Original Article

Comparative Study of Antimicrobial Activities of TiO_2 and CdO Nanoparticles against the Pathogenic Strain of *Escherichia coli*

Saeed Rezaei-Zarchi[1], Aisha Javed[2], Madiha Javeed Ghani[3], Safieh Soufian[4], Fatemeh Barzegari Firouzabadi[1], Abdolmajid Bayanduri Moghaddam[5], Seyed Hossein Mirjalili[6]

1. Dept. of Biology, Payam-e-Noor University, Yazd, Iran
2. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Dept. of Bioinformatics, Government College University, Faisalabad, Pakistan
4. Dept. of Biology, Payam-e-Noor University, Arak, Iran
5. Medical Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran- Iran
6. Dept. of Chemistry, Yazd University, Yazd, Iran

ABSTRACT

Background: and Objectives: **The aim of this study was to detect the antibacterial properties of 0.01, 0.5 and 1% nano-TiO_2 and -CdO against *E. coli*.**

Materials and Methods: ***E. coli*** **was cultured in liquid and agar nutrient medium to evaluate the antibacterial effects of 0.01, 0.05 and 1% of both nano-TiO_2 and -CdO via the optical density (OD) and log CFU/ml measurements.**

Results: **Non-significant effect was seen for 0.01% of both nano-specimens. While, 0.05 and 1% of both nanoparticles showed considerably decreased bacterial number. A 4.5 and 1.9 times decrease in the OD value was found in the presence of 1 and 0.5% nano-TiO_2, respectively ($P< 0.001$). 1.5 and 3.3 times decreased OD was seen in the presence of 0.5 and 1% nano-CdO, respectively, as compared to control ($P<0.001$). In the second study, 6.3 log CFU/ml of *E. coli* were present in the cultures treated with 1% nano-TiO_2 and CdO at 4 ºC in water. Control *E. coli* cells survived for 12 days while complete cell death was seen when 1% nano-TiO_2 was applied for 13 hours as compared to 1% nano-CdO, which showed complete cell death after 15 hours. In the third study, *E. coli* was grown in the agar medium with and without both nanoparticles and suppressed growth (4.5 and 5.6 times; $P<0.001$) was seen in the presence of 1% nano-CdO and -TiO_2, respectively.**

Conclusion: **In spite of the fact that both nanoparticles showed bactericidal activity, nano-TiO_2 has proven to be more efficient antibacterial agent as compared to nano-CdO.**

Keywords: Nanoparticle, P(2-HMC)-TiO2, CDO-MeB, Bacterial count, Antibacterial Agent, *Escherichia coli*

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Address communications to: Dr. Saeed Rezaei-Zarchi, Department of Biology, Payam-e-Noor University, Yazd, Iran
Email: srezaei@ibb.ut.ac.ir

Introduction

Metal nano particles have various functions that are not observed in bulk phase (1,2). Antibacterial agents, used in textile industry, are divided into two parts: the organic and inorganic matters. The organic antibacterial materials have been used as insecticides and bactericides for many years. Unfortunately, high temperatures in manufacturing process reduce their antibacterial properties. However, inorganic antibacterial agents show excellent resistance against the bacterial and thermal stability (3). Over the past few decades, inorganic nanoparticles, whose structures exhibit significantly novel and improved physical, chemical, and biological properties and functionality due to their nano-scale size, have elicited much interest.

Nano-structured materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical applications (4-6). At present, the use of nano-structured materials is becoming more widespread and a major advantage over either organic or inorganic nanoparticles offers many possibilities of applications in the areas of physics, chemistry, pharmacy, surface coating agents, textile sizing, agriculture, biochemistry and so on (4-9). It has been demonstrated that specially formulated metal oxide nanoparticles have good antibacterial activity, and antimicrobial formulations comprising nanoparticles could be effective bactericidal materials (10). Nano-materials are called "a wonder of modern medicine". It is stated that antibiotics kill perhaps a half dozen different disease-causing organisms but nano-materials can kill some 650 cells (11). Resistant strains fail to develop if we apply nanoparticle-based formulations in their culture media. In laboratory tests with nanoparticles, the bacteria, viruses, and fungi are killed within minutes of contact.

