Mutation Research 742 (2012) 61–65

Contents lists available at SciVerse ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Characterization of synthesized silver nanoparticles and assessment of its genotoxicity potentials using the alkaline comet assay

Natesan A.L. Flower[a], Balusamy Brabu[a], Murugan Revathy[b], Chandrasekaran Gopalakrishnan[a], Savarimuthu V.K. Raja[a], Siva S. Murugan[b,c], Tirukalikundram S. Kumaravel[b,c,*]

[a] Nanotechnology Research Center, SRM University, Chennai, India
[b] GLR Laboratories Private Limited, Madhavaram, Chennai, India
[c] GLR Laboratories Private Limited, Willingham, Cambridge, UK

ARTICLE INFO

Article history:
Received 10 June 2011
Received in revised form 10 October 2011
Accepted 3 December 2011
Available online 13 December 2011

Keywords:
Silver nanoparticle
Genotoxicity
Comet assay
Hydrogen peroxide
Free radicals

ABSTRACT

Nano-silver (Nano-Ag) particles were synthesized and then characterized using transmission electron microscopy (TEM) and X-ray diffractometry. TEM showed that Nano-Ag were spherical in shape and their size ranged from 40 to 60 nm. X-ray diffractometry indicated that the sample was crystalline and had a face centered cubic structure of pure silver. Genotoxicity of this Nano-Ag was evaluated in human peripheral blood cells using the alkaline comet assay. Results indicated that Nano-Ag (50 and 100 μg/mL) caused DNA damage following a 3 h treatment. Subsequently, a short treatment of 5 min also showed DNA damage. In conclusion, we have shown that the synthesized Nano-Ag induced DNA damage in human peripheral blood cells as detected by the alkaline comet assay. Results further indicated that treatment of cells with Nano-Ag in the presence of hydrogen peroxide did not induce any DNA damage.

1. Introduction

Biologically active nanomaterials are expected to open new avenues to fight and prevent disease using atomic scale tailoring of materials. Nanomaterials with biological activities have been used as antibacterials, drug delivery, radio-contrast agents and others. Among the most promising nanomaterials with antibacterial properties are metallic nanoparticles, which exhibit increased chemical activity due to their large surface to volume ratios and crystallographic surface structure. The antibacterial activity exhibited by silver in a range of studies [1–5] has resulted in the widespread use of nano-silver (Nano-Ag) in bedding, washing machines, water purification, toothpaste, shampoo and rinse, nipples and nursing bottles, fabrics, deodorants, filters, kitchen utensils, toys, and humidifiers [6], where the internal surface of the product is mixed or coated with germ-resistant Nano-Ag to prevent the growth of fungi and bacteria. Despite their widespread use, there is limited data on the safety, toxicology and exposure of Nano-Ag, especially those relating to its interaction with the DNA.

We synthesized Nano-Ag particles and characterized those using transmission electron microscope (TEM) and X-ray diffraction studies. Genotoxic potentials of these Nano-Ag particles were investigated using the alkaline comet assay [7,8]. Since nanoparticles have a large surface area to mass ratio, they are highly reactive and when they are exposed to aqueous media or cellular components, free radicals will be released. The released free radicals cause DNA damage which can be detected using the comet assay.

Release of free radicals by nanoparticles is well documented [9–11]. AshaRani et al. [12] and Piao et al. [13] and references cited therein have clearly demonstrated that Nano-Ag releases free radicals, that causes damage to DNA and other cellular components.

Furthermore, it is speculated that due to their high reactivity, these nanoparticles may also react with or trap other free radicals and may thus quench their activities. It is therefore highly possible that nanoparticles may exhibit dual nature in relation to generation as well as quenching of free radicals. In this manuscript, we also investigated whether or not Nano-Ag quenched free radicals released by hydrogen peroxide (H_2O_2), by determining if Nano-Ag prevented H_2O_2 induced DNA damage.

