Inhibition of growth of *S. epidermidis* by hydrothermally synthesized ZnO nanoplates

C Abinaya, J. Mayandi, J. Osborne, M. Frost, C Ekstrum, J M Pearce

► To cite this version:

C Abinaya, J. Mayandi, J. Osborne, M. Frost, C Ekstrum, et al.. Inhibition of growth of *S. epidermidis* by hydrothermally synthesized ZnO nanoplates. *Materials Research Express*, 2017, 4 (7), pp.075401. 10.1088/2053-1591/aa796d . hal-02111412

HAL Id: hal-02111412

<https://hal.science/hal-02111412>

Submitted on 26 Apr 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Inhibition of growth of *S. epidermidis* by hydrothermally synthesized ZnO nanoplates

C. Abinaya ^a, J. Mayandi* ^{a, b}, J. Osborne ^c, M. Frost ^c, C. Ekstrum ^b, J. M. Pearce ^{b, d, *}

^a Department of Materials Science, School of Chemistry, Madurai Kamaraj University, Madurai- 625 021, Tamil Nadu, India.

^b Department of Materials Science and Engineering, Michigan Technological University, Houghton - 49931 1295, Michigan, USA.

^c Department of Biomedical Engineering, Michigan Technological University, Houghton - 49931 1295, Michigan, USA.

^d Department of Electrical & Computer Engineering, Michigan Technological University, Houghton - 49931 1295, Michigan, USA.

***Corresponding authors**

1400 Townsend Drive, Houghton, MI 49931-1295, 906-487-1466., pearce@mtu.edu

Department of Materials Science, School of Chemistry, Madurai Kamaraj University, Madurai- 625 021, India, jeyanthinath@yahoo.co.in

Abstract

The antibacterial effect of zinc oxide (ZnO#1) as prepared and annealed (ZnO#2) at 400°C, Cu doped ZnO (CuZnO), and Ag doped ZnO (AgZnO) nanoplates on *Staphylococcus epidermidis* was investigated for the inhibition and inactivation of cell growth. The results shows that pure ZnO and doped ZnO samples exhibited antibacterial activity against *S. epidermidis* as compared to tryptic soy broth (TSB). Also it is observed that *S. epidermidis* was extremely sensitive to treatment with ZnO nanoplates and it is clear that the effect is not purely depend on Cu/Ag. Phase identification of a crystalline material and unit cell dimensions were studied by X-ray powder diffraction (XRD). The scanning electron microscopy (SEM) provides information on sample's surface topography and the EDX confirms the presence of Zn, O, Cu and Ag. X-ray photo-electron spectroscopy (XPS) was used to analyze the elemental composition and electronic state of the elements that exist within the samples. These studies confirms the formation of nanoplates and the presence of Zn, O, Ag, Cu with the oxidation states +2, -2, 0 and +2 respectively. These results indicates promising antibacterial applications of these ZnO-based nanoparticles synthesized with low-cost hydrothermal methods.

Keywords: zinc oxide; *Staphylococcus epidermidis*; bactericide; nanoplate; nanotechnology

1. Introduction:

Bacteria and viruses are the most common cause for many infections such as food poisoning, meningitis, wound infections, pneumonia, strep throat etc., Millions of bacteria are living all over the world and a vast majority are non-infectious and helpful in obtaining good health. These types are called good or healthy bacteria. There are some bacteria which are dreadful and causes many infections and diseases called pathogenic bacteria. These types have evolved various strategies to initiate infections even in sterile tissues (1). These causes the most illness, hospitalizations and death all over the world. Some of the bacterial species that possess include the following: *E.coli* (Food poisoning), *H. alvei* (diarrhea in humans), *P. aeruginosa* (severe opportunistic infections in humans), *H. pylori* (Gastritis and Ulcer), *N.gonorrhoeae* (Gonorrhea), *S. aures* (Boils, pneumonia, abscesses), *S. epidermidis* (nosocomial infections), *Yersinia spp.* (Plague and gastroenteritis), and *S. enterica* (Typhoid fever and gastroenteritis) (2). *Staphylococcus epidermidis* is one of the gram positive bacteria and nowadays it is seen as an important opportunistic pathogen (3). Although *S. epidermidis*

is not usually pathogenic, in recent times patients with low immunity are at a risk of developing it. It has the capability of forming colonies which is the successful outcome of bacterial interactions with the living and nonliving components of the environment. *S. epidermidis* is a permanent and ubiquitous colonizer of human skin, it has the ability to form bio-film on the surface of plastic devices implanted resulting high probability of device contamination (4). Mostly people with catheters or surgical implants acquiring this bacteria at hospitals (5).