The effect of nanoparticles on bacteria is very important since they constitute the lowest level and hence enter the food chain of the ecosystems (5, 8). Recent studies have demonstrated that specifically formulated nanoparticles demonstrate good antipcterial activity and constitute the antimicrobial formulations (12-14). As, nano-silver has been used for imparting antibacterial properties (14,15), nano-TiO_2 and the oxides of other nanomaterials like CdO and ZnO have also been reported for antibacterial properties (16-22). Metal oxide nanoparticles are more preferable than nano-silver because of cost considerations. In fact, both cadmium oxide and titanium dioxide are non-toxic and chemically stable under exposure to both high temperatures and capable of photo catalytic oxidation (20, 22).

This study aimed to investigate the potent long-lasting antibacterial activity of nano-TiO_2 and CdO toward the gram-negative bacterium *E. coli*, which is known as diarrhea-causing organism.

Materials and Methods

Chemicals, growth media, and bacterial Strain

E. coli (ATCC 25922) was used for the present experiment. Nutrient Broth (BD234000; Becton Dickinson & Company, MD, USA) was used in growing and maintaining the bacterial cultures as per supplier's protocol. The chemicals such as; ascorbic acid, sodium citrate tribasic dehydrate, ammonium sulphate, ethanol and cetyltrimethyl ammonium bromide (CTAB) were purchased from Sigma and were of the highest purity available. These reagents were used as received without further purification. TiO_2 and CdO nanoparticles, with the particle-size of 60 nm, were used throughout the experiment. The particles were suspended in sterile water and sonicated for 15 min before use (11).

Bacterial susceptibility to nanoparticles

To examine the susceptibility of *E. coli* to nano-TiO_2 and -CdO, three different estimation methods were used with three tiles repetition.

Bacterial growth in the presence of nano-TiO_2 and CdO in liquid medium

In the first method, the bacteria were grown in nutrient broth (NB). To start the growth, 2 mL of the overnight-cultured *E. coli* stock was added to 100 mL NB, containing 0.12% glucose with and without 0.01, 0.5 and 1% nano-TiO_2 and CdO, separately. The bacteria were aerobically cultured at 30 ºC for 24 hours. Optical density (OD) measurements were taken at 600 nm to monitor the bacterial concentration.

Bacterial killing in the presence of nano-TiO_2 and CdO in liquid medium

In the second method, the culture solution was centrifuged and the cells were washed and re-suspended in distilled water, reaching a final concentration of 6.3 *log* CFU/ml in each of the sample flasks and incubated at 4 ºC. The final concentration of the *E. coli* suspensions was made in 100 ml distilled water. Different amounts of nano-TiO_2 and -CdO (0.01, 0.5 and 0.1%) were then separately added to the bacterial suspensions to keep in contact with the bacterial cells and shaken at 4 ºC for 48 hours. Optical density (OD) was measured to obtain the results. Aliquots of 0.1 ml of the growth mixtures (water + bacterial cells + nanoparticles) were sampled every two hour. The number of resulting bacterial cells was noted after every two hours of incubation. Bacterial number was determined by measuring the optical density (OD) at 600 nm. The OD values were converted into the *E. coli* concentration as *log* CFU/ml (13).

Bacterial growth in the presence of nano-TiO_2 and CdO in agar medium

In the third method, the same bacteria strain was grown on a solid NB containing 0.12% glucose, 2% agar (control plates) alone or in the presence of 1% nano-TiO_2 and -CdO. Bacterial cells were grown at 30 ºC for 48 hours. Afterwards, the plates were visually estimated and bacterial colonies counted. The pictures were taken by an Olympus C2020Z digital camera. The data obtained in all tests were compared with the control. Student's *t*-test was used to evaluate the significance of experimental results ($P<0.05$).

Results

Effect of nano-TiO_2 and CdO on the growth of E. coli in liquid medium

In the first study, we investigated the effect of different concentrations of both nanoparticles in liquid culture of *E. coli.* The optical density of the medium was investigated as the number of bacteria after contact with the nanoparticles. Fig. 1 shows the effect of different 0.01, 0.5 and 1% nano-TiO_2 on the in growth and killing of *E. coli*. As demonstrated by the figure, 0.01% nano-TiO_2 did not have antibacterial efficiency on *E. coli* but the concentrations of 0.5 and 0.1% nano-TiO_2 inhibited the bacterial growth. Fig. 1 shows that 0.5% nano-TiO_2 showed 1.9 times decrease the optical density of bacterial cultures ($P<0.05$) as compared to the control. While, in the presence of 1% nano-TiO_2, the optical density of *E. coli* cultures decreased 4.5 times as compared to the control experiment. Fig. 2 shows the effect of different concentrations of nano-CdO on the growth of *E. coli*. As demonstrated in this figure, 0.01% nano-CdO did not have antibacterial effect while, 0.5 and 1% nano-CdO was highly efficient in inhibiting the *E. coli* growth as compared to control group. This figure shows that the presence of 0.5% nano-CdO caused a 1.5 times decrease in the optical density of bacterial cultures ($P<0.05$) as compared to control. As shown in Fig. 2, the presence of 1% nano-TiO_2 caused a 3.3 times decrease in the optical density of *E. coli* as compared to the control group. Results of the Figs. 1 and 2 show that nano-TiO_2 have more efficient antibacterial property for *E. coli* in comparison with nano-CdO.