2. Materials and methods

2.1. Nano-silver synthesis and characterization

Nano-Ag were synthesized by reduction of silver nitrate ($AgNO_3$; RFCL Limited, Delhi, India) using sodium borohydrate ($NaBH_4$; SISCO Research Laboratories, Mumbai, India) as a reducing agent. An aqueous solution of 200 mM $NaBH_4$ in deionized (DI) water was added drop-by-drop to an aqueous solution of 100 mM $AgNO_3$ kept

* Corresponding author at: GLR Laboratories Private Limited, Plot 18-19, Ganesh Nagar, Asisi Nagar, Madhavaram, Chennai 600 060, India. Tel.: +91 9500064248.
E-mail address: kumaravelts@glrlabs.com (T.S. Kumaravel).

1383-5718/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrgentox.2011.12.003

in an ice bath under constant stirring using a magnetic stirrer. The grey solutions formed were then stirred for approximately 5 min [14]. The reaction mixture was then centrifuged, washed several times with DI water and finally with alcoholic water to dissolve any impurities present. The Nano-Ag particles were then dried at room temperature.

The shape and the size of the nanoparticles were characterized by transmission electron microscopy (TEM) using JEOL 2010 high resolution electron microscope operated at an accelerating voltage of 200 kV. The sample was prepared by placing a drop of Nano-Ag sample on to a copper grid and examined under TEM.

The crystal structure and the phase purity of the synthesized Nano-Ag particles were examined by X-ray diffractometry using PANalytical's X'Pert PRO Powder XRD with filtered CuKα (λ = 1.5406 Å) at a load of 45 kV tension and 40 mA current, at 2θ ranging 30–90° with a step size of 0.05° at a scan time of 3 s per step.

2.2. Alkaline comet assay

Alkaline comet assay was performed on peripheral blood samples obtained from apparently healthy human volunteers [7,15–17]. Briefly, heparinized peripheral blood samples were collected, mixed with phosphate buffer saline (PBS; 9 mL of PBS for each mL of blood), and treated with appropriate agents for the required durations. All treatments were carried out in 15 mL centrifuge tubes, with a final volume of 10 mL. During the treatment period, the tubes were regularly shaken in a rotating platform to prevent aggregation of cells at the bottom of the centrifuge tube. Since Nano-Ag particles are insoluble in commonly used solvents, pre-weighed quantities of Nano-Ag were added directly to the treatment cultures. For H_2O_2 treatments, the cells were centrifuged and resuspended in appropriate concentrations of H_2O_2 in a final volume of 10 mL.

After treatments, the tubes were spun and cell pellets and collected. The slides were prepared by mixing 5–10 μL of cell suspension on to 100 μL of 0.7% low melting point agarose and layered on to microscope slides. The microscope slides were then placed in lysis solution (2.5 M sodium chloride (NaCl), 100 mM ethylene diamine tetra acetic acid (EDTA), 10 mM Trizma base, 10% dimethyl sulfoxide (DMSO) and 1% Triton X-100) at 4 °C for a minimum period of 1 h to remove cellular proteins. The slides were then immersed in electrophoresis buffer [300 mM NaOH, 1 mM EDTA (pH > 13)] for unwinding the DNA for 20 min, and subjected to electrophoresis (18 V; 100 mA) for 30 min. Following electrophoresis, the slides were neutralized with PBS for 10–15 min and dried overnight at room temperature. Neutralized and dehydrated slides were stained with ethidium bromide (2 ng/mL) before scoring. The slides were scored using fluorescence microscope fitted with appropriate filters.

Around 1000 cells were scored for each sample and DNA damage classified using the visual scoring method described by Collins et al. [18]. One-thousand cells on each slide were scored visually as belonging to one of five classes according to tail intensity and given a value of 0, 1, 2, 3, or 4 (from undamaged 0, to maximally damaged, 4). Thus, the total score for 1000 cells could range from 0 (all undamaged) to 4000 (all maximally damaged). The arbitrary values for DNA migrations were calculated as follows:

$$\text{DNA migration} = (0 \text{ times } N_0) + (1 \text{ times } N_1) + (2 \text{ times } N_2) + (3 \text{ times } N_3) + (4 \text{ times } N_4)$$

where N_0, N_1, N_2, N_3 and N_4 are the number of cells in grades 0, 1, 2, 3 and 4 of DNA migration.

