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# The Toxic Effects of Silver Nanoparticles on Blood Mononuclear Cells

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## Abstract

**Background:** Nanoparticles have become one of the leading technologies over the past two years. The extensive use of nanoparticles has raised great concern about their occupational fate and biological effects. With an increase in the production and use of nanomaterial, it is more likely to get exposed to them occupationally and environmentally.

**Objective:** To assess the toxicity of silver nanoparticles on human mononuclear cells.

**Methods:** In this *in vitro* experimental study, suspensions of blood mononuclear cells from 10 young healthy men were incubated with 10-nm silver nanoparticles in different concentrations (range: 1–500 µg/mL) for 6 and 24 hours by MTT assay. Positive and negative controls were used for comparison.

**Results:** After 6 hours of exposure, 10.9% to 48.4% of the cells died. After 24 hours of exposure, the rate ranged from 56.8% to 86.3%. Regardless of the exposure time, the maximum cytotoxicity was observed at the concentration of 500 µg/mL of silver nanoparticles. By increasing the exposure time to 24 hours, the cytotoxicity of nanoparticles substantially increased at all concentrations. Cell death was significantly higher when compared to the controls ( $p < 0.01$ ).

**Conclusion:** Silver nanoparticles possess both time- and dose-dependent cytotoxicity and can thus be considered as very toxic for mononuclear cells.

**Keywords:** Leukocyte, mononuclear; Nanoparticles; Toxicity; Tetrazolium salts; Silver

## Introduction

Workplace is changing due to accelerating improvements in technology. As a consequence of the progress achieved, the safety, health and conditions of many workers have faced new dilemmas.

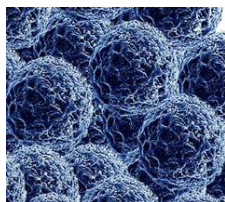
Nanoparticle chemicals that have at least one dimension in the range of 1–100 nm, have become one of the leading tech-

nologies over the past two years and are widely used in a variety of industries.<sup>1</sup>

Extensive application of various nanoparticles, however, raises concern about their biological effects. Various nanoparticles have had extensive applications in biomedical and biotechnological fields.<sup>2,3</sup> The effects of many particles have been well documented, but it is likely that the biological effects of nanoparticles are quite different from their microparticles.<sup>2,4</sup>

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Many studies indicated that systemic sclerosis, rheumatoid arthritis, lupus erythematosus, and chronic renal disease are caused by exposure to nanoparticles.<sup>5,6</sup>

Some studies have demonstrated that nanoparticles cause proinflammatory stimulation of endothelial cells and fibrogenesis.<sup>4,7-9</sup> The cytotoxicity, inflammatory, developmental toxicity, and genotoxicity characteristics of various nanoparticles (*eg*, silver nanoparticles) have also been demonstrated.<sup>7,10-16</sup>

*In vitro* toxicity assessment of nanoparticles has also become widely used in the recent studies; however, published data are still inadequate to earn a full understanding of the potential effects of the particles. Although the occupational and health impact of nanoparticles, due to their unique physical and chemical characteristics, are of great interest, less attention has been paid to the cytotoxic effects of nanoparticles on human cells. The present study was therefore conducted to assess the cytotoxicity of silver nanoparticles on human blood mononuclear cells.

## Materials and Methods

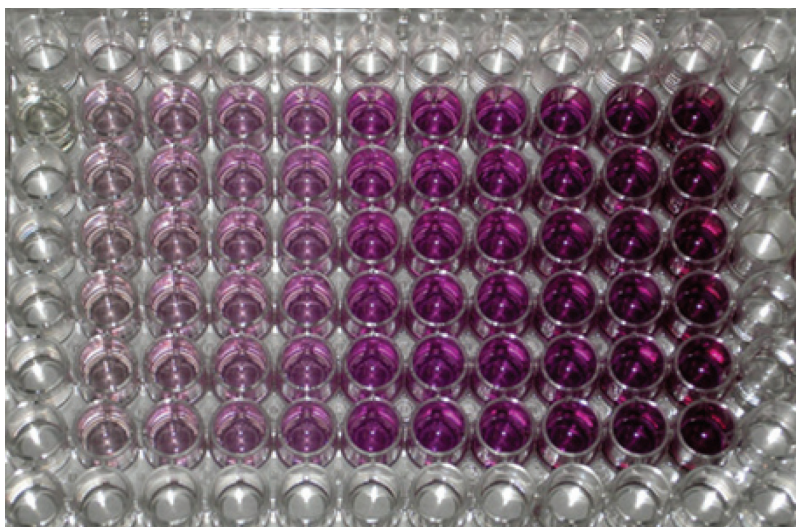
In this *in vitro* experimental study, we prepared different concentrations of 10-nm silver nanoparticles and suspensions of blood mononuclear cells from 10 healthy young men with a mean age of 25 years. All subjects gave a written informed consent prior to participation in the study.

