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Antibacterial Effects of Nanosil on Some Gram Positive and Gram Negative Bacteria

[1]*Maryam Ekhtelat and* [2]*Ali Fazlara*

[1]Department of Microbiology, Faculty of Veterinary Medicine,
Shahid Chamran University of Ahvaz, Iran
[2]Department of Food Hygiene, Faculty of Veterinary Medicine,
Shahid Chamran University of Ahvaz, Iran

Abstract: The antibacterial properties of silver are well known and silver nanoparticles are now incorporated into a wide variety of consumer products for microbial control. In attention to extent uses of disinfectants, it is necessary to evaluate and certificate the efficacy of disinfectants and employing the minimum effective dosages. In this research the antibacterial effects of a disinfectant solution (Nanosil) of hydrogen peroxide and nanosilver particle were evaluated on six important foodborne pathogens including three Gram positive (*Staphylococcus aureus, Listeria monocytogenes* and *Bacillus cereus*) and three Gram negative bacteria (*Salmonella typhimurium, Escherichia coli* and *Pseudomonas aeruginosa*). According to the results after three times conducing experiments, *Salmonella typhimurium* and *Listeria monocytogenes* were the most sensitive and resistant studied bacteria with MIC and MBC equal to 0/02 and 0/025% and 0/08 and 0/1% respectively. In attention to obtained results, the used disinfectant has good antibacterial effects and also was more effective on Gram negative than Gram positive bacteria. Further studies are needed to verify if the bacteria develop resistance toward the nanoparticles and examine the cytotoxicity of nanosilvers towards human cells.

Key words: Disinfectant · Hydrogen peroxide · Nanosilver · Bacteria

INTRODUCTION

Human beings are often infected by microorganisms such as bacteria, moulds, yeasts and viruses in the living environment. Research using antibacterial material containing various natural and inorganic substances has been reported in literature [1]. Medicinal and preservative properties of silver have been known for over 2,000 years [2]. Silver ion has been known as a metal ion that exhibits anti-mould, anti-microbial and anti-algal properties for a long time[3], for example in the current clinical use for the treatment of various wounds, including burns [4-6], chronic ulcers [7] and pemphigus [8]. It has been also used in modulation of cytokine production [9], suppression of the expression of tumor necrosis factor-α (TNF-α) and interleukin-12 (IL-12) and induction of apoptosis of inflammatory cells [10].

Nanotechnology is the most promising field for generating new applications in medicine. The most prominent nanoproduct is nanosilver. Nanosilver particles are generally smaller than 100 nm and contain 20-15000 silver atoms [11, 12]. Due to its strong antibacterial activity, the current silver nanoparticles are widely used as antibacterial/antifungal agents in a diverse range of consumer products like air sanitizer sprays, detergents, toothpastes, air filters, food storage containers, etc. [13]. Silver nanoparticles are also employed in products for water disinfection and food stabilization, such as plastics used to fabricate food containers, refrigerator surfaces and storage bags, under the pretext of preserving foods longer by inhibiting microorganism growth [12, 14].

The toxicity of nanosilvers to bacteria is greatly influenced by silver nanoparticles size, shape [15] and concentration of active free silver ions [16]. Nanotechnology has facilitated the production of smaller silver particles with increasingly large surface area-to-volume ratios, greater efficacy against bacteria [17, 18] and most importantly, lower toxicity to humans

Corresponding Author: Maryam Ekhtelat, Department of Microbiology,
Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran.
Tel: +989161130232, Fax: +986113333936, E-mail: maryam_ekhtelat@yahoo.com.

