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Full Length Research Paper

Effects of Silver Nanoparticles on the Structural Integrity of Yeast Cell Walls

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Silver nanoparticles are increasingly finding applications in health care and consumer products. Although the bactericidal properties of silver nanoparticles are well documented, there is scant data available on their effect on eukaryotic cells. We report results from experiments concerning the model organism *Saccharomyces cerevisiae*. A novel method of nanoparticle synthesis in yeast malt media was achieved. Characterization of particles by electron microscopy and XRD revealed a mean particle size of 8.6 nm. Micrographs of cells treated with nanoparticles revealed deposition on and extensive damage to cell wall/membranes. Observations on membrane damage were confirmed by an *in vitro* cell permeability assay.

Keywords: Silver nanoparticle, *Saccharomyces cerevisiae*, Transmission electron microscopy.

INTRODUCTION

Over the past few decades, inorganic nanoparticles, which exhibit significantly novel physical, chemical and biological phenomena and functionality due to their nanoscale size, have elicited much interest. In the last few years silver, in the form of silver nanoparticles, has made a remarkable comeback as an antibacterial, antiviral and antimicrobial compound. Among the products listed in the Inventory of Nanotechnology Consumer Products compiled by the US Woodrow Wilson Center for International Scholar's Project on Emerging Nanotechnologies, silver nanoparticles are now the most prevalent nanomaterial used in consumer products (313 products or 24 percent of the inventory). As nanoparticles become more common and widely produced, the chances of unplanned events leading to their dissemination and accumulation in the environment increase, and could lead to unforeseen changes to biological systems (Williams *et al.,* 2006).

Silver nanoparticles are typically used in the size range of 1-50nm. At this very small size, the particles' surface area is large comparative to its volume. The comparatively large surface area of nanoparticles increases their reactivity, which in many instances also increases toxicity. It is this increased surface area that is credited with enabling the destruction of bacteria and other microbes.

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The actual mechanism by which silver nanoparticles interfere with microorganisms is as yet unclear. Some researchers suggest that silver nanoparticles damage bacterial cells by destroying the enzymes that transport the cell nutrient and weakening the cell membrane or cell wall (Elechiguerra *et al.,* 2005). The bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. A study of *E. coli* bacteria, (Sondi and Salopek-Sondi 2007) found that nanosilver damaged and pitted the bacteria's cell walls, leading to increased cell permeability and ultimately cell death.

While there has been numerous studies on the anti-microbial properties of silver nanoparticles on pathogenic bacteria (Rai *et al.,* 2009), their effect on eukaryotes are less well investigated.

We report findings, from a larger study, on the effect of silver nanoparticles (Ag NPs) on the yeast cell wall. The yeast *Saccharomyces cerevisiae* is used as a model system representing a simple eukaryote.

MATERIALS AND METHODS

S. cerevisiae (MTCC 36) was procured from the Microbial Type Culture Collection, IMTECH, Chandigarh, India, as an active culture on slant. Cells were maintained on yeast malt (YM) agar (HiMedia Labs) at 30˚C, with an incubation time of 48h, then maintained at refrigerated

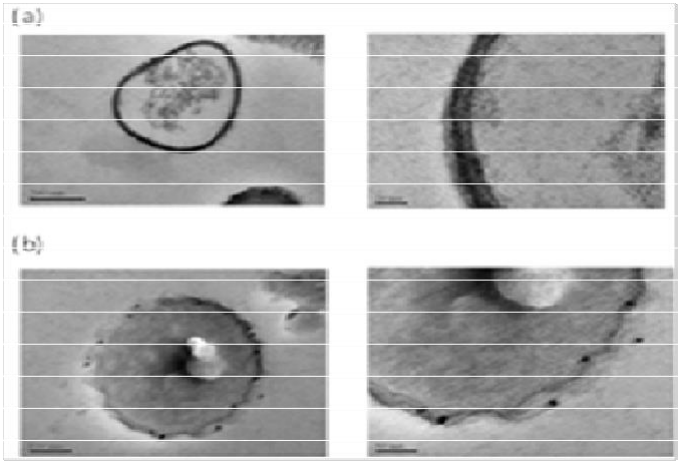


Figure 1. Electron microscopy of yeast cells. (a) Growth in YM media, figure on right show a magnified view of cell wall. Scale bars: 200 nm (left) and 20 nm (right). (b) Growth in YM media having 70.07 µg/ml Ag NPs. Nanoparticles are deposited on the outer layers and intactness of cell wall is missing. Scale bars: 100 nm (left) and 50 nm (right).

Temperature and routinely sub-cultured at 30 days intervals.

Ag NPs were prepared by reducing $AgNO_3$ with $NaBH_4$ (Gogoi *et al.,* 2006 and Kim *et al.,* 2007) in liquid yeast malt (YM) media. Varying concentrations (10^{-3} to 10^{-4} M) of $AgNO_3$ in conjunction with 10^{-2} M $NaBH_4$ were used to prepare Ag NPs in the media. In a typical synthesis reaction, dehydrated media was dissolved in solutions of $NaBH_4$ (30 ml), cooled at 4°C for 30 min and placed in an ice bath over a magnetic stirrer. $AgNO_3$ solutions (10 ml) was then added drop-wise using a burette under constant stirring. The synthesis and characterization (shape & size distribution) of Ag NPs in YM media were verified using a transmission electron microscope (Jeol, JSM-100CX) operating at 100kV.