A separate set of slides were prepared and treated in parallel along with alkaline comet assay experiments. These additional slides were used to assess cytotoxicity. Briefly, these slides were lysed for 1 h, neutralized with PBS, stained with 2 ng/mL of ethidium bromide and scored for number of highly diffused cells [15,19–21]. The percentage of highly diffused cells on a slide indicates the percentage of cells with cytotoxicity.

2.3. Treatment groups

The first experiment was designed to investigate whether or not Nano-Ag induced DNA damage to peripheral blood cells. In this experiment, the peripheral blood cells were treated with 50 or 100 μg/mL of Nano-Ag particles. In the absence of any standard guidelines for assessing genotoxicity of nanoparticles, the doses selected for this study were based on the current guidelines and recommendations for bulk chemicals [22]. Concentrations higher than 100 μg/mL resulted in cytotoxicity and could potentially affect genotoxicity results. Therefore, 100 μg/mL was selected as a suitable top dose for this study [17]. AshaRani et al. [12] have also shown that Nano-Ag exhibits dose related increase in DNA migration on comet assay from 25 to 400 μg/mL.

Table 1 summarizes the various experimental conditions used to investigate the genotoxicity of Nano-Ag particles.

Based on standard recommendations for in vitro genotoxicity testing [17,22], we carried out comet assay experiments following a 3 h exposure of cells to Nano-Ag. Comet assay following a 3 h treatment is expected to pick up the majority of the types of DNA damage induced by Nano-Ag [15,17].

The second set of comet assay experiments were designed to investigate the interaction of Nano-Ag with H_2O_2. In these experiments, cells were treated with Nano-Ag, Nano-Ag plus H_2O_2 or H_2O_2 alone for 5 min. A short time course treatment was selected based on comet assay experiments with H_2O_2. H_2O_2 releases free radicals, which produces DNA strand breaks and oxidative damage. The kinetics of DNA strand break repair is extremely fast and can only be detected using a short time course comet assay experiments [23–25]. Based on our previous experience and that of others, 250 μM of H_2O_2 gave a clear increase in the DNA migration on the comet assay and followed a short time course kinetics that was very consistent with DNA strand break repair. We therefore used 250 μM of H_2O_2 in this study. Table 2 summarizes the various experimental conditions used to investigate the genotoxicity of Nano-Ag particles.

Table 1
Experimental conditions used to the investigate genotoxicity of nano-silver particles.

Treatments	Duration of treatment	Number of replicates
Untreated control	3 h	3
Nano-Ag (50 μg/mL)	3 h	3
Nano-Ag (100 μg/mL)	3 h	3
Untreated control	5 min	3
Nano-Ag (50 μg/mL)	5 min	3
Nano-Ag (100 μg/mL)	5 min	3

3. Results and discussion

Sufficient quantities of Nano-Ag was synthesized and characterized. All experiments were carried out on a single batch of the Nano-Ag.

3.1. Characterization of silver nanoparticle

Fig. 1A shows the TEM image of the Nano-Ag that are almost spherical in shape and slightly agglomerated with the size ranging from 40 to 60 nm with the scale bar of 20 nm. Fig. 1B shows the X-ray diffraction pattern of the silver nanoparticles. All the prominent peaks at respective 2θ positions 38.25°, 44.442°, 64.62°, 77.58° and 81.70° which are indexed to (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) planes, indicate that the sample is crystalline and has a cubic structure of pure silver (JCPDS # 87-0597). Cubic system is conferred with the space group Fm-3m with the calculated unit cell parameters ($a = b = c = 4.088$ Å). A mathematical analysis of the Bragg's peaks was done to calculate the particle size using Scherrer formula, $D = k\lambda/\beta\cos\theta$, where D is the crystalline size, k is a constant ($k = 0.94$, assuming the grain to be spherical), λ is the wavelength of the incident X-ray radiation (λ = 1.5406 Å), β is the intrinsic full width at half maximum of the peak expressed in radians (obtained after the correction of instrumental error), θ is the Bragg's diffraction angle of the respective diffraction peak.

3.2. Comet assay

Alkaline comet assay was performed to investigate whether or not Nano-Ag induced DNA damage to peripheral blood cells.