The field of clinical microbiology has expanded rapidly over a decade and literally thousands of research papers were published in the destruction of bacteria. Even though a large number of antibiotic drugs are commercially available, many of them become ineffective in eradicating the bacterial diseases. The failure of these available antibiotics is mainly due to the adaptive nature of bacteria (6). As a consequence of this alarming increase in the number of multidrug resistant (MDR) microorganisms there is an emergence in the usage of antibiotics and it exemplifies the adaptive abilities of bacteria. Resistance to conventional antibiotics for human pathogens has created a crisis (7), for which nanoparticles have been proposed as a potential solution (8). The U.S. FDA has already recognized ZnO as one of the safest materials, making it an ideal potential candidate as an antimicrobial agent as it has a broad antimicrobial activity against bacteria (9), fungus (10) and virus (11). ZnO is already used in a number of common products such as baby powders, barrier and calamine lotions, anti-dandruff shampoos and antiseptic creams (12). Thus, there is considerable promise in nano-engineering ZnO to be a more effective human pathogen fighter. It has numerous attractive characteristics not only a good transparency, high electron mobility, wide bandgap, strong room-temperature luminescence but also it acts as a noteworthy antibacterial material. It is known from literature that the ZnO nano material has bacteriostatic or bacteriocidal activities. Part of the appeal of ZnO is that there are many scalable, low-cost methods to synthesize it including: hydrothermal (13-15), spray pyrolysis (16), sol-gel (17), solvothermal (18), precipitation (19) and co precipitation (20). Of these methods, the hydrothermal method is appealing for high-purity nanoscale ZnO synthesis as it offers control over the purity, composition, size and the crystal shape of the powder without any need for high temperature calcination processes and crushing, thus saving both energy and cost. In addition, it avoids both expensive solvents and toxic chemicals (21).

Previous work showed that ZnO particles prepared by a chemical synthesis method had antibacterial properties (22). In addition to zinc, both silver and copper have been proposed as an alternative to conventional antimicrobials for some applications (23). Following an open source nanotechnology approach (24) recent work (25) has tested hydrothermal synthesized nanoscale ZnO plates for efficacy as a bactericide for *E. coli*, *S. typhi* and *S. aureus* along with Cu and Ag doped ZnO powders (Cu:ZnO and Ag:ZnO). The materials were well characterized with the effect of doping Cu and Ag on ZnO quantified for the structural and optical properties and the efficacy for killing human bacterial pathogens was promising (25). However, it was not clear that the effect was due to ZnO, doped ZnO or simply that the Cu or Ag were the cause for antibacterial effects observed.

To answer that question and expand the range of microorganisms that could be killed by these nanomaterials a semi-quantitative testing method was designed to test the antibacterial properties for topical applications of the ZnO nanoparticle materials against *Staphylococcus epidermidis* (*S. epidermidis*), which is an opportunist pathogen that is the most frequent cause of hospital-acquired infections (3). But it was also reported that ZnO nanorods synthesized by hydrothermal method showed remarkable antibacterial activity against *E. coli* when its pH is subject to change [26]. Taking this into account we have tried to kill the bacteria by annealing and incorporating dopants. Thus, *S. epidermidis* was used to test the antibacterial properties of the materials synthesized by the low-cost hydrothermal method including: ZnO#1 (as prepared), ZnO#2 (annealed at 400°C), CuZnO, and AgZnO. The results of the synthesized nanoplates and their impact on the ZnO ability to kill the

bacteria at low concentrations are compared to the same densities of metal Zn, Ag and Cu and are discussed below.