The silver nanoparticles were purchased from Lolitech, Germany. The size of the nanoparticles were determined by scanning electron microscopy (Hitachi model S-2400, Japan) at 15 kV. First, nanoparticles were dried on an aluminum plate, and coated with a thin layer of gold by sputtering with low vacuum mode (Fig 1). Cells were taken from 10 volunteers. One mL of their venous blood was taken, placed in heparinized propylene plastic

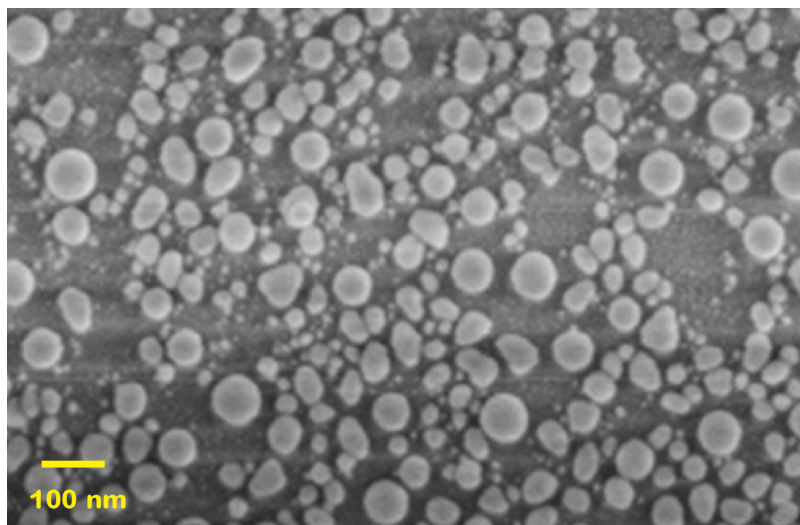
### TAKE-HOME MESSAGE

- Workplace is changing due to accelerating improvements in technology.
- Nanoparticles have become one of the leading technologies over the past two years and are widely used in a variety of industries.
- In this study, we assess the cytotoxicity of silver nanoparticles on human blood mononuclear cells.
- Cell vitality depends on time and concentration of the nanoparticles, with the lowest viability observed at a concentration of 500  $\mu\text{g}/\text{mL}$  of silver nanoparticles. Both lower and higher doses had lower toxicity.

tubes, and continuously shaken for 5 min. Then, 3 mL of Ficoll (70%) was gently added, and centrifuged at 5000 rpm for 15 min. So, the mononuclear cells were isolated and washed with physiological serum. Finally, the concentration of mononuclear cells was adjusted to  $5 \times 10^3$  cells/mL by RPMI-1640. The cells were harvested, and after three times washing with physiological serum, they were resuspended at  $5 \times 10^3$  cells/mL in RPMI-1640 medium. Suspensions of mononuclear cells (100  $\mu\text{L}$ , almost 500 cells) were distributed in a 96-well



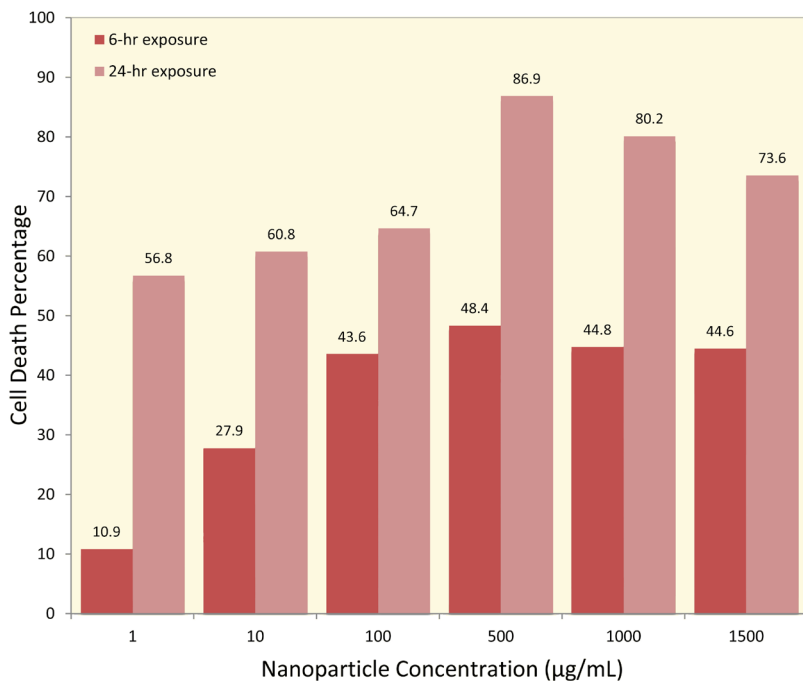
**Figure 1:** The 96-well microplate after addition of MTT