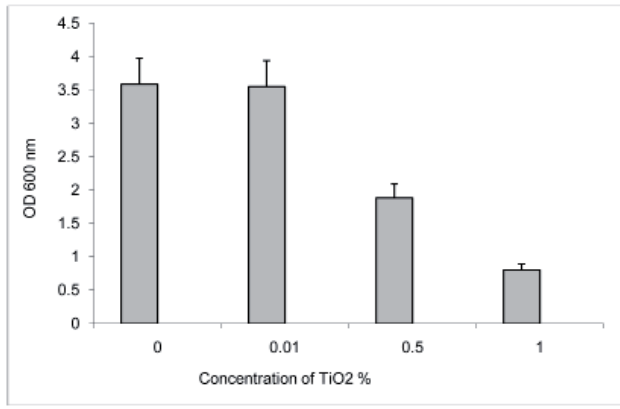

Fig. 1: *E. coli* concentration dependence upon different concentrations of TiO_2 in the culture medium.

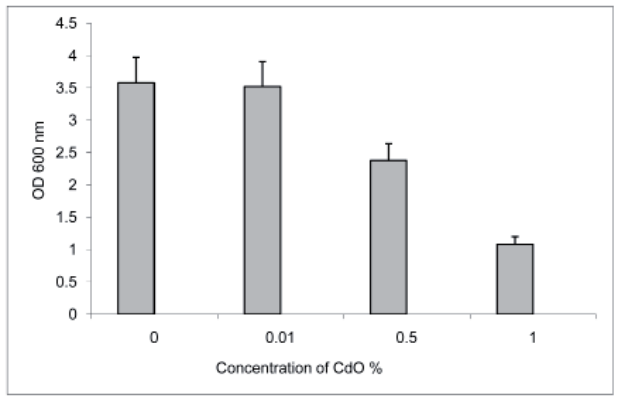

Fig. 2: *E.coli* concentration dependence upon different concentrations of CdO in the culture medium.

Bactericidal effect of nano-TiO_2 and CdO on E. coli in liquid medium

In the second study, estimation of the number of viable *E. coli* cells in contact with 1% TiO_2 and CdO

was carried out in water at 4 ºC for different contact time intervals. Our result showed the reduction of *E. coli* cells from 6.3 *log* CFU/ml to undetectable levels after 12 days (Data have not been shown). While, upon the addition of these nano-materials to the bacterial culture showed decreased survival rate within 2 days as compared to that of 12-day experiment for control group. Fig. 3 represents the number of viable *E. coli* cells in contact with 1% nano-TiO_2 and –CdO, separately, suspended in water at 4 ºC for different contact times. From the figure, it can be clearly observed that nano-TiO_2 and -CdO exhibited different antibacterial properties. After the *E. coli* were suspended in water alongwith TiO_2, the number of microbial cells reached zero after 26 hours. While, 1% CdO showed complete bacterial killing after 30 hours of their contact with 1% nano-CdO. These results demonstrate a stronger antibacterial effect of nano-TiO_2 on the *E. coli* as compared to nano-CdO. As compared to the control group where survival was seen upto a culture period of 12 hours, the administration of nano-materials to thebacterial cultures killed the bacteria in less than 2 days. These results demonstrate that nano-TiO_2 and CdO have a high antibacterial efficiency against *E. coli*.