2. Experimental details

2.1 Hydrothermal Synthesis of ZnO nanoplates

In this work ZnO was prepared by the hydrothermal method using Zinc acetate dihydrate, Sodium hydroxide pellets, silver nitrate and copper (II) acetate monohydrate and double distilled water (Borosil). All the chemicals and reagents were purchased from Merck (analytical grade) and employed without further purification.

The synthesis experiments were done by an open source hydrothermal synthesis in an aqueous solution in a mixture of zinc acetate dihydrate, sodium hydroxide and silver nitrate/copper (II) acetate monohydrate. 3 g of zinc acetate dihydrate and 2.4 g of sodium hydroxide dissolved in 75 ml of double distilled water separately using a magnetic stirrer. The NaOH solution was slowly dripped into the zinc acetate solution with the immediate formation of a white gelatinous precipitate. This solution was further dissolved under constant stirring for 30 minutes. It was then sealed in an autoclave and annealed at a temperature of 160 °C for 5 h. The precipitation was collected and repeatedly rinsed with double distilled water and ethanol. At last, the product was dried at 120° C for 1 hour using a hot plate to obtain ZnO nanopowder.

For doping, 0.11 g and 0.13 g (5 wt %) of silver nitrate and copper (II) acetate monohydrate was added as dopants along with the 2.88 g and 2.87 g of zinc acetate dihydrate in 75 ml of distilled water. The process is summarized in Figure 1. In addition a second set of ZnO was prepared by similar procedure and annealed at 400°C for 2 hours in the air.

2.2 Methodologies

All the samples were characterized by an X-ray diffractometer (Model: Bruker AXS D8 Advance using Cu Wavelength 1.5406 nm), scanning electron microscope (Model: Hitachi S - 4700), electron dispersive X-ray spectroscopy (FEI Philips XL instrument) and X-ray photo-electron spectroscopy (Model: Thermofisher scientific, UK using K-alpha surface analyser).

2.3 Antibacterial Activity Quantification

S. epidermidis was cultured in tryptic soy broth (TSB). *S. epidermidis* (ATCC 12228) LYFO DISK pellets were purchased from Microbiologics, Inc. (St. Cloud, MN), and TSB #2 dehydrated culture media was purchased from Hardy Diagnostics Inc. (Santa Maria, CA). In addition to the above materials, CuCl₂ which was purchased from Alfa Aesar (Ward Hill, MA) and AgCl which was purchased from Sigma Aldrich (St. Louis, MO) were tested as controls to eliminate the metals as the cause for antibacterial effects. *S. epidermidis* was cultured for 24 hours in 2ml vials each with a different material suspended in TSB. After the 24 hours, streak testing was completed to quantify bacterial density in solution based on urine-streak testing method (27). For each material an N=3 was used.

Material stock solutions (80 g/ml) were prepared in VWR Super-clear 50ml centrifuge tubes purchased from VWR International (Radnor, PA). For CuCl₂ and AgCl solutions, the mass of compound was based on the weight percentage found in its corresponding zinc based material. A stock *S. epidermidis* solution (108 colony-forming units (CFU)/ml) based on an optical density of 0.2 at 600 nm (28). This solution was further diluted to 0.1% (v/v) solution with sterile TSB. In sterile 2ml Cryo.s Cryogenic Storage Vials purchased from VWR International (Radnor, PA), 0.5ml of material stock solution was pipetted for each material along with control vials which had 0.5ml of sterile TSB pipetted into them. After materials were pipetted into all the appropriate vials, the vials had 0.5ml of the 0.1% bacterial solution added to them to bring the overall material concentration in

each vial down to 40 µg/ml. The vials were then loosely capped and placed on a VWR Mini Shaker 15 in the incubator at 37°C for 24 hours.