Table 2
Experimental conditions used to investigate the interaction of nano-silver particles with H_2O_2.

Treatments	Duration of treatment	Number of replicates
Untreated control	5 min	3
Nano-Ag (50 μg/mL)	5 min	3
Nano-Ag (100 μg/mL)	5 min	3
Nano-Ag (50 μg/mL PLUS H_2O_2[a,b])	5 min	3
Nano-Ag (100 μg/mL PLUS H_2O_2[a,b])	5 min	3
H_2O_2[b]	5 min	3

[a] Sequence of adding H_2O_2 and Nano-Ag did not alter the results in preliminary trials. Therefore, in this study we added Nano-Ag first followed immediately by H_2O_2.
[b] Concentration of H_2O_2 used was 250 μM.

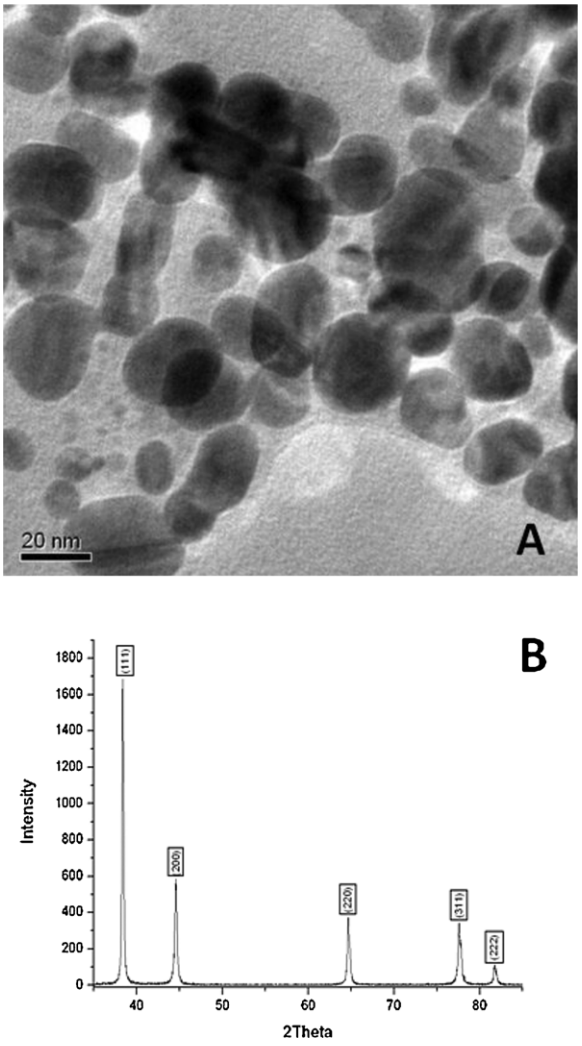

Fig. 1. Transmission electron microscopic (TEM) image (A) and X-ray diffraction (XRD) pattern (B) of the synthesized silver nanoparticles.

Treatment of cells with 50 and 100 μg/mL of Nano-Ag for 3 h resulted in a significant increase in DNA migration (1399 ± 217 and 1496 ± 217 arbitrary values, respectively) compared to concurrent untreated control cells (282 ± 99 arbitrary values; see Fig. 2). This

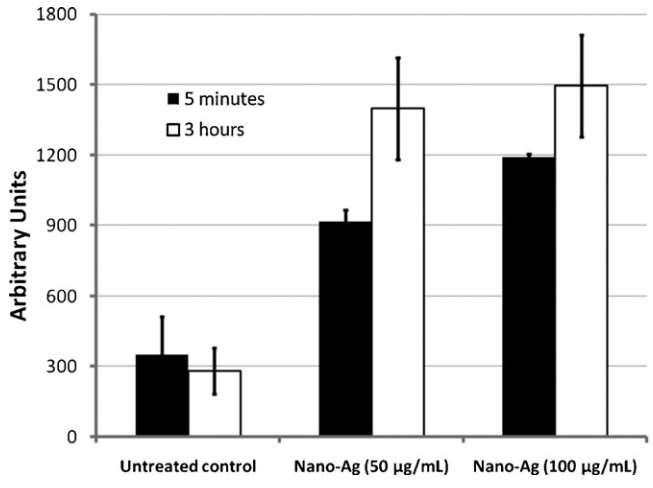

Fig. 2. DNA migration (in arbitrary units) following treatment of human peripheral blood cells with silver nanoparticles (50 or 100 μg/mL) for either 5 min or 3 h.