[19]. When nanosilver particles are small and release many silver ions, the antibacterial activity is dominated [20]. Data on silver nanoparticles anti-microbial activity suggest that silver nanoparticles exert their anti-microbial effects through the interaction with proteins thiol groups, which leads to the inactivation of the proteins [1, 21]. Also, it is revealed that bulk silver in an oxygen-charged aqueous media catalyzes the complete destructive oxidation of microorganisms [22]. It is believed that silver ions interact with three main components of the bacterial cell to produce the bactericidal effect: the peptidoglycan cell wall [23] and the plasma membrane [24]; bacterial (cytoplasmic) DNA [25, 26]; and bacterial proteins [23, 25], especially enzymes involved in the vital cellular processes such as the electron transport chain. Nanosilver has intrinsic antibacterial properties that do not depend on the elution of silver ions. Nanosilver extensively interacts with bacterial cell walls and it has been proposed that it causes its lysis [23, 27]. Silver ions are also able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur-and phosphorus-containing compounds such as DNA [16].

Antibiotic multiresistant bacteria have become an important problem but bacterial resistance to elemental silver is extremely rare [28], emphasizing the presence of multiple bactericidal mechanisms acting in synergy [29]. The traditional belief is that except some minor problems, silver is relatively non-toxic to mammalian cells. Silver poisoning only occurs among workers who have chronic history of silver exposure [12]. Thus, long-term exposure to silver nanoparticles in aerosols or food packaging might pose toxicity problems in humans [29]. Moreover, it has been demonstrated that, in low concentrations, silver is non toxic to human cells [15, 30]. However, systemic toxicity of ingested nanosilver is scarcely seen. This situation may probably be accounted for by the presence in the gastrointestinal tract of a complex mixture of compounds including ingested food, digestive enzymes, electrolytes and intestinal microbial flora, etc. [31].

Hydrogen peroxide is a natural disinfectant. Hydrogen peroxide is created from oxygen and hydrogen molecules. Its oxygen is responsible for the killing of germs. When hydrogen peroxide is applied to a surface, free oxygen radicals are released. These radicals create oxidation, a chemical process in which oxygen combines with another substance to break down or change the function of the molecules and through oxidation, the bacteria will decompose. Adding peewee amount of hydrogen peroxide to nanosilver was carried on in order to enhance both benefits associated with silver and hydrogen peroxide [32].

The ideal properties of an antibacterial nanoparticle include prolonged activity, high levels of bactericidal and bacteriostatic activity, ability to act against a wide spectrum of bacteria, biocompatibility and low *in vivo* toxicity. In addition, its production should be inexpensive, reproducible and disposable to minimize environmental damage [29]. In recent years, extensive studies have been undertaken on the use of antimicrobial properties of silver.

The aim of this study was to evaluate the antibacterial activity of commercial Nanosil by determination of its minimum inhibitory concentrations (MIC) and minimal bactericidal concentration (MBC) against some important foodborne Gram positive (*Staphylococcus aureus, Listeria monocytogenes* and *Bacillus cereus*) as well as Gram-negative (*Salmonella typhimurium, Escherichia coli* and *Pseudomonas aeruginosa*) pathogens.

MATERIALS AND METHODS

Materials, Bacterial Strains and Culture Conditions: Commercial Nanosil (containing 50% Hydrogen peroxide and 0.05% nanosilver) was bought from the market. *Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes* (ATCC 19118) and *Bacillus cereus* (ATCC 11778) as Gram-positive and *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922) and *Pseudomonase aeruginosa* (ATCC 25619) as Gram-negative bacteria were used. For the antimicrobial activity measurement, first of all active cultures were generated by inoculating single colony of each bacterium into 5 ml sterile nutrient broth (Merck) and incubated at 37°C for 24 h. Freshly synchronized cultures of bacterial strains form initial inoculums were prepared after two times overnight cultures (24 h) of each bacterium by successively transferring 100 µl of the vegetative cells into tryptic soy broth (TSB, Merck). Then the optical densities of the active freshly synchronized cultures were adjusted at 600 nm to a cell density equivalent to 10^6 CFU/mL [33, 34].