**Figure 2:** Electron microscopy of silver nanoparticles used in this study

plate, twice.

The cytotoxicity of nanoparticles was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, a yellow tetrazole) assay—a colorimetric assay for measuring the activity of enzymes



**Figure 3:** Mean of two readings of the cell death percentage after exposure to silver nanoparticles.

that reduce MTT.<sup>11</sup> Silver nanoparticles (3 g) were suspended directly in de-ionized water; concentrations of 3000 µg/mL were prepared and dispersed by ultrasonic vibration for 5 min. Similarly, suspension concentrations of 2, 20, 200, 1000, 3000, and 4000 µg/mL were prepared. Then, 100 µL of various concentrations of the nanoparticles were added and final concentrations of 1, 10, 100, 500, 1000, and 1500 µg/mL were prepared. After incubating mononuclear cells at 37 °C with 5% CO<sub>2</sub> for 3 hrs, 25 µL MTT solution (5 mg/mL) was added into each well. The plates were then placed in an incubator at 37 °C for 6 and 24 hrs. Then, 50 µL isopropanol 70% (Merck, Germany) was added to each well. Controls for this study included negative and positive controls made with RPMI-1640 medium and NaCl 0.9%, respectively. The absorbance was measured by a spectrophotometer (ELICO, India) at the wavelength of 490 nm. NOAEC (no observable adverse effect concentration) and IC<sub>50</sub> (50% inhibitory concentration) values were derived by graphical extrapolation based on the plotted absorbance data.

Cell death rate was expressed as mean±SD. SPSS® for Windows® ver 15 was used for data analysis. Means of two groups were compared by *Student's t* test for independent samples. A p value <0.05 was considered statistically significant.

## Results

The electron microscopy demonstrated that the structure of our 10-nm silver nanoparticles was amorphous; the nanoparticles were approximately spheres with diameters ranging from 10 to 50 nm (Fig 2).

The cell death rate was significantly (p<0.01) different in the treatment groups than the controls. The viability of MNCs treated with NaCl 0.9%, as the negative control, was 100%. The effect of various

concentrations of silver nanoparticles on the cell death after 6- and 24-hour exposure is presented in Figure 3. After six hours of exposure, 10.9% to 48.4% of the cells died. After 24 hours of exposure, the rate ranged from 56.8% to 86.3%. Regardless of the exposure time, the maximum cytotoxicity was observed at the concentration of 500 µg/mL of silver nanoparticles (Fig 3).

The NOAEC of silver nanoparticles was “not available” for both 6- and 24-hour exposures; *ie*, no observable adverse effects were seen at any concentrations and time exposure. IC<sub>50</sub> was also “not available” after six hours of exposure. However, it was 1 mg/mL after 24 hours—50% of the cells were killed after 24 hours of exposure.

## Discussion

In the current study, the effect of various doses of silver nanoparticles on healthy human mononuclear cells was evaluated. Previous studies on human were conducted on lung cancer cells and isolated cells.<sup>7,10-16</sup> To determine the cytotoxicity of nanoparticles, it is necessary, first, to assess their effect on normal cells.

Other researchers studied different methods, nanoparticles, concentrations, sizes, and other cells. Therefore, it is difficult to compare our results with others'. For both six and 24 hours of exposure, when mononuclear cells get into contact with nanoparticles, the highest toxicity was achieved at 500 µg/mL of silver nanoparticles. By increasing the exposure time, the cytotoxicity of nanoparticles increased substantially for all concentrations.

Our results are consistent with the findings of another study conducted on human skin fibroblasts where showed that the cell vitality depends on time and concentration of nanoparticles.<sup>7</sup> The results of this study are also in agreement with previously reported data showing that differ-

ent nanoparticles are cytotoxic to macrophages.<sup>14-17</sup> The preliminary results of the current study could be used for further studies on the cytotoxic effects of nanoparticles.

**Conflicts of Interest:** None declared.

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