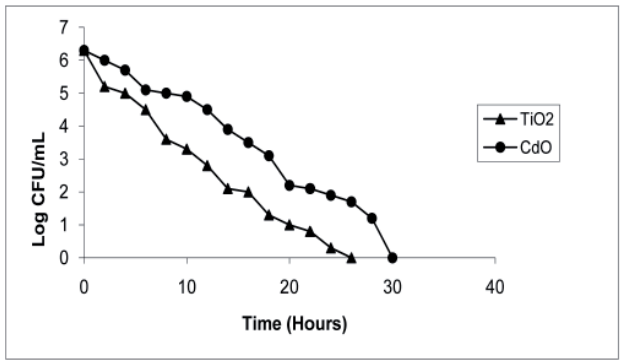

Fig. 3: Comparative killing kinetics of 1% TiO_2 (▲) and CdO (●) on the *E. coli* cultures

Effect of nano-TiO_2 and CdO on the E. coli growth in agar medium

In the third investigation, *E. coli* was grown on agar medium without (control) or with 1% nano-TiO_2 and -CdO, separately. Distinct bacterial colonies were observed in 10^5 times dilution. The visual estimation and bacterial colony counts were performed at this dilution. In Fig. 4, we can see smaller number of *E. coli* colonies on the agar medium with nano-CdO (plate B) and -TiO_2 (plat C) as compared to the control group (plate A). In the control plates, 802 ± 75 bacterial colonies were obtained while, in the experimental plates with 1% nano-TiO_2, 63 ± 19; and with 1% nano-CdO, 195 ± 32 bacterial colonies were seen. Thereby, nano-TiO_2 suppresses the bacterial growth 12.7 times (P<0.05) in the agar medium as compared to that of 4.1 times decrease in the presence of nano-CdO.

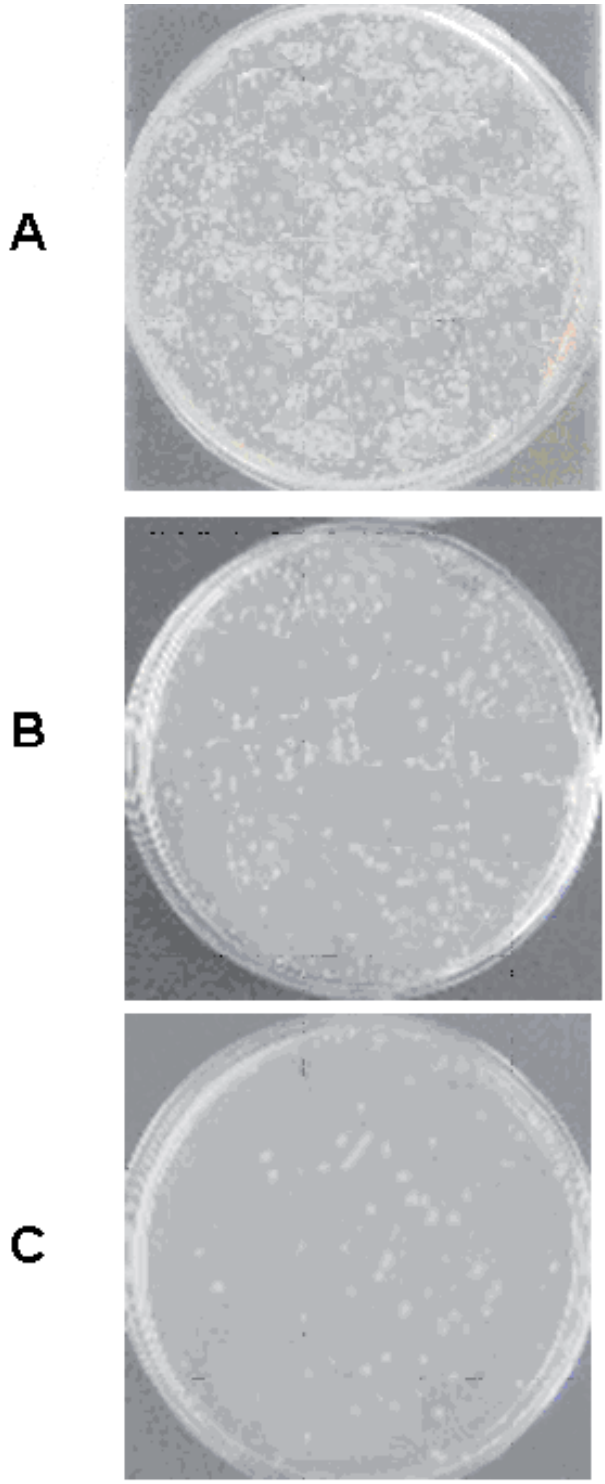

Fig. 4: *E.coli* growing on the agar medium without TiO_2/CdO nano-particles (A) and with 1% nano-CdO (B) and nano-TiO_2 (C)

Discussion

The antibacterial activities of different concentrations of nano-TiO_2 and CdO were investigated during the recent analysis. *E. coli* (ATCC 25922) was used as the test organism during the experiments. Good growth-inhibition results were observed when the bacterial cells were incubated with both kinds of nanoparticles during the liquid and solid cultures. The quantitative examination of bacterial activity was estimated by the survival ratio as calculated from the number of viable cells, which formed colonies on the nutrient agar plates (23). Another study states the nano-TiO_2 as a strong and effective bactericidal agent (24).

