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Antifungal Effect of Silver Nanoparticles on Dermatophytes

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Spherical silver nanoparticles (nano-Ag) were synthesized and their antifungal effects on fungal pathogens of the skin were investigated. Nano-Ag showed potent activity against clinical isolates and ATCC strains of *Trichophyton mentagrophytes* and *Candida* species (IC_{80}, 1–7 μg/ml). The activity of nano-Ag was comparable to that of amphotericin B, but superior to that of fluconazole (amphotericin B IC_{80}, 1–5 μg/ml; fluconazole IC_{80}, 10–30 μg/ml). Additionally, we investigated their effects on the dimorphism of *Candida albicans*. The results showed nano-Ag exerted activity on the mycelia. Thus, the present study indicates nano-Ag may have considerable antifungal activity, deserving further investigation for clinical applications.

Keywords: Silver nanoparticles, antifungal effect, *Trichophyton mentagrophytes*, *Candida* species

Skin infections caused by fungi, such as *Trichophyton* and *Candida* species, have become more common in recent years [19]. In particular, fungal infections are more frequent in patients who are immunocompromised because of cancer chemotherapy, or organ or human immunodeficiency virus infections [11]. This upward trend is concerning, considering the limited number of antifungal drugs available because prophylaxis with antifungals may lead to the emergence of resistant strains. Therefore, there is an inevitable and urgent medical need for novel antifungals.

Since ancient times, it has been known that silver and its compounds are effective antimicrobial agents [6, 14, 15]. In particular, because of the recent advances in research on metal nanoparticles, nano-Ag has received special attention as a possible antimicrobial agent [1, 7, 9, 16]. Therefore, the preparation of uniform nanosized silver particles with specific requirements in terms of size, shape, and physical and chemical properties is of great interest in the formulation of new pharmaceutical products [3, 10]. Many studies have shown their antimicrobial effects, but the effects of nano-Ag against fungal pathogens of the skin are mostly unknown. In this study, nano-Ag was synthesized and its antifungal effects on clinical isolates and ATCC strains of *Trichophyton mentagrophytes* and *Candida* species were investigated.

Preparation of Nano-Ag

One-hundred g of solid silver was dissolved in 100 ml of 100% nitric acid at 90°C, and then 1 l of distilled water was added. By adding sodium chloride to the silver solution, Ag ions reduced and clustered together to form monodispersed nanoparticles in the aqueous medium. Because the final concentration of colloidal silver was 60,000 ppm, this solution was diluted, and then samples of different

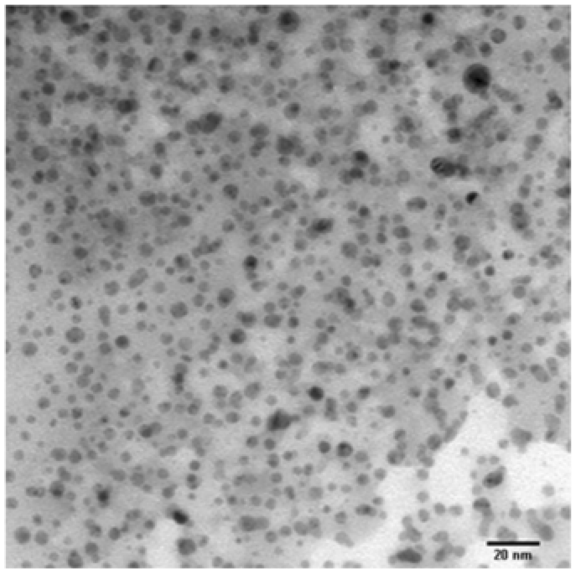

Fig. 1. Transmission electron micrograph of the nano-Ag used in this work.

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concentrations were used to investigate the antifungal effect of nano-Ag. The sizes and morphology of nano-Ag were examined by using a transmission electron microscope (H-7600; Hitachi, Ltd.). The results showed that nano-Ag was a spherical form and its average size was 3 nm (Fig. 1).

Determination of Antifungal Susceptibility

A total of 44 strains of 6 fungal species was used in this study. *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida parapsilosis* (ATCC 22019), and *Candida krusei* (ATCC 6258) were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, U.S.A.). Clinical isolates of *Candida* spp. were obtained from the Department of Laboratory Medicine, Chonnam National University Medical School (Gwangju, Korea), and clinical isolates of *Trichophyton mentagrophytes* were obtained from the Institute of Medical Mycology, Catholic Skin Clinic (Daegu, Korea). *Candida* spp. and *Trichophyton mentagrophytes* were cultured in a Sabraud dextrose agar (SDA) and a potato dextrose agar (PDA) at 35°C, respectively.

