

Original research

Effect of CuO and SiO₂ nanoparticles on growth of *Candida albicans*

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Introduction

Nanotechnology has created new hopes for diagnosis, treatment and targeted drug delivery. Nanoparticles have different characteristics compared with their bulk material, due to their extreme small size, and have shown various applications in industry, agriculture, military weapons, and medicine. It also has played an important role in detection of infectious agents and development of new antimicrobial drugs(1-6). Numerous metal nanoparticles and metal oxides with antimicrobial action are known(7). Their exact mechanisms of action are not yet well-determined, but there are several hypotheses in this regard, including 1) destruction of cell wall, 2) destruction of selective permeability of cell membrane, 3) derangement in cellular respiration, and 4) damage to DNA and cellular proteins(8).

Silver nanoparticles are one of the antimicrobial agents that have been in use since a long time before. There have been wide usages of them in the material science, physics, and medicine. Nowadays these nanoparticles are used in sterile pads, BUMS, catheters, and also in dental alloys, only to name some(8). Titanium Oxide nanoparticles also have antimicrobial properties, by free radical formation in the presence of ultraviolet light(9). Nano particles of ZnO reveal antibacterial features through photocatalytic properties, similar to TiO₂.(10) MgO nanoparticle has superior actions compared with TiO₂, especially on some human pathogens like *S.aureus* and *B.subtilis*(11). Previous research has shown antibacterial effects of CuO(12) and SiO₂(13),but no study about the fungicidal action of these compounds has been done. In the current study, we evaluated the effects of CuO and SiO₂ on growth of *Candida albicans* ATCC number 10231 to determine their MIC (minimum inhibitory concentration).

Effect of CuO and SiO₂ nanoparticles on growth of *Candida albicans*

Since some fungal species show drug resistance, antifungal agents usually have low solubility in blood, and most of them are considerably toxic, it seems very important to find antifungal nanoparticles with low adverse effects.

Materials and methods:

Preparation of CuO and SiO₂ suspensions & fungal isolate:

Different concentrations (1.4, 2.9, 5.8, 11.7, 23.4, 46.8, 93.7, 187.5, 375, 750, 1500 and 3000 mg/L) of the nanoparticles of CuO (60 nm, 80 m²/g, from Lolitech, Germany) and SiO₂ (10 nm, 600 m²/g, from Lolitech, Germany) in deionized water were prepared.

Candida albicans ATCC 10231 standard strain was obtained from the fungal/bacterial collection center of the Iranian Scientific & Industrial Research Organization, cultured on Sabouraud's dextrose agar (Merck, Germany), and incubated at 25°C for 2 days. A few fresh colonies of the yeast were added to 50 mL of distilled water and mixed to yield a suspension of 0.5 McFarland turbidity, which is equivalent to a concentration of 10⁶/mL.

Preparation of aqueous fungal culture medium for determination of MIC:

According to the manufacturer's instructions, 31 grams of the powder medium of Sabouraud's dextrose broth (Quelab, UK) was added to one liter of distilled water, heated for 15 minutes on flame until clearing, and sterilized by autoclaving.

MIC determination:

We added 100 µL of the liquid medium, 100 µL of the yeast suspension and 100 µL of nanoparticles to a 96-well microplate (in duplicate), incubated it at 37°C for 48 hours, and read the optical density (OD) of suspensions on an ELISA (enzyme-linked immunosorbent assay) reader

Effect of CuO and SiO₂ nanoparticles on growth of *Candida albicans*

(Awareness, USA) at 405 nanometers wavelength at 6, 12, 24, 36 and 48 hours. Final concentrations of the nanoparticles in each well were as follows: 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 1.9, 0.9 and 0.4 mg/L.

Positive and negative controls:

The positive control wells (all in duplicate) contained yeast suspension and culture medium. The 4 negative control wells (all in duplicate) included 1) yeast suspension and culture medium and nystatin; 2) yeast suspension only; 3) culture medium only; and 4) all of the different suspensions of both nanoparticles only. For comparison with the controls, t-test was used.