suggests that Nano-Ag produced DNA damage in peripheral blood cells. Fig. 3 gives the distribution of cells in the various DNA damage categories. The majority of the cells in the untreated control group fell in category 0, with very few cells in the other categories. Whereas, following Nano-Ag treatment, the cells shifted from category 0 to other categories, confirming that Nano-Ag induced DNA migration. It should be noted that increase in DNA migration can also result from cytotoxicity, apoptosis or necrosis. The number of highly diffused cells from the cytotoxicity assessments on these slides ranged between 1 and 4%. It is therefore highly unlikely that cytotoxicity/necrosis events contributed to the DNA migration on these slides. Furthermore, since DNA fragmentation following apoptosis is not an immediate consequence and it takes several hours to develop, it is unlikely that apoptosis contributed to the DNA migration seen on these slides. Therefore, it is concluded that Nano-Ag induced DNA damage in peripheral blood cells. It should also be noted that Nano-Ag induced some cytotoxicity, but is not further investigated in this paper.

Alkaline comet assay experiments following a 3-h treatment detect many types of DNA damage, i.e. strand breaks, alkali labile sites, and incomplete excision repair sites [15–17]. It is therefore possible that Nano-Ag induces some or all of these types of DNA damage. The mechanism of action by which Nano-Ag causes DNA damage is not fully understood from these results. One of the plausible mechanisms by which Nano-Ag may cause DNA damage is via the generation of free radicals. Nanosilver has been shown to induce a variety of toxic effects, including generation of reactive oxygen species and oxidative stress [26].

Free radicals are extremely reactive and thus short lived. Consequently, free radicals are not easily amenable to direct assay and free radical activity is usually assessed by indirect methods such as measurement of the various end products of reactions with lipids, proteins and DNA. Free radicals produce DNA strand breaks that can be detected using the comet assay. Since Nano-Ag is expected to release free radicals, we also investigated the DNA damaging effects of Nano-Ag following a 5 min treatment.

Treatment of cells with 50 and 100 μg/mL of Nano-Ag for 5 minutes resulted in a statistically significant increase in DNA migration (915 ± 52 and 1191 ± 14 arbitrary values, respectively) compared to concurrent untreated control cells (352 ± 162 arbitrary values; see Fig. 2). As discussed in previous paragraph, cytotoxicity, necrosis and apoptosis is unlikely to affect the comet assay results in these experiments. The chances of Nano-Ag producing damage to DNA by direct interaction are also highly unlikely following a 5 min treatment. Therefore the most likely mechanism by which Nano-Ag produces DNA damage following a short treatment regime is via the generation of free radicals. Generation of free radicals by nanoparticles is well documented [13,27–29]. It should be noted that Nano-Ag induced reactive oxygen species generation and reduction of glutathione activity in human Chang liver cells [13]. The generated reactive oxygen species by Nano-Ag resulted in damage to various cellular components, DNA breaks, lipid membrane peroxidation, and protein carbonylation [13].

Since nanoparticles have a large surface area to mass ratio, they are highly reactive. It is therefore possible that nanoparticles may adsorb free radicals on to their surface in addition to releasing free radicals. Such dual nature of gold nanoparticles has been demonstrated by Ionita et al. [30]. In this paper, we investigated if Nano-Ag quenches the free radicals released from H_2O_2. In these experiments, cells were treated with Nano-Ag, Nano-Ag plus H_2O_2 or H_2O_2 alone for 5 min. The results from these experiments are summarized in Fig. 4.