Measurement of Antibacterial Activity of Nanosil: In order to evaluate the antimicrobial activity, Nanosil stock solution was serially diluted with TSB to obtain test solutions containing required concentrations (from 0/005% until 0/6%) of Nanosil. Then aliquots of 1ml

from each bacterium (about 10^6 CFU/mL) were added to the tubes containing 1 mL of the prepared concentrations of Nanosil solutions separately. At the end of contact time (24 h incubation at 37°C), the tubes were examined for growth and then the amount of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The MIC is defined as the lowest concentration of Nanosil showing no visible bacterial growth after the incubation time [35, 36]. Then in order to measure bacterial viability, 100 µL from tubes that showed no visible growth were spread on the standard plate count agar plates and incubated for 24 h at 37°C. The MBC is defined as the lowest concentration of Nanosil at which no visible bacterial growth (colony) is observed after sub-culturing [35]. All the above mentioned assays were run three times in parallel with negative and positive controls.

RESULTS

According to the obtained results as shown in Table 1, *Salmonella typhimurium* showed the maximum sensitivity to Nanosil among the studied bacterial species with MIC and MBC equal to 0/02 and 0/025% respectively. *Pseudomonas aeruginosa* was proved to be the second sensitive Gram-negative microorganism with MIC and MBC equal to 0/025 and 0/03% respectively. Table 2 shows that *Listeria monocytogenes* was the most resistant bacteria in this study with MIC and MBC equal to 0/08 and 0/1%, respectively. The results of present study showed that Nanosil in 0/1% (1000 mg/L) concentration was able enough to kill all target bacteria during 24 h contact time.

Table 1: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Nanosil for the Gram-negative bacteria

Gram-negative bacteria	*S. typhimurium*	*E. coli*	*P. aeruginosa*
MIC	0/02%	0/03%	0/025%
MBC	0/025%	0/04%	0/03%

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Nanosil for the Gram-positive bacteria

Gram-positive bacteria	*S. aureus*	*L. monocytogenes*	*B. cereus*
MIC	0/04%	0/08%	0/035%
MBC	0/06%	0/1%	0/05%

DISCUSSION

The Environmental Protection Agency (EPA) [37, 38] has approved the use of hydrogen peroxide as a sanitizer. This active ingredient prevents and controls bacteria and fungi that cause serious diseases. Very little is known regarding the toxic properties of hydrogen peroxide. Therefore, caution is advised in the use of hydrogen peroxide until levels which are safe to host tissues are established. In the interim, the possibility of reducing the toxicity and increasing the efficacy of hydrogen peroxide by combined or targeted chemotherapeutic strategies can be explored [32].

In this study, the combination of hydrogen peroxide and nanosilver (Nanosil) was evaluated for their antibacterial activity against Gram positive and Gram negative bacteria. Nanosilver stabilized with hydrogen peroxide showed strong antibacterial activity. This combination relates primarily to a novel process for the manufacture of silver oxide nano materials as highly effective and useful antibacterial agents in the treatment of wounds, preservation of crops and food protection and other similar applications because of its wide spectrum of activity [39].

In present work, the antibacterial effect of Nanosil on some important foodborne bacterial pathogens was investigated. According to obtained results, the studied Gram negative bacteria were more sensitive than Gram positive ones. Many studies have investigated Nanosil and its influence on various microorganisms in different environments; Nabizadeh *et al.* [40] reported the application of Nanosil containing hydrogen peroxide and silver ions in disinfecting swimming pool water and its environment. Heterotrophic plate count, thermotolerant coliforms, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were monitored as the target microorganisms. According to their results, *Pseudomonas aeruginosa* (ATCC 9027) showed the maximum sensitivity to Nanosil among the studied bacterial species. Also they reported that *Staphylococcus aureus* (ATCC 29737) was the most resistant among the studied bacteria. They showed that Nanosil in 2% (20000 mg/L) concentration was enough to kill all target bacteria in 15 minutes [40]. While in our research, the Nanosil in 0/1% (1000 mg/L) concentration killed all target bacteria during 24 h contact time. Also Maliszewska and Sadowski [30] reported that nanosilvers effectively inhibited *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and

