Au–Ag–Cu nanoparticles alloys showed antifungal activity against the antibiotics-resistant Candida albicans

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Abstract
Formation of Au–Ag–Cu ternary alloy nanoparticles (NPs) is of particular interest because its trimetallic process that includes miscible (Au–Ag and Au–Cu) and immiscible (Ag–Cu) system. So there is a possibility of phase segregation in this ternary system. At this challenge, it was an attempt to use the synthetic technique to generate such trimetallic alloy nanoparticles by exploding wire technique. This work aims to develop an effective antifungal treatment against the pathogenic fungus Candida albicans using Au–Ag–Cu nanoparticles alloys. In this work, three metals alloys (A, B, and C) containing Au-Ag-Cu nanoparticles were prepared as a physical methods by exploding wire with different ratio of each elements (A: 50% Au 25% Ag 25% Cu; B: 25% Au 50% Ag 25% Cu; C: 15% Au 60% Ag 25% Cu). The antifungal activity of the alloy nanoparticles was tested against pathogen fungi, Candida albicans grown on a solid medium using disk diffusion method. The highest antifungal activity against Candida albicans was observed on alloy B that was prepared by 160 A and concentrations 12.5 μg.ml\(^{-1}\). The treatment with this alloy showed more than 85% inactivation of the fungus growth.

Keywords: Exploding Wire, Au–Ag–Cu alloy nanoparticles, Candida albicans, Antibiotic-resistant, Growth rate.

1. Introduction
Nanotechnology is a multidisciplinary and fast growing scientific field in physics, chemistry, and biotechnology sciences [1]. Hybrid and multifunctional nanoparticles that constructed from more than one metallic phase have attracted much interest because of their unique catalytical, optical, electrical, and magnetically properties compared to the individual monometallic metals and bimetallic [2]. This notion leads toward the concept and possibility of personalized medicine for the potential of early detection of diseases and most importantly, molecular targeted therapy. The application of nanotechnology to biomedicine or biological designated as nanomedicine has dramatically accelerated the diagnosis, imaging, and treatment of many diseases.
Living organisms are built of cells that are typically 10 μm across. However, the cell parts are much smaller and are in the sub-micron size domain and proteins typical size is just 5 nm which is comparable with the dimensions of the smallest humanmade nanoparticles [3]. Functionalities can be added to nanomaterials by interfacing them with biological molecules or structures. Because the size of nanomaterials is similar to that of most biological molecules and structures, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications. Thus far, the integration of nanomaterials with biological processes has led to the development of physical therapy applications, and drug delivery vehicles [4]. The nanoparticles are produced by using various techniques like chemical methods, physical methods, and mechanical methods. One of the physical processes is the electrical explosion wire (EEW) technique for producing nanoparticles, which has recently gained immense importance [5]. EEW method has become one of the promising ways for producing metal nanoparticles because it is ecologically safe, simple, effective, and cost-effective. [6,7]. Therefore, these nanoparticles can be used in almost any environment with no risk of rapid chemical reactions. Furthermore, it has a great potential in a wide range of applications with great interest from both scientific and technological points of view [8]. It is important to note that the metallic nanoparticles are most promising application as they show good antimicrobial properties and therefore it has a great interest from researchers in the field of microbial and fungi resistance against metal ions and antibiotics [9].

Candida albicans is one of the many fungi which live in and on all of us [10]. It is most common fungal pathogen presents in the oral cavity and is best known for causing thrush in the mouths of babies. It also causes the sore white and moist plaques in the mouth, on the tongue and the upper respiratory tract, as well as in some parts of gastrointestinal, blood, and genital tracts of the human body. In certain conditions, this fungus causes many types of diseases, for example, dermatocandidiasis, and may also lead to morbidity and mortality of immuno-compromised (e.g., via HIV infection) patients [11]. Recently, the development of antibiotic-resistant pathogens has become a world concern. For instance, Candida albicans has become resistant to antifungal drugs such as fluconazole andazole [12]. Thus, the current treatment of Candida albicans with the commonly used antifungal agents such as fluconazole or other azoles has become unsuccessful [13]. For this reason, new antifungal therapies are of utmost importance for effective treatment of Candida albicans infection. Importantly, using the trimetallic Alloy nanoparticles such as (Ag, Au, Cu) as antifungal agent has attracted much interest [14]. Therefore, this study was designed to investigate the antifungal treatment of Au–Ag–Cu nanoparticles alloys against the pathogenic fungus Candida albicans.