Treatment of cells with Nano-Ag (50 and 100 μg/mL) or H_2O_2 (250 μM) resulted in a significant increase in DNA damage (915 ± 52 and 1191 ± 14 arbitrary units for 50 or 100 μg/mL Nano-Ag; or 1055 ± 178 arbitrary units for H_2O_2). However,

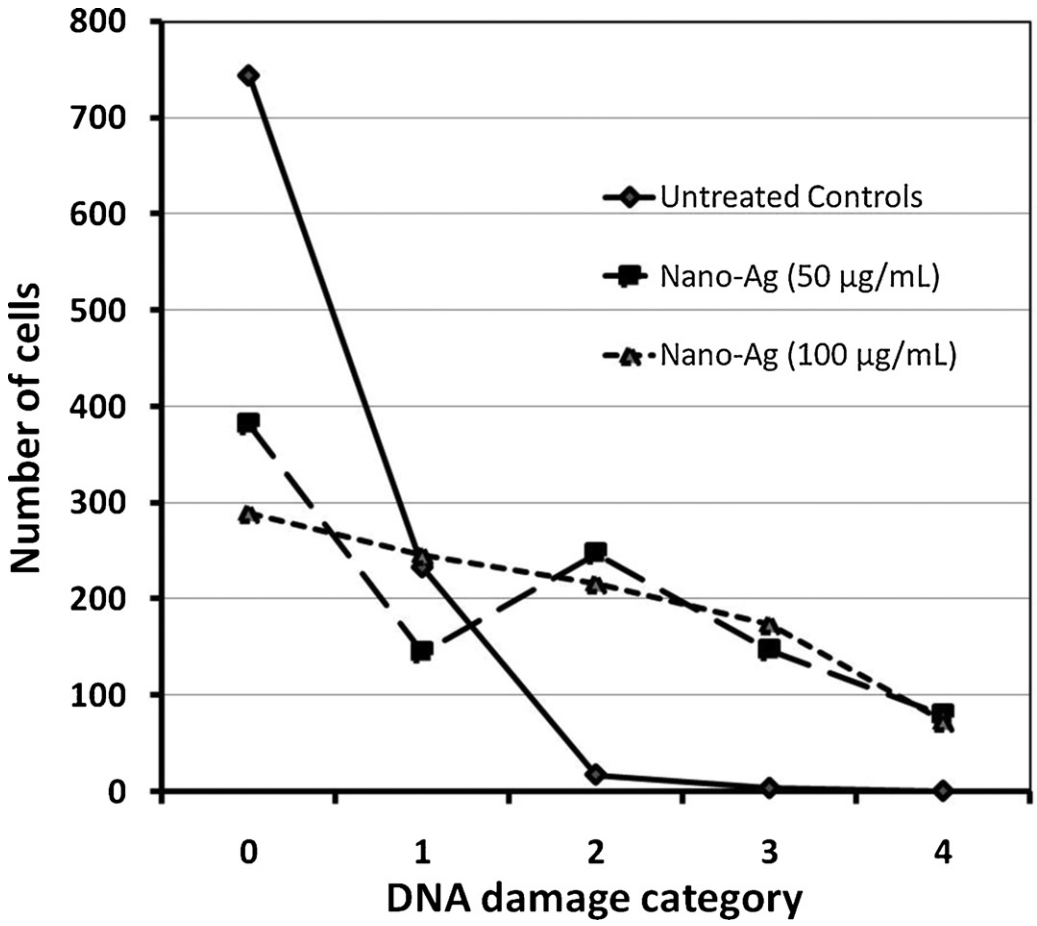

Fig. 3. Cell distribution in various DNA damage categories following treatment of human peripheral blood cells with silver nanoparticles (50 or 100 μg/mL) for 3 h.

treatment of cells with both Nano-Ag (50 or 100 μg/mL) and H_2O_2 (250 μM) resulted in very little or no DNA damage (453 ± 188 or 510 ± 130 arbitrary units for 50 or 100 μg/mL Nano-Ag) compared to untreated cells (352 ± 162 arbitrary units; see Fig. 4). Sequence of adding H_2O_2 and Nano-Ag did not alter the results in preliminary trials. Therefore, in this study we added Nano-Ag first followed immediately by H_2O_2. These results indicated that Nano-Ag may quench the free radicals produced by H_2O_2. Also in the process, Nano-Ag may lose its ability to release free radicals, the mechanism of which is unknown. Thus Nano-Ag may exhibit dual behavior both as generators and scavengers for free radicals, very similar to that reported for gold nanoparticles [30].