The present data demonstrate that a formulation made with the biologically stabilized TiO_2 and CdO nanoparticles can be useful in the treatment of infectious diseases caused by *E. coli*. A strong binding of nanoparticles to the outer membrane of *E. coli* causes the inhibition of active transport, dehydrogenase and periplasmic enzyme activity and eventually the inhibition of RNA, DNA and protein synthesis, which finally leads to cell lysis as was seen for *E. coli* during the present study (25). Such effective and less-time consuming formulations can be useful in the clinical practices where *E. coli* causes urinary tract infections (UTIs). It has been known that nano-materials exhibit strong inhibitory effects towards a broad spectrum of bacterial strains (26).

During the present study, different concentrations of nano-scale TiO_2 and CdO were tested to find out the best concentration that can have the most effective antibacterial property against the *E. coli* culture. Our data is in accordance with the previous studies, dealing with the antibacterial effects of nano-materials (26-28). Several investigations have suggested the possible mechanisms involving the interaction of nano-materials with the biological macromolecules. It is believed that microorganisms carry a negative charge while metal oxides carry a positive charge. This creates an "electromagnetic" attraction between the microbe and treated surface. Once the contact is made, the microbe is oxidized and dead instantly. Generally, it is believed that nano-materials release ions, which react with the thiol groups (-SH) of the proteins present on the bacterial cell surface. Such proteins protrude through the bacterial cell membrane, allowing the transport of nutrients through the cell wall. Nano-materials inactivate the proteins, decreasing the membrane permeability and eventually causing the cellular death (27). Nano-materials also retard the bacterial adhesion and bio-film formation (29).

Antimicrobial modification to prevent the growth of detrimental microorganisms is a highly desired objective. Microbial cell growth and colonization result in the formation of a compact bio-film matrix, capable of protecting the underlying microbes from antibiotics and host defense mechanisms. Microbial infestation can result in serious infection (30, 31). Such infections are also implicated in food spoilage, spread of food-borne diseases, and bio fouling of materials (32). Hence, there is a significant interest in the development of antimicrobial materials and surfaces for applications in the health, biomedical, food and personal-hygiene industry. The nanomaterials, based on the metal ions, exhibit broad-spectrum biocidal activity towards different bacteria, fungi, and viruses (31). Nanomaterials are known to deactivate cellular enzymes and DNA by coordinating to electron-donating groups such as thiols, carboxylates, amides, imidazoles, indoles, hydroxyls, and so forth. They cause pits in bacterial cell walls, leading to increased permeability and cell death (32).

Conclusion

The present study reveals that the antibacterial effect of nano-TiO_2 was stronger than that of nano-CdO, which can be because of the fact that the nano-composition of TiO_2 contained consequently more antibacterial active sites than CdO nano-particles.

Acknowledgements

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References

1.Sosa IO, Noguez C, Barrera RG. Optical properties of metal nanoparticles with arbitrary shapes. J Phys Chem B

2003;107(26):6269–75.

2. Sun YG, Mayers B, Herricks T, Xia YN. Polyol synthesis of uniform silver nanowires: a plausible growth mechanism and the supporting evidence. Nano Lett 2003;3(7):955–60.

3. Te-Hsing W, Yi-Der T, Lie-Hang S. The novel methods for preparing antibacterial fabric composites containing nano-material. Solid State Phenomena 2007;124:1241-44.

4. Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, *et al*. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol 2003;21(1):41–6.

5. Fortner JD, Lyon DY, Sayes CM, Boyd AM, Falkner JC, Hotze EM, Alemany *et al*. C-60 in water: Nanocrystal formation and microbial response. Environ Sci Technol 2005;39(11):4307-16.

6. Li P, Li J, Wu C, Wu Q, Li J. Synergistic antibacterial effects of lactam antibiotic combined with silver nanoparticles. Nanotechnol 2005;16(9):1912–17.

7. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TM, Kim JO. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J Biomed Mater Res 2000;52(4):662–68.

8. Herrera M, Carrion P, Baca P, Liebana J, Castillo A. In vitro antibacterial activity of glass-ionomer cements. Microbios 2001;104(409):141–48.