Results:

Figure 1 shows the OD vs. different concentrations of CuO nanoparticles. There is no obvious OD at 6 and 12 hours, but it shows considerable OD due to yeast growth after 24, 36 and 48 hours, the lowest of which is in the presence of 1000 mg/L of CuO (that c).

Figure 2 reveals the OD vs. different concentrations of SiO₂ nanoparticles. There is again no considerable OD at 6 and 12 hours, but it shows considerable OD afterwards, the lowest of them being after 24 hours (at 1000 mg/L concentration, being its MIC) and 36 and 48 hours (in the presence of 0.9 mg/L of SiO₂, which is its MIC).

T-test revealed significant differences ($p=0.01$) between the control samples and these concentrations at 48 hours of incubation.

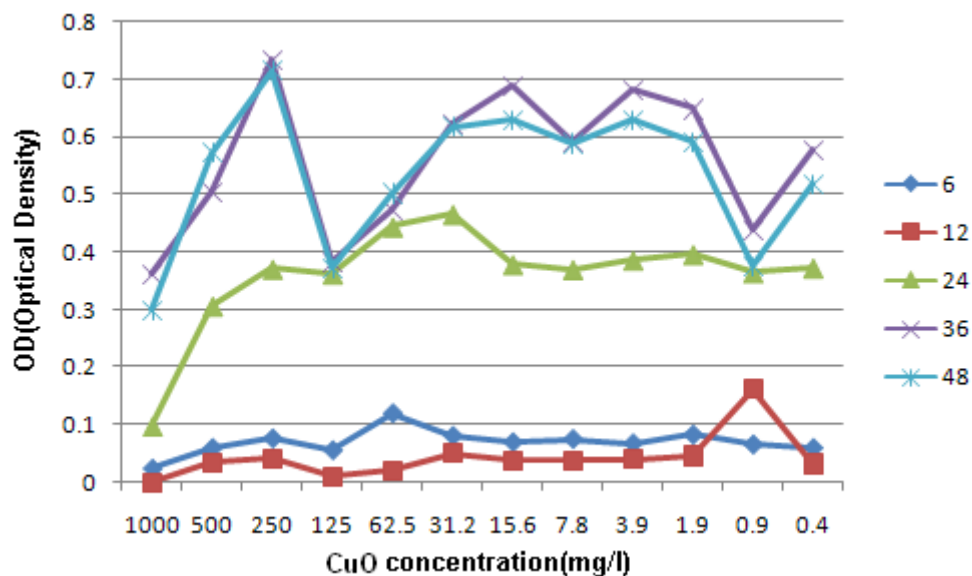


Figure 1. Various concentrations of CuO vs. OD at different times.