After the incubation period, streak plates were made using Mueller-Hinton 28ml filled 15x100 mm media plates and disposable 1 µl loops purchased from Hardy Diagnostics Inc. (Santa Maria, CA). Streaks plates were made by taking a 11 loopful of known 1:10 dilution of the incubated test vials and making a single streak across the diameter of the media plate; and then without flaming or changing the loop, streaks in a zigzag pattern are made at a 90° angle to the original streak to evenly disperse the bacteria over the entire plate. Plates were then sealed with parafilm and incubated at 37°C for 24 hours before isolated bacterial colonies were imaged and counted.

3. Results and Discussion

3.1 XRD analysis

The X-ray diffraction patterns of the samples ZnO#1, ZnO#2, CuZnO and AgZnO are shown in Figure 2. XRD patterns of undoped and doped ZnO exhibited the characteristic peaks of hexagonal wurtzite structure. The pattern is in good agreement with standard JCPDS-89-0510 with space group P6₃mc and no other crystalline phases were detected. But for the silver addition (5wt% Ag), the XRD pattern show additional peaks at 38.02° revealing the presence of Ag (JCPDS 04-0783) in addition to ZnO diffraction peaks which is assigned to elemental silver. The crystallite size of the samples were estimated by Scherrer formula as ZnO#1 (35 nm), ZnO#2 (31 nm), CuZnO (28 nm) and AgZnO (31 nm).

3.2 SEM analysis

Figure 3 shows the typical SEM micrographs of ZnO#1, ZnO#2, CuZnO and AgZnO that all the samples have the morphology as nanoplates. The synthesized materials exhibit the formation of nano plates with the thickness varies from 60 nm to 70 nm and diameter from 200 to 300 nm. The elemental composition of the samples were examined using EDX and are summarized in Table 1. The existence of carbon is due to the carbon grid. The presence of Cu and Ag in ZnO is confirmed and it is evident that there is no other impurities. This shows the quality of samples synthesized by this simple hydrothermal method.

3.3 XPS analysis

Figure 4 shows the deconvoluted high-resolution XPS spectra of Zn (2p), O (1s), Cu (2p) and Ag (3d), respectively. According to spectra, the Zn (2p) consists of two peaks positioned at 1022.1 and 1045.2 eV for Zn (2p_{3/2}) and Zn (2p_{1/2}), in agreement with the previously reported binding energies (BE) of ZnO (29). The splitting of Zn-2p states was about 23 eV which was induced from the powerful spin-orbit coupling. These peaks confirm the wurtzite structure and Zn²⁺ mode of Zn atom on the samples. The O 1s peak was deconvoluted into two lorentzian components. The O (1s) BE's (530.9 and 532.7 eV) are also in agreement with the previously reported values for ZnO with the oxidation state 2- (29). The peaks with BE of 369.1 and 375.2 eV correspond to Ag (3d_{5/2}) and Ag (3d_{3/2}), respectively, which is a characteristic of silver (Ag⁰) in the sample (30). Photoelectron peaks corresponding to the Cu 2p_{3/2} and Cu 2p_{1/2} core levels were observed at 933.8 eV and 953.7 eV and (31) as Cu²⁺. Also the shake-up satellite structures for Cu 2p_{3/2} and Cu 2p_{1/2} at 942.4 eV and 962.4 eV confirms the formation of Cu²⁺ on the surface [34].

3.4 Bacterial Density

Figure 5 represents streak plate for all six materials such as ZnO #1 as prepared, ZnO #2 as annealed, CuZnO, CuCl₂, AgZnO, AgCl, and TSB as the control. The results of all three sets of plates are summarized in Table 2 shows the bacterial density numerical results.

As can be seen by Figure 5, the hydrothermally synthesized pure ZnO when annealed and doped ZnO samples exhibited antibacterial activity against tested human pathogenic bacteria as compared to ZnO as prepared and TSB. In addition, it is clear that the effect is not purely the Cu and Ag

as the metals that were not processed into hydrothermal doped ZnO allowed the growth of *S. epidermidis*.