To conclude, we have shown that the synthesized Nano-Ag induced DNA damage in human peripheral blood cells as detected by the alkaline comet assay. Results further indicated that treatment of cells with Nano-Ag in the presence of hydrogen peroxide did not induce any DNA damage.

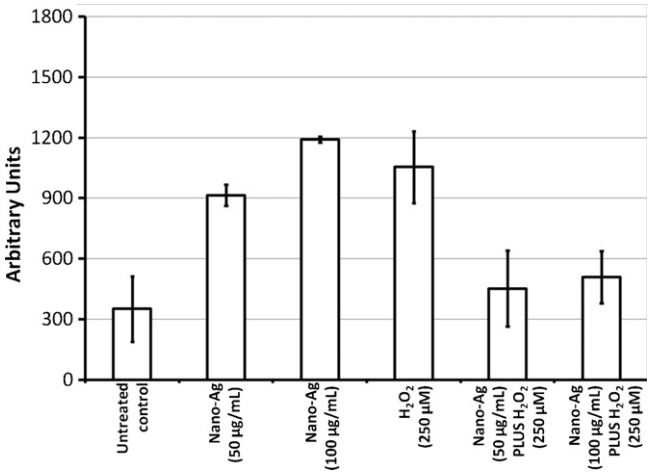

Fig. 4. DNA migration (in arbitrary units) following treatment of human peripheral blood cells with silver nanoparticles (50 or 100 μg/mL) and/or H_2O_2 (250 μM) for 5 min.

Conflict of interest statement

None declared.

Acknowledgments

The authors, ALF, BB, CG and SVKR would like to thank Department of Biotechnology (DBT), Ministry of Science and Technology, Govt. of India for their financial support.

References

[1] I. Sondi, B. Salopek-Sondi, Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria, J. Colloid Interface Sci. 275 (2004) 177–182.
[2] K.H. Cho, J.E. Park, T. Osaka, S.G. Park, The study of antimicrobial activity and preservative effects of nanosilver ingredient, Electrochim. Acta 51 (2005) 956–960.
[3] J.H. Ji, G.N. Bae, S.H. Yun, J.H. Jung, H.S. Noh, S.S. Kim, Evaluation of a silver nanoparticle generator using a small ceramic heater for inactivation of *S. epidermidis* bioaerosols, Aerosol Sci. Technol. 41 (2007) 786–793.