The MICs for *Candida* spp. and *T. mentagrophytes* were determined by a broth microdilution method based on the National Committee for Clinical Laboratory Standards (NCCLS; now renamed as Clinical and Laboratory Standards Institute, CLSI, 2000) method outlined in documents M-27A [12] and M-38P [13], respectively. An RPMI 1640 medium buffered to pH 7.0 with 3-(*N*-morpholino) propanesulfonic acid (MOPS) was used as the culture medium, and the inoculum size of *Candida* spp. was 0.5×10^3 to 2.5×10^3 cells/ml, and that of *T. mentagrophytes* was 0.4×10^4 to 5×10^4 cells/ml. The microdilution plates inoculated with fungi were incubated at 35°C, and the turbidity of the growth control wells was observed every 24 h. The 80% inhibitory concentration (IC_{80}) was defined as the lowest concentration that inhibited 80% of the growth as determined by a comparison with the growth in the control wells. The growth was assayed with a microplate reader (Bio-Tek Instruments, Winooski, VT, U.S.A.) by monitoring absorption at 405 nm. In the current study, amphotericin B and fluconazole were used as a positive control toward fungi; amphotericin B is a fungicidal agent widely used in treating serious systemic infections [4], and fluconazole is used in the treatment of superficial skin infections caused by dermatophytes and *Candida* species [2]. Nano-Ag, in an IC_{80} range of 1–7 μg/ml, showed significant antifungal activity against *T. mentagrophytes* and *Candida* species. Toward all fungal strains, nano-Ag exhibited similar activity with amphotericin B, showing IC_{80} values of 1–5 μg/ml, but more potent activity than fluconazole, showing IC_{80} values of 10–30 μg/ml. However, this compound exhibited less potent activity than amphotericin B, showing IC_{80} values of 2–4 μg/ml for *C. parapsilosis* and *C. krusei* (Table 1).

Table 1. Antifungal activity of nano-Ag.

Fungal strains (no. of strains)	IC_{80} (μg/ml)		
	Nano-Ag	Amphotericin B	Fluconazole
C. albicans (4)	2–4	5	10–16
C. tropicalis (2)	7	2–4	13
C. glabrata (4)	1–7	2	10–16
C. parapsilosis (3)	4–25	2	13
C. krusei (1)	13	4	13
T. mentagrophytes (30)	1–4	1–2	20–30

Effect of Nano-Ag on the Dimorphic Transition

C. albicans cells were maintained by periodic subculturing in a liquid yeast extract/peptone/dextrose (YPD) medium. Cultures of yeast cells (blastoconidia) were maintained in a liquid YPD medium at 37°C. To induce mycelial formation, cultures were directly supplemented with 20% of a fetal bovine serum (FBS). The dimorphic transition in *C. albicans* was investigated from cultures containing 2 mg/ml of nano-Ag (at the IC_{80}), which were incubated for 48 h at 37°C [5, 17, 18]. The dimorphic transition to mycelial forms was detected by phase contrast light microscopy (Nikon, Eclipsete300, Tokyo, Japan). The dimorphic transition of *C. albicans* from yeast form to mycelial form is responsible for pathogenicity, with mycelial shapes being predominantly found during the invasion of host tissue. A mycelial form can be induced by temperature, pH, and serum [8]. As shown in Fig. 2, the serum-induced mycelia

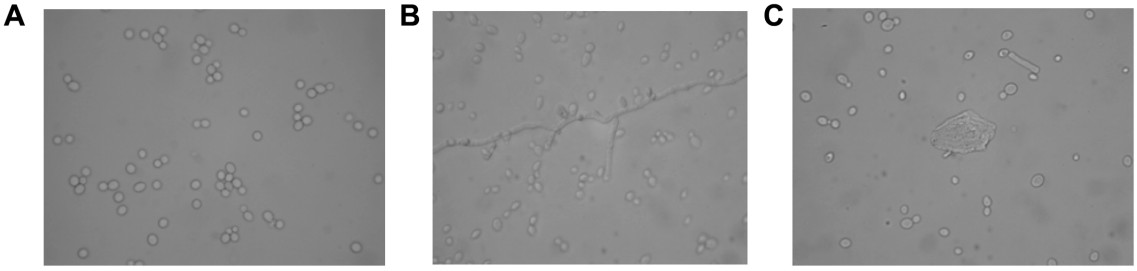

Fig. 2. The effect of nano-Ag on the dimorphic transition in *C. albicans*.
Yeast control without 20% FBS and nano-Ag (**A**), without treated nano-Ag (**B**), or with 2 μg/ml of nano-Ag (**C**).

were significantly inhibited from extending and forming in the presence of nano-Ag (Fig. 2C), but the mycelia formed was normal in the absence of nano-Ag (Fig. 2B). These results suggested that nano-Ag is a potential compound in the treatment of fungal infectious diseases.

Many studies have shown the antimicrobial effects of nano-Ag [6, 14, 15], but the effects of nano-Ag against fungal pathogens of the skin including clinical isolates of *T. mentagrophytes* and *Candida* species are mostly unknown. The primary significance of this study is the observation that nano-Ag could inhibit the growth of dermatophytes, which cause superficial fungal infections. To our knowledge, this is the first study to apply nano-Ag successfully to dermatophytes. Secondly, the fact that preparation method of nano-Ag described here is cost-effective is also of importance. Therefore, it could be expected that nano-Ag may have potential as an anti-infective agent for human disease caused by dermatophytes.

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K.-J. Kim and W.S. Sung contributed equally to this work and should be considered co-first authors.

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