Pseudomonas aeruginosa. According to their results nanosilver was found to have wider antimicrobial activity than those described in earlier reports [41, 42]. It can be expected that the presence of a high specific surface area and fraction of surface atoms of nanosilver, could lead to high antimicrobial activity compared to bulk silver metal [43]. In another study, the antimicrobial activity of silver nanoparticles on *Staphylococcus aureus* and *Escherichia coli* was investigated by measuring the MIC. The growth of Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria were inhibited by silver nanoparticles. The MIC of silver nanoparticles for *Staphylococcus aureus* (KCTC 1928) and *Escherichia coli* (KCTC1041) were 10 and 5 ppm, respectively [22]. In a research by Dabbagh *et al.* [43] in which the "serial dilution method" was used to determine the MIC of nanosilver, on a mixture of microorganisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The MIC of nanosilver solution for this bacterial mixture was equal to 15.12 µg/m1.

In another study by Pedahzur *et al.* [44] where the synergistic effect of silver and hydrogen peroxide was studied on the viability of *Escherichia coli* K-12 and up to 3 logs of reduction was obtained. Many researches have showed that silver ions like silver nanoparticles are strongly adsorbed to bacterial cells [27]. A comparative mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus* showed that slighter morphological changes of *Staphylococcus aureus* compared with *Escherichia coli* was found as a defense system of *Staphylococcus aureus* against the inhibitory effects of silver ions. The slight morphological changes of *Staphylococcus aureus* might be due to its structural characters. Gram positive and Gram negative cells differ markedly in their cell walls. Obviously, the peptidoglycan in the cell walls of Gram positive cells is much thicker than that in the Gram negative ones. The thicker cell wall of *Staphylococcus aureus* is of immense practical importance in protecting the cell from penetration of silver ions into the cytoplasm. When the silver ions penetrate into the cell cytoplasm, they turn the DNA into a condensed form which at the same time reacts with proteins. All these phenomena lead to the damage or even the death of the microorganisms [45]. The above mentioned mechanism is in accordance with the obtained results in the present study for MIC and MBC of Nanosil on Gram positive and Gram negative bacteria (Tables 1 and 2). Further studies have be done to verify if the bacteria develop resistance toward the nanoparticles and examine the cytotoxicity of nanosilvers towards human cells.

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REFERENCES

1. Chen, X. and H.J. Schluesener, 2008. Nanosilver: A nanoproduct in medical application. Toxicol. Lett., 176: 1-12.
2. Klasen, H., 2000. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. Burns, 26: 131-138.
3. Kheybari, S., N. Samadi, S.V. Hosseini, A. Fazeli and M.R. Fazeli, 2010. Synthesis and antimicrobial effects of silver nanoparticles produced by chemical reduction method. DARU, 18: 168-172.
4. Huang, Y., Y. Huang, X. Li, Z. Liao, G. Zhang, Q. Liu, J. Tang, Y. Peng, X. Liu and Q. Luo, 2007. A randomized comparative trial between acticoat and SD-Ag in the treatment of residual burn wounds, including safety analysis. Burns, 33: 161-166.
5. Chen, J., C.M. Han, X.W. Lin, Z.J. Tang and S.J. Su, 2006. Effect of silver nanoparticle dressing on second degree burn wound. Zhonghua Wai Ke. Za Zhi., 44: 50-52.
6. Vlachou, E., E. Chipp, E. Shale, Y.T. Wilson, R. Papini and N.S. Moiemen, 2007. The safety of nanocrystalline silver dressings on burns: a study of systemic silver absorption. Burns, 33: 979-985.
7. Sibbald, R.G., J. Contreras-Ruiz, P. Coutts, M. Fierheller, A. Rothman and K. Woo, 2007. Bacteriology, inflammation and healing: a study of nanocrystalline silver dressings in chronic venous leg ulcers. Adv. Skin Wound, Care, 20: 549-558.
8. Yang, J.Y., C.Y. Huang, S.S. Chuang and C.C. Chen, 2007. A clinical experience of treating exfoliative wounds using nanocrystalline silver-containing dressings (Acticoat1). Burns, 33: 793-797.
9. Shin, S.H., M.K. Ye, H.S. Kim and H.S. Kang, 2007. The effects of nanosilver on the proliferation and cytokine expression by peripheral blood mononuclear cells. Int Immunopharmacol., 7: 1813-1818.