[4] J.R. Morones, J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, M.J. Yacaman, The bactericidal effect of silver nanoparticles, Nanotechnology 16 (2005) 2346–2353.
[5] P. Li, J. Li, C. Wu, Q. Wu, J. Li, Synergistic antibacterial effects of β-lactam antibiotic combined with silver nanoparticles, Nanotechnology 16 (2005) 1912–1917.
[6] J.H. Sung, J.H. Ji, J.D. Park, J.U. Yoon, D.S. Kim, K.S. Jeon, M.Y. Song, J. Jeong, B.S. Han, J.H. Han, Y.H. Chung, H.K. Chang, J.H. Lee, M.H. Cho, B.J. Kelman, I.J. Yu, Subchronic inhalation toxicity of silver nanoparticles, Toxicol. Sci. 108 (2009) 452–461.
[7] Comet Assay Interest Group website. <www.cometassay.com>, (accessed April 2011).
[8] M. Ahamed, M. Karns, M. Goodson, J. Rowe, S.M. Hussain, J.J. Schlager, Y. Hong, DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells, Toxicol. Appl. Pharmacol. 233 (2008) 404–410.
[9] N.J. Dodd, A.N. Jha, Titanium dioxide induced cell damage: a proposed role of the carboxyl radical, Mutat. Res. 660 (2009) 79–82.
[10] W.F. Vevers, A.N. Jha, Genotoxic and cytotoxic potential of titanium dioxide (TiO_2) nanoparticles on fish cells in vitro, Ecotoxicology 17 (2008) 410–420.
[11] N.J. Dodd, A.N. Jha, Photoexcitation of aqueous suspensions of titanium dioxide nanoparticles: an electron spin resonance spin trapping study of potentially oxidative reactions, Photochem. Photobiol. 87 (2011) 632–640.
[12] P.V. AshaRani, G. Low Kah Mun, M.P. Hande, S. Valiyaveettil, Cytotoxicity and genotoxicity of silver nanoparticles in human cells, ACS Nano 3 (2009) 279–290.
[13] M.J. Piao, K.A. Kang, I.K. Lee, H.S. Kim, S. Kim, J.Y. Choi, J. Choi, J.W. Hyun, Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis, Toxicol. Lett. 201 (2011) 92–100.
[14] I. Dragieva, S. Stoeva, P. Stoimenov, E. Pavlikianov, K. Klabunde, Complex formation in solutions for chemical synthesis of nanoscaled particles prepared by borohydride reduction process, Nanostruct. Mater. 12 (1999) 267–270.
[15] T.S. Kumaravel, A.N. Jha, Reliable comet assay measurements for detecting DNA damage induced by ionising radiation and chemicals, Mutat. Res. 605 (2006) 7–16.
[16] T.S. Kumaravel, B. Vilhar, S.P. Faux, A.N. Jha, Comet assay measurements: a perspective, Cell Biol. Toxicol. 25 (2009) 53–64.
[17] R.R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu, Y.F. Sasaki, Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutagen. 35 (2000) 206–221.
[18] A.R. Collins, M. Dusinska, A. Horska, Detection of alkylation damage in human lymphocyte DNA with the comet assay, Acta Biochim. Pol. 48 (2001) 611–614.
[19] D.W. Fairbairn, P.L. Olive, K.L. O'Neill, The comet assay: a comprehensive review, Mutat. Res. 339 (1995) 37–59.
[20] A.R. Collins, A.A. Oscoz, G. Brunborg, I. Gaivao, L. Giovannelli, M. Kruszewski, C.C. Smith, R. Stetina, The comet assay: topical issues, Mutagenesis 23 (2008) 143–151.
[21] P. Sestili, The fast-halo assay for the assessment of DNA damage at the single-cell level, Methods Mol. Biol. 521 (2009) 517–533.
[22] OECD guidelines for the testing of chemicals. Section 4. Health effects.
[23] E. Horváthová, D. Slamenová, L. Hlincíková, T.K. Mandal, A. Gábelová, A.R. Collins, The nature and origin of DNA single-strand breaks determined with the comet assay, Mutat. Res. 409 (1998) 163–171.
[24] E. Horváthová, D. Slamenová, A. Gábelová, Use of single cell gel electrophoresis (comet assay) modifications for analysis of DNA damage, Gen. Physiol. Biophys. 18 (1999) 70–74.
[25] F. Azevedo, F. Marques, H. Fokt, R. Oliveira, B. Johansson, Measuring oxidative DNA damage and DNA repair using the yeast comet assay, Yeast 28 (2011) 55–61.
[26] W. Liu, Y. Wu, C. Wang, H.C. Li, T. Wang, C.Y. Liao, L. Cui, Q.F. Zhou, B. Yan, G.B. Jiang, Impact of silver nanoparticles on human cells: effect of particle size, Nanotoxicology 4 (2010) 319–330.
[27] T. Prevenslik, Nanoparticle induced DNA damage. <www.nanoqed.org>, 2008 (accessed May 2011).
[28] K. Donaldson, A.P. Craig, P.F.S. Roel, Possible genotoxic mechanisms of nanoparticles: criteria for improved test strategies, Nanotechnology 4 (2010) 414–420.
[29] J.F. Reeves, S.J. Davies, N.J. Dodd, A.N. Jha, Hydroxyl radicals (*OH) are associated with titanium dioxide (TiO_2) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells, Mutat. Res. 640 (2008) 113–122.
[30] P. Ionita, F. Spafiu, C. Ghica, Dual behavior of gold nanoparticles, as generators and scavengers for free radicals, J. Mater. Sci. 43 (2008) 6571–6574.