Yeast cells (~10^6) from a log phase culture were inoculated in YM media (40 ml) having pre-formed Ag NPs at concentrations of 26.95, 48.51 and 70.07 µg/ml, along with a negative control (i.e. without Ag NP). Cultures were grown overnight in a shaker incubator set at 30°C/100rpm. Changes in cell membrane morphology and localization of nanoparticles were studied using a high resolution transmission electron microscope (HRTEM). Cells were first harvested from log phase cultures (with and without Ag NPs). Primary fixation was done in Karnovsky's reagent for 1 h at 4°C, after which the cells were washed thrice with sodium cacodylate buffer. Cells were then dehydrated in acetone and embedded in Araldite CY212 resin overnight at 50°C. Staining was done by a double staining technique using uranyl acetate and lead citrate. Micrographs of ultra-thin sections were captured with an HRTEM (Jeol, JEM-2100) operating at 200kV.

Damage to cell membrane/wall integrity was verified by a simple *in vitro* assay (Yphantis *et al.,* 1967). Briefly, 2.0 mg cells/ml (wet weight) were incubated in Ag NP solutions at 30°C for 1 h. Cells were spinned down at 3000 rpm for 10 min to collect the supernatant. Absorbance of the supernatant fluid was recorded at 260/280 nm to account for the UV-absorbing compounds released from the cells due to Ag NP treatment. The data was adjusted to account for spontaneous release of UV-absorbing compounds by cells in de-ionised water. Total extractable compounds were estimated by incubating 2.0mg cells/ml in 1.0 N $HclO_4$ at 100°C for 1h.

RESULTS AND DISCUSSION

Silver nanoparticles were readily synthesized in YM media without the use of any additional stabilizing agents. Electron micrographs showed the presence of well dispersed spherical particles with a mean size of 8.6 nm. Particle size was also calculated from XRD spectra (data not shown). Synthesis of nanoparticles in growth media was previously reported by Gogoi *et al.,* (2006). This process circumvents the stringent conditions often employed for nanoparticle synthesis and also simultaneously minimizes the 'artifact effect' which chemical agents might have on the test organism. We propose that direct synthesis in growth media is an efficient way of studying nanoparticle-cell interactions as the particles are smaller in size and poly-dispersed leading to a more robust contact (Das and Ahmed 2011, unpublished manuscript).

Alterations in cell wall integrity are clearly visible in electron micrographs (Fig. 1). Cells grown in absence of Ag NPs (Fig. 1a) show an intact outer layer and homogenous distribution of chromatin, whereas deformities in the cell wall are

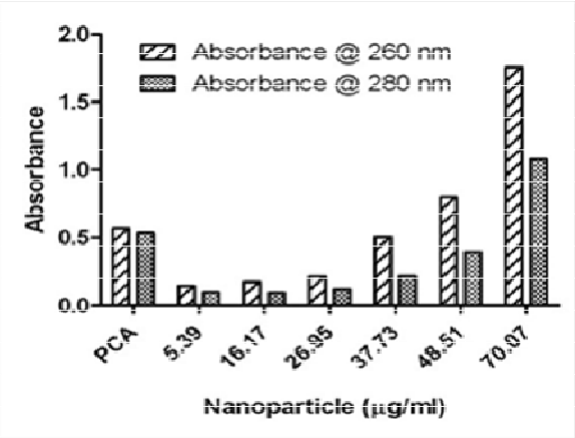


Figure 2. Cell permeability assay – release of UV-absorbing compounds on treatment with silver nanoparticles and perchloric acid (PCA).

observed for those cells grown in presence of Ag NPs (Fig. 1b). Dark spots, construed to be nanoparticles, were deposited on the outer layers as well as in the cytoplasm indicating deposition and penetration of the particles inside cells. It is likely that the interaction between the particles and the membrane is non-specific rather than specific between the nanoparticles and a particular component of the membrane such as a surface expressed protein (Williams *et al.,* 2006). A recent study (Despax *et al.,* 2011) reported sulfur-containing silver clusters preferentially located at the cell wall periphery together with nodules composed of silver, sulfur and phosphorus all over the cell. Electron-dense regions and apparent destruction of chromatin are visible inside cells treated with Ag NPs. The *in vitro* membrane permeability assay confirmed the damage caused by Ag NPs to cell membrane. The release of UV-absorbing compounds from cells increased in proportion to the concentration of nanoparticles (Fig.2) present in the growth media. Nanoparticles of 70.07µg/ml mediated the release of metabolites which were >2.5 times than that released after digestion in 1N $HClO_4$ at 100°C for 1 hour. These observations on the yeast cell suggest that silver nanoparticles are potent cytotoxic agents and have a profound impact on their cell wall leading to altered diffusion across membranes and cell death.

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