9. Hranisavljevic J, Dimitrijevic NM, Wurtz GA, Wiederrecht GP. Photoinduced charge separation reactions of J-aggregates coated on silver nanoparticles. J Am Chem Soc 2002;124(17):4536–37.

10. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. Langmuir 2002;18(17):6679–86.

11. Sungkaworn T, Triampo W, Nalakarn P, Triampo D, Tang IM, Lenbury Y, *et al*. The effects of TiO_2 nanoparticles on tumor cell colonies: fractal dimension and morphological properties. Int J Biomed Sci 2007;2(1):67-74.

12. Matsumura Y, Yoshikata K, Kunisaki S, Tsuchido T. Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. Appl Environ Microbiol 2003;69(7):4278–81.

13. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. J Colloid Interface Sci 2004;275(1):177–82.

14. Lee HJ, Yeo SY, Jeong SH. Antibacterial effect of nanosized silver colloidal solution on textile fabrics. J Mater Sci 2003;38(10):2199–2204.

15. Dura´n N, Marcato PD, De Souza GIH, Alves OL, Esposito E. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. J Biomed Nanotechnol 2007;3(2):203–8.

16. Xin JH, Daoud WA, Kong YY. A new approach to UV-blocking treatment for cotton fabrics. Text Res J 2004;74(2):97–100.

17. Fei B, Deng Z, Xin JH, Zhang Y, Pang G. Room temperature synthesis of nanorods and their applications on cloth. Nanotechnol 2006;17(8):1927–31.

18. Qi K, Chen X, Liu Y, Xin JH, Mak CL, Daoud WA. Facile preparation of anatase/SiO_2 spherical nanocomposites and their application in self cleaning textiles. J Mater Chem 2007;17:3504–3508.

19. Baglioni P, Dei L, Fratoni L, Lo Nostro P, Moroni M. Preparation of nano- and micro-particles of group II and transition metals oxides and hydroxides and their use in the ceramic, textile and paper industries. Patent 2003;8:827-42.

20. Wang RH, Xin JH, Tao XM, Daoud WA. ZnO nanorods grown on cotton fabrics at low temperature. Chem Phys Lett 2004;398:250–55.

21.Vigneshwaran N, Kumar S, Kathe AA, Varadarajan PV, Prasad V. Functional finishing of cotton fabrics using zinc oxide-soluble starch nanocomposites. Nanotechnol 2006;17(1-3):5087–95.

22.Fu G, Vary PS, Lin C. Anatase TiO_2 nanocomposites for antimicrobial coatings. J Phys Chem B 2005;109(18):8889-98.

23. Tsuang YH, Sun JS, Huang YC, Lu CH, Chang WH, Wang CC. Studies of photokilling of bacteria using titanium dioxide nanoparticles. Artif Organs. 2008;32(2):167-74.

24. Power EGM. Aldehydes as biocides. Prog Med Chem 1995;34:149–201.

25.Russell AD, Hugo WB. Antimicrobial activity and action of silver. Prog Med Chem 1994;31:351-70.

26.Clement JL, Jarrett PS. Antibacterial Silver. Met Based Drugs. 1994;1(5-6):467-82.

27.Zhang H, Chen G. Potent antibacterial activities of Ag/TiO_2 nanocomposite powders synthesized by a one-pot sol-gel method. Environ Sci Technol 2009;43(8):2905-10.

28.Cook G, Costerton JW. Direct confocal microscopy studies of the bacterial colonization in vitro of a silver-coated heart valve sewing. Cuff Int J Antimicrob Agents

2000;13(3):169-73.

29.Raad, II, Hanna HA, Boktour M, Chaiban G, Hachem RY, Dvorak T, *et al*. Vancomycin-resistant Enterococcus faecium: catheter colonization, esp gene, and decreased susceptibility to antibiotics in biofilm. Antimicrob Agents Chemother 2005;49(12):5046−50.

30. Jones GL, Muller CT, O'Reilly M, Stickler DJ. Effect of triclosan on the development of bacterial biofilms by urinary tract pathogens on urinary catheters. J Antimicrob Chemother 2006;57(2):266−72.

31.Greenberg CB, Steffek C. Bio-adhesion to thin films in relation to cleaning. Thin Solid Films 2005;484(2): 324−327.

32.Holt KB, Bard AJ. Interaction of silver (I) ions with the respiratory chain of *Escherichia coli*: An electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag. Biochemistry 2005;44(39):13214−23.