Previous work had shown dose dependent activity of the nanoparticles on for *E. coli* (MTTC443) *S. aureus* (MTCC3160) and *S. typhi* (MTCC3216) and it was found to be significant even at lowest concentration (25). These early results even compared favorably compared to a commercial antibiotic used as control (DMSO) (25). The results reported here in Figure 5 and Table 2 show that hydrothermal synthesis of doped and undoped ZnO is effective against gram-positive bacteria (*S. epidermis*). There are two possibilities for the destruction of bacterial cell. The obtained reactive oxygen species such as $\bullet\text{OH}$ and $\bullet\text{O}^{2-}$ can induce oxidative degradation of lipids in membranes that leads to DNA damage (32). Another mechanism is the inhibition of bacterial cells by interaction between positively charged ZnO with the negatively charged bacterial cell wall.

Unlike the previous results for *E. coli*, *S. aureus* and *S. typhi* where the nanoparticle Ag:ZnO showed remarkable antibacterial activity against tested panel of pathogens compared to the others (25), in this study all three materials (including the annealed ZnO) were effective at killing *S. epidermis* at the concentrations used and a interaction between metallic Ag or Cu and the as prepared semiconductor ZnO was not observable. Also it is reported that the photoinactivation of *E. coli* bacteria by Cu nanoparticles is better than CuO nanoparticles in both light and dark condition [33]. Under both dark and illumination Cu and Ag based oxide materials show efficient bacterial inactivation. [34-36]. The results showed that annealed ZnO (ZnO#2) nanoparticles exhibited enhanced bactericidal effects under visible light prevailing in the laboratory. When the same pathogens were tested for ZnO#1, ZnO#2, CuZnO and AgZnO under dark condition, using similar environment to those described previously but with the suspensions prepared in the dark and the cultures grown in the dark the antibacterial activity was slightly attenuated. This suggests that the visible light enhanced the antibacterial activity for all the samples.

Most remarkably the annealed ZnO and doped ZnO was more effective at killing than silver/copper alone. Therefore, it is possible that the efficacy of the synthesized materials depends on the activated ZnO. As for the CuZnO and AgZnO the release of Ag^0 and Cu^{2+} was highly impressive when it is doped in ZnO that implies that the antibacterial activity depend on both the photo active ZnO and the plasmonic effect of copper and metallic silver.

Comparing with pure silver nanoparticles Ag doped ZnO have many advantages such as low toxicity and cost effective. The inhibitory concentration of the AgZnO nanoparticle was found to be similar (25) to that of the MIC value of the approved antibiotics (37). Ag with materials such as TiO_2 , SiO_2 , Silica glass and multi walled carbon nanotubes also showed remarkable activity against various gram positive and gram negative bacteria's [38-44]. However, pure ZnO is even more cost effective. It should be pointed out that these chemically and thermally modified hydrothermal synthesized ZnO nanoparticles will be applicable for various industrial or medicinal applications such as the deposition of antimicrobial coatings and food packaging applications. As the process is open source, common challenges associated with proprietary nanotechnology can be avoided (24).

The further investigation of annealed pure ZnO is clearly warranted to explain the role of annealing on microstructure changes and their effects on the antimicrobial efficacy and mechanism involved in microbe death. In addition, a sensitivity analysis on the effects of annealing on doped ZnO materials should be studied in detail to attempt to increase the efficacy against multidrug resistant bacteria. In the future, clinical trials can be carried out using the optimized hydrothermal synthesized doped and annealed ZnO to overcome the resistance of bacteria to avoid growing global public health problems.

4. Conclusion

The hydrothermally synthesized ZnO nanoplates with a low-temperature, aqueous solution-based, low-cost and industrially-scalable process without a calcination process exhibited antibacterial activity against *S. epidermis* as compared to TSB. This open source hydrothermal synthesis method

also enables relatively easy incorporation of Cu and Ag dopants into ZnO. The structural and morphological studies were carried out by XRD and SEM. The absence of impurities and the presence of Cu and Ag were evidenced by EDX and XPS. In addition, it is clear that the effect is not purely the Cu and Ag as the metals that were not processed into hydrothermal doped ZnO allowed the growth of *S. epidermis*. These results represents a major potential benefit in the battle against antibiotic resistant pathogens for a large number of medical and health applications.