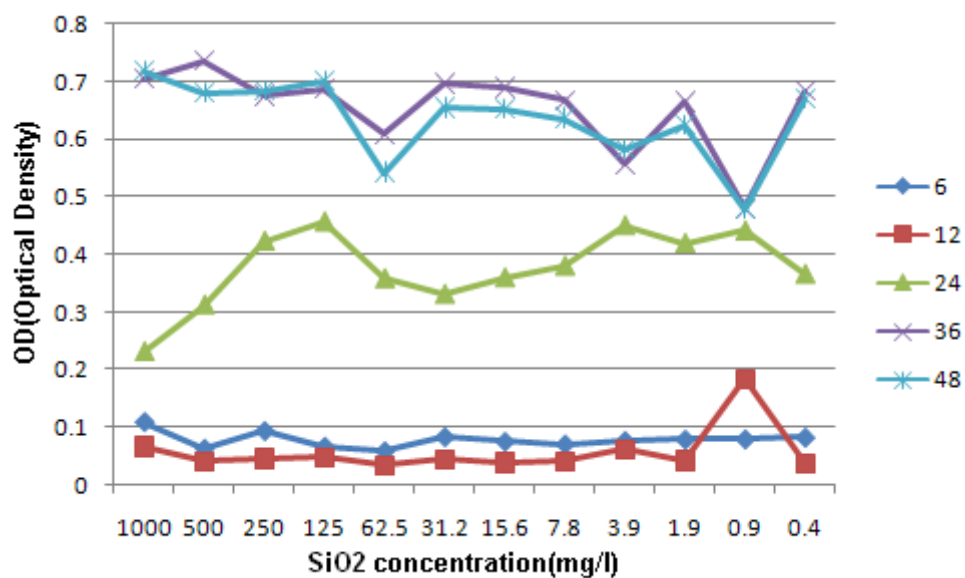


Figure 2. Various concentrations of SiO₂ vs. OD at different times.

In figures 3 and 4, SEM (scanning electron micrographs) of CuO and SiO₂ are demonstrated, respectively.

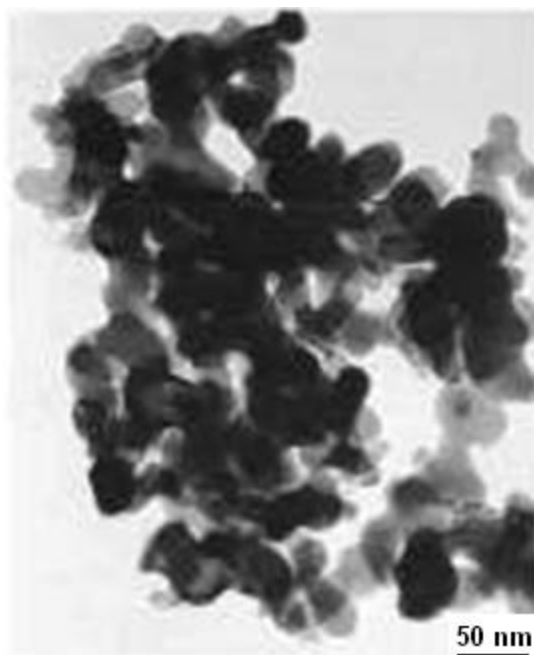


Figure 3. TEM of CuO nanoparticles.

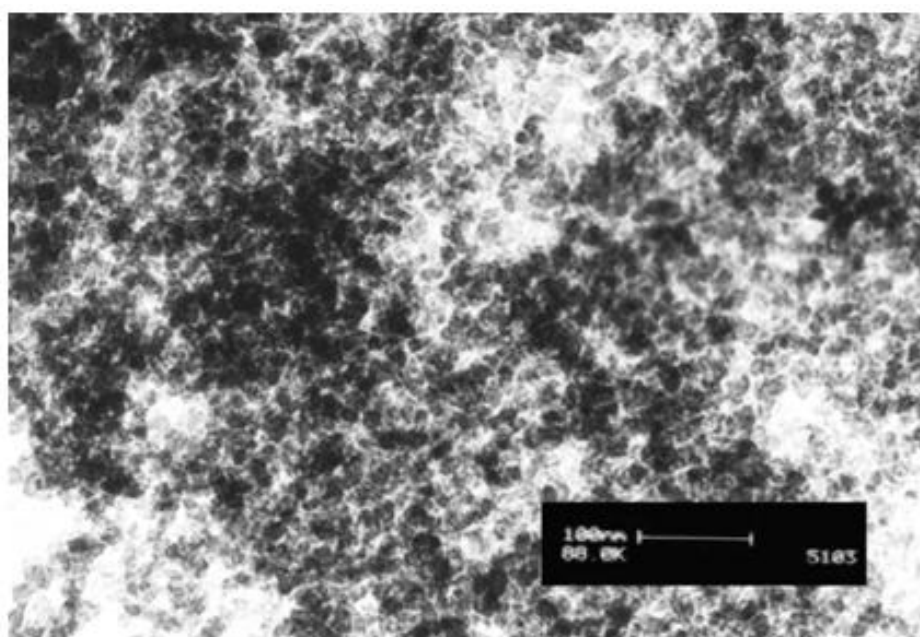


Figure 4. TEM of SiO₂ nanoparticles.

