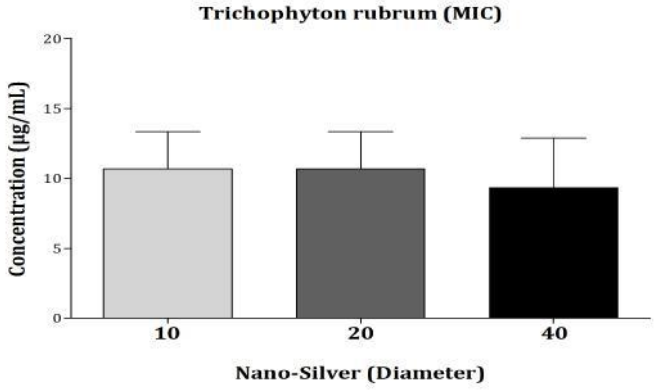

Diagram 9. The effect of different size of Nano –Ag on *Tricophyton .rubrum*

Conclusion

Nano – Ag, in an range of 4-16 µg/ml, showed significant antifungal activity against fungi investigated, whereas the other antifungal drugs has impression in higher concentration[13]. These results show the efficiency of nano-Ag as an antifungal drug to treatment fungal infection diseases. There are substantial difficulties to use antifungal again, because of fungal resistance and bad affect

on kidneys, liver toxicity and stopping in testosterone synthesis. So that requiring new drugs with less side effects[3,15].

Many studies have shown the antifungal effects of nano-Ag [2,9,10,11] on *C. albicance* and *T. rubrum* [14], but the effects effect of nano-Ag against Asp. fumigatus species are mostly unknown. The primary significant of this study is the observation that the nano-Ag could inhibit in extent area on fungal infection caused by fungi above mentioned. Based on our information this is the first study to apply nano- Ag on successfully to *Asp. fumigatus*. Secondly, it shows that, the size of nano- Ag is a determinative factor in their antifungal activity.

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