Acknowledgments

CA thanks the DST-INSPIRE New Delhi, India for the financial assistance as fellowship. JM, thanks the DST-SERB for the partial support through the project SERB/F/1829/2012-13. The authors would like to acknowledge helpful comments from J. Bow.

Compliance with Ethical Standards

Funding: This study was funded by DST-INSPIRE New Delhi, India and DST-SERB for project SERB/F/1829/2012-13.

Conflict of Interest: All authors have no conflict of interest.

Ethical approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Natural History of Infectious Disease By Macfarlane Burnet, David O. White, Fourth Edition, Cambridge University Press, 1972.
2. <https://www.healthgrades.com/conditions/bacterial-diseases>
3. M. Otto, *Staphylococcus epidermidis* — the 'accidental' pathogen, *Nature Reviews Microbiology* 7 (2009) 555-567
4. J. W. Costerton, P. S. Stewart, E. P. Greenberg, Bacterial biofilms: a common cause of persistent infections, *Science* 284 (1999) 1318-22.
5. J. P. Guggenbichler, O. Assadian, M. Boeswald, and A. Kramer, Incidence and clinical implication of nosocomial infections associated with implantable biomaterials – catheters, ventilator-associated pneumonia, urinary tract infection, *GMS Krankenhaushygiene Interdisziplinär* 6 (2011) ISSN 1863-5245
6. J. Blazquez, A. Oliver, J-M. Gomez-Gomez, Mutation and Evolution of Antibiotic Resistance: Antibiotics as Promoters of Antibiotic Resistance?, *Current Drug Targets*, 3(2002) 345-349
7. H. C. Neu, The crisis in antibiotic resistance, *Science* 257 (1992) 1064-1073
8. D. Mandal, M. E. Bolander, D. Mukhopadhyay, G. Sarkar, P. Mukherjee, The use of microorganisms for the formation of metal nanoparticles and their application, *Appl. Microbiol. Biotechnol.* 69 (2006) 485-492
9. L. Zhang, Y. Jiang, Y. Ding, M. Povey, D. York, Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids), *J. Nanopart. Res.* 9 (2007) 479-489
10. A. Lipovsky, Y. Nitzan, A. Gedanken, R. Lubart, Antifungal activity of ZnO nanoparticles—the role of ROS mediated cell injury, *Nanotechnology* 22 (2011) 105101-105106
11. J. You, Y. Zhang, Z. Hu, Bacteria and bacteriophage inactivation by silver and zinc oxide nanoparticles, *Colloids Surf. B* 85 (2011) 161-167

12. O. Akhavan, E. Ghaderi, Enhancement of antibacterial properties of Ag nanorods by electric field, *Sci Technol Adv. Mater.* 10 (2009) 015003-015007
13. C. X. Xu, A. Wei, X. W. Sun, Z. L. Dong, Aligned ZnO nanorods synthesized by a simple hydrothermal reaction, *J. Phys. D: App. Phys.* 39 (2006) 1690-1693.
14. D. Polsongkram, P. Chamninok, S. Pukird, L. Chow, O. Lupan, G. Chai, H. Khallaf, S. Park, A. Schulte, Effect of synthesis conditions on the growth of ZnO nanorods via hydrothermal method, *Physica B* 403 (2008) 3713-3717
15. J. Song, S. Baek, S. Lim, Effect of hydrothermal reaction conditions on the optical properties of ZnO Nanorods, *Phys. B: Condens. Matter* 403 (2008) 1960-1963.
16. E. Andrade, M. Miki-Yoshida. Growth, structure and optical characterization of high quality ZnO thin films obtained by spray pyrolysis. *Thin Solid Films* 350(1) (1999) 192-202.
17. L. Spanhel, M. A. Anderson, Semiconductor clusters in the sol-gel process: quantized aggregation, gelation, and crystal growth in concentrated zinc oxide colloids, *J. Am. Chem. Soc.* 113 (1991) 2826-2833.
18. J. Zhang, L. D. Sun, J. L. Yin, H. L. Su, C. S