10. Bhol, K.C. and P.J. Schechter, 2005. Topical nanocrystalline silver cream suppresses inflammatory cytokines and induces apoptosis of inflammatory cells in a murine model of allergic contact dermatitis. Br. J. Dermatol., 152: 1235-1242.
11. Cheng, D., J. Yang and Y. Zhao, 2004. Antibacterial materials of silver nanoparticles application in medical appliances and appliances for daily use. Chin. Med. Equip. J., 4: 26-32.
12. Zhang, Y. and J. Sun, 2007. A study on the bio-safety for nano-silver as anti-bacterial materials. Chin. J. Med. Instrumen., 31: 35-38.
13. Buzea, C., I.I. Pacheco Blandino and K. Robbie, 2007. Nanomaterials and nanoparticles: sources and toxicity. Biointerphases, 2: MR17-MR71.
14. Chaudhry, Q., M. Scotter, J. Blackburn, B. Ross, A. Boxall, L. Castle, R. Aitken and R.Watkins, 2008. Applications and implications of nanotechnologies for the food sector. Food Addit. Contam., 25: 241-258.
15. Pal, S., Y.K. Tak and J.M. Song, 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study o the gram-negative bacterium *Escherichia coli*. Appl. Environ. Microbiol., 73: 1712-1720.
16. Khaydarov, R.R., R.A. Khaydarov, Y. Estrin, S. Evgrafova, T. Scheper, C. Enders and S.Y. Cho, 2009. Silver nanoparticles, Environmental and Human Health Impacts. Nanomaterials: Risks and Benefits. Springer Scie. Business Media B.V., pp: 287-296.
17. Sladkova, M., B. Vlckova, K. Siskova, I. Pavel, M. Moskovits and M. Slouf, 2009. Surface-enhanced Raman scattering from a single molecularly bridged silver nanoparticle aggregate. J. M. Struct., 924-926: 567-570.
18. Choi, O., K.K. Deng, N.J. Kim, L.J. Ross, R.Y. Surampalli and Z. Hu, 2008. The inhibitory effects of silver nanoparticles, silver ions and silver chloride colloids on microbial growth. Water Res., 42: 3066-3074.
19. Foldbjerg, R., P. Olesen, M. Hougaard, D.A. Dang, H.J. Hoffmann and H. Autrup, 2009. PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes. Toxicol. Lett., 190: 156-162.
20. Sotiriou, G.A. and S.E. Pratsinis, 2010. Antibacterial activity of nanosilver ions and particles. Environ. Sci. Technol., 44: 5649-5654.
21. Jose, R.M., L.E. Jose, C. Alejandra, H. Katherine, B.K. Juan, T.R. Jose and J.Y. Miguel, 2005. The bactericidal effect of silver nanoparticles. Nanotechnology, 16: 2346-2353.
22. Cho, K.H., J.E. Park, T. Osakab and S.G. Park, 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochimica Acta., 51: 956-960.
23. Yamanaka, M., K. Hara and J. Kudo, 2005. Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. Appl. Environ. Microbiol., 71: 7589-7593.
24. Jung, W.K., H.C. Koo, K.W. Kim, S. Shin, S.H. Kim and Y.H. Park, 2008. Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. Appl. Environ. Microbiol., 74: 2171-2178.
25. Shrivastava, S., T. Bera, R. Roy, G. Singh, P. Ramachandrarao and D. Dash, 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology, 18: 225103-225112.
26. Yang, W., C. Shen, Q. Ji, H. An, J. Wang, Q. Liu and Z. Zhang, 2009. Food storage material silver nanoparticles interfere with DNA replication fidelity and bind with DNA. Nanotechnology, 20: 085102.
27. Morones, J.R., J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramirez and M.J. Yacaman, 2005. The bactericidal effect of silver nanoparticles. Nanotechnology, 16: 2346-2353.
28. Silver, S., 2003. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiol. Rev., 27: 341-353.
29. Chaloupka, K., Y. Malam and A.M. Seifalian, 2010. Nanosilver as a new generation of nanoproduct in biomedical applications. Trend in Biotechnology, 28: 580-588.
30. Maliszewska, I. and Z. Sadowski, 2009. Synthesis and antibacterial activity of silver nanoparticles. J. Physics. Ser., 146: 012024.
31. Almofti, M.R., T. Ichikawa, K. Yamashita, H. Terada and Y. Shinohara, 2003. Silver ion induces a cyclosporine a-insensitive permeability transition in rat liver mitochondria and releas of apoptogenic cytochrome. C.J. Biochem., 134: 43-49.
32. Miyasaki, K.T., R.J. Genco and M.E. Wilson, 1986. Antimicrobial properties of hydrogen peroxide and sodium bicarbonate individually and in combination against selected oral, gram-negative, facultative bacteria. J. Dent. Res., 65: 1142-1148.
33. Fazlara, A., H. Najafzadeh and E. Lak, 2008. The potential application of plant essential oils as natural preservatives against *Escherichia coli* O157:H7. Pak. J. Bio. Sci., 11: 2054-2061.