Discussion:

Antibacterial properties of nanosilver and titanium oxide nanoparticles have already been explained(7-9), and there are a few reports on such activities regarding other nanoparticles including MgO, ZnO, CuO and SiO₂(10-13). However, no data are available about antifungal effects of those nanoparticles. So, we aimed at finding possible antifungal activity of CuO and SiO₂ against *Candida albicans*. Results show that during 48 hours of incubation, a concentration of 1000 mg/L from CuO and also a concentration of 0.9 mg/L of SiO₂ will exert maximum inhibitory effect on the yeast.

Since the antifungal activity of SiO₂ was superior to CuO, it may be useful for in vitro applications. If proved (through further research) to be harmless to body cells and non-toxic, then it may be evaluated for possible use in treatment of cutaneous, mucosal and systemic candidal infections in the future. It is suggested to perform complementary studies regarding the effects of these 2 nanoparticles on other strains of *Candida* and also important molds such as dermatophytes.

References:

- 1.Zrazhevskiy P, Sena M, Gao X. Designing multifunctional quantum dots for bioimaging, detection, and drug delivery.Chem Soc Rev. 2010 Nov;39(11):4326-54.
- 2.Tolaymat TM, El Badawy AM, Genaidy A, Scheckel KG, Luxton TP, Suidan M. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: a systematic review and critical appraisal of peer-reviewed scientific papers.Sci Total Environ. 2010 Feb 1;408(5):999-1006.

Effect of CuO and SiO₂ nanoparticles on growth of *Candida albicans*

3. Rhyner MN, Smith AM, Gao X, Mao H, Yang L, Nie S. Quantum dots and multifunctional nanoparticles: new contrast agents for tumor imaging. *Nanomedicine (Lond)*. 2006 Aug;1(2):209-17.
4. Huo Q. A perspective on bioconjugated nanoparticles and quantum dots. *Colloids Surf B Biointerfaces*. 2007 Sep 1;59(1):1-10.
5. Montañez MI, Ruiz-Sanchez AJ, Perez-Inestrosa E. A perspective of nanotechnology in hypersensitivity reactions including drug allergy. *Curr Opin Allergy Clin Immunol*. 2010 Aug;10(4):297-302.
6. Behari J. Principles of nanoscience: an overview. *Indian J Exp Biol*. 2010 Oct;48(10):1008-19.
7. Nowack B, Krug HF, Height M. 120 Years of Nanosilver History: Implications for Policy Makers. *Environ Sci Technol*. 2011 Jan 10.
8. Tang B, Wang J, Xu S, Afrin T, Xu W, Sun L, Wang X. Application of anisotropic silver nanoparticles: Multifunctionalization of wool fabric. *J Colloid Interface Sci*. 2011 Jan 22.
9. Shi Z, Xiang Y, Zhang X, Yao S. Photocatalytic Activity of Ho-doped Anatase Titanium Dioxide Coated Magnetite. *Photochem Photobiol*. 2011 Jan 5.12-13.
10. Xiong D, Fang T, Yu L, Sima X, Zhu W. Effects of nano-scale TiO₂, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. *Sci Total Environ*. 2011 Feb 4.
11. Necula BS, Fratila-Apachitei LE, Berkani A, Apachitei I, Duszczyk J. Enrichment of anodic MgO layers with Ag nanoparticles for biomedical applications. *J Mater Sci Mater Med*. 2009 Jan;20(1):339-45.
12. Ren G, Hu D, Cheng EW, Vargas-Reus MA, Reip P, Allaker RP. Characterisation of copper oxide nanoparticles for antimicrobial applications. *Int J Antimicrob Agents*. 2009 Jun;33(6):587-90.

Effect of CuO and SiO₂ nanoparticles on growth of *Candida albicans*

13. Jia H, Hou W, Wei L, Xu B, Liu X. The structures and antibacterial properties of nano-SiO₂ supported silver/zinc-silver materials. *Dent Mater*. 2008 Feb;24(2):244-9.