2. Experimental Setup and Procedures

2.1 produce the nanoparticles

In this work the process was achieved by exploding wire (EEW) technique of producing nanoparticles as we described previously [15].

2.2 Fungi Preparation

Candida albicans. The fungi isolate were obtained from Central Teaching laboratories in the City of Medicine, Teaching Hospital, Baghdad. The culture of Candida albicans was prepared under the following conditions: First, the fungi were cultured in the Sabouraud Dextrose Agar Medium that was prepared according to manufacturer protocol. In brief, the amount of 65g agar (40 g dextrose, 10 g peptone,15g agar ) was dissolved in 1L distilled water. The medium PH was adjusted to 6.8 and sterilized by autoclaving. It was cooled down to 45-50°C then poured in sterile Petri dishes and left for 30 min at 37 °C to solidify. The most viable cellular colonies selected for the treatment (inoculum) by a sterile loop.
The sterile loop was streaked on the whole surface of the medium plates containing Sabouraud Dextrose Agar, and then kept stored in the refrigerator at 4°C and used as needed.

2.3 Antifungal activity test of alloys nanoparticles by Disk-Diffusion Method

The antifungal activity of synthesized in a distilled water solution of different alloys (A,B,C) NPs where don by exploding wire a different current (100A,160A) and with four concentration (6.25, 12.5, 25 and 50 μg ml⁻¹) are tested against pathogen fungi of Candida albicans. Were examined using the disk diffusion method as ( NCCLS document M44-A) [16] per the modified method of against of Candida albicans were taken from fresh stock cultures (24 hours) by the loop, then suspended in normal saline, and adjusted by comparison using standard 0.5 McFarland turbidity (5x10⁶ cell ml⁻¹) tubes. These pathogens were absorbed on the surface of sterile cotton swab by dipping into the suspension. The sterile cotton swab was streaked on the whole surface of the medium plates containing Muller Hinton Agar. Then each test sample (50 μℓ) was soaked separately on a paper disc (6 mm diameter) made up from Whatman filter paper, and should wait until it completely dry. These impregnated paper discs were ascetically transferred to the surface of the petri dish media. The inoculated plates were then safely incubated at a temperature of 35 °C for 24 hours in the case of fungi. The clear zone around the disc was considered as the zone of inhibition of microbial growth. The diameter of inhibition zones was estimated using a meter ruler and the average value for each organism was recorded. All tests were carried out in triplicate. Plates containing Muller Hinton Agar with fungi cultures(untreated) were used as a control. The diameter of the inhibition zone was precisely determined to calculate the antifungal activity of the test samples.

3. Results and discussion

3.1 Inhibition of Fungus Growth by applying different alloys NPs

The antifungal activity of different alloys NPs (A, B, C) was tested against the pathogen fungi Candida albicans. Figures 1,2 and 3 shows the inhibition zone (IZ) of Candida albicans grown in solid medium (Muller Hinton agar plates) using disk diffusion methods. Different alloys nanoparticles (A, B and C) were applied at increasing concentrations (6.25, 12.5, 25 and 50 μg ml⁻¹). Figures 1,2 and 3 illustrated the inhibition zone at a varied concentration of nanoparticles ranged from 6 mm to 40 mm. The best inhibition of growth rate was observed after applying (160 A with 12.5 μg.ml⁻¹) to alloy B, that produced 40 mm zone diameter as showed in Figure 2. It is most likely that the small size of NPs compared to fungi facilitates the penetration through the cell wall and causes uncontrolled mass transfer through membranes. The cell wall is the primary target of the nanoparticles whereby the small size of these particles facilitates the penetration of these particles through the cell wall and interferes with the intracellular processes. Another effect of the nanoparticles as antifungal is the ability to induce cellular toxicity through the release of reactive oxygen species (ROS) [17]. Additionally, to achieve higher antifungal efficacy of nanoparticles, moderation, attenuation or erosion of the cell wall probably will enhance the effect of nanoparticles and facilitate their interaction with cellular components [9]. The antifungal effect of alloy NPs could be due to NPs toxicity. The high antifungal activity of NPs could be through a combination of membrane damage and oxidative stress. The antimicrobial mechanisms of NPs can be explained in three stages: Primary NPs fungi contact, membrane perturbation, and membrane oxidation in an electronic structure[18]. These results indicated that direct physical membrane disruption of the fungi cells by the NPs is an important cytotoxic mechanism for effective fungi cell inactivation. Besides, metals alloy have also been demonstrated to be active agents in killing fungi cells through the generation of ROS that can induce oxidative stress within the pathogenic cells [9, 19]. The nanoparticles are likely to improve the
directionality of the propagation of nanoparticles towards diseased sites within a tissue. Furthermore, the Au-Ag-Cu alloy consists of three elements, it is unlikely the fungus produced a resistant mutant to the nanoparticles compared with the single element nanoparticle.

![Figure 1](image_url)

**Figure 1.** Growth inhibition of fungal *Candida albicans* grown in solid medium, samples in Petri dishes. (A) A-100A and (B) A-160-A at concentrations of 6.25 μg.ml⁻¹. (C) A-100A and (D) A-160A at concentration of 12.5 μg.ml⁻¹. (E) A-100A and (F) A-160A at concentration of 25 μg.ml⁻¹. (G) A-100A and (H) A-160A at concentration of 50 μg.ml⁻¹. The alloy (A) nanoparticles were done by exploding wire currents (100A,160A) respectively. (I) The statistical analysis of the differences between A-100 and A-160 showed significant difference at the concentration 12.5 μg/ml. Two-way ANOVA with Bonferroni post-test. **p<0.01.
Figure 2. Growth inhibition of fungal *Candida albicans* grown in solid medium, samples in Petri dishes. (A) A-100A and (B) A-160-A at concentrations of 6.25 μg.ml\(^{-1}\). (C) A-100A and (D) A-160A at concentration of 12.5 μg.ml\(^{-1}\). (E) A-100A and (F) A-160A at concentration of 25 μg.ml\(^{-1}\). (G) A-100A and (H) A-160A at concentration of 50 μg.ml\(^{-1}\). The alloy (B) nanoparticles were done by exploding wire currents (100A, 160A) respectively. (I) The statistical analysis of the differences between A-100 and A-160 showed no significant differences between A-100 and A-160. Two-way ANOVA with Bonferroni post-test. **p<0.01.**
Figure 3. Growth inhibition of fungal *Candida albicans* grown in solid medium, samples in Petri dishes. (A) A-100A and (B) A-160-A at concentrations of 6.25 μg.ml⁻¹. (C) A-100A and (D) A-160A at concentration of 12.5 μg.ml⁻¹. (E) A-100A and (F) A-160A at concentration of 25 μg.ml⁻¹. (G) A-100A and (H) A-160A at concentration of 50 μg.ml⁻¹. The alloy (C) nanoparticles were done by exploding wire currents (100A,160A) respectively. (I) The statistical analysis of the differences between A-100 and A-160 showed no significant differences between A-100 and A-160. Two-way ANOVA with Bonferroni post-test. **p<0.01.

4. Conclusions

The outcome of this study showed that the alloys nanoparticles inhibited the growth rate of the pathogenic fungus *Candida albicans*. The best antifungal activity against pathogen fungi of *Candida albicans* was for a concentration 12.5 μg.ml⁻¹ of alloy B prepared by 160 A that showed more than 85% growth inhibition after the treatment. The enhanced penetration by nanoparticles offers a spatial selectivity that could improve their safe use as a therapeutic strategy.

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