34. Akhoondzadeh Basti, A. and V. Razavilar, 2004. Growth response and modeling of the effects of selected factors on the time-to-detection and probability of growth initiation of *Salmonella Typhimurium*. Food Microbiol., 21: 431-438.
35. Mohsenzadeh, M., 2007. Evaluation of antibacterial activity of selected Iranian essential oils against *Staphylococcus aureus* and *Escherichia coli* in nutrient broth medium. Pak. J. Bio. Sci., 10: 3693-3697.
36. Delaquis, P.J., K. Stanich, B. Girard and G. Mazza, 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int. J. Food Microbiol., 74: 101-109.
37. United States Environmental Protection Agency, 2002. Hydrogen peroxide (Hydrogen dioxide) (000595) fact sheet-Pesticides: regulating pesticides. Washington, DC: EPA. Available from: http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_000595.htm.
38. Marriot, N., 2006. Sanitation Equipment. Principles of Food Sanitation. 5th ed. New York, Springer, pp: 158-189.
39. Hussain, S.T. and M. Mohammed, 2009. Novel method of manufacture of silver oxide nanoparticles. United States Patent Application Publication, Pub. No.: US 2009/0068089 A.1, pp: 1-7.
40. Nabizadeh, R., N. Samadi, Z. Sadeghpour and M. Beikzadeh, 2008. Easibility study of using complex of hydrogen peroxide and silver for disinfecting swimming pool water and its environment. Iran. J. Environ. Health. Sci. Eng., 5: 235-242.
41. Mukherjee, P., M. Roy, B.P. Mandal, G.K. Dey, P.K. Mukherjee, J. Ghatak, A.K. Tyagi and S.P. Kale, 2008. Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. Nanotechnology, 19: 075103.
42. Martinez-Castanon, G.A., N. Nino-Martinez, F. Martinez-Gutierrez, J.R. Martinez-Mendoza and F. Ruiz, 2009. Antibacterial effect of silver nanoparticles against *Streptococcus mutans*. Materials Lett., 63: 2603-2606.
43. Dabbagh, M.A., E. Moghimipour, A. Ameri and N. Sayfoddin, 2008. Physicochemical characterization and antimicrobial activity of nanosilver containing hydrogels. Iran. J. Pharmaceutical Res., 7: 21-28.
44. Pedahzur, R., H.I. Shuval and S.A. Ulitzur, 1997. Silver and hydrogen peroxide as potential drinking water disinfectant: their bactericidal effects and possible modes of action. Water Sci. Technol., 35: 87-93.
45. Feng, Q.L., J. Wu, G.Q. Chen, F.Z. Cui, T.N. Kim and J.O. Kim, 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J. Biomed. Mater. Res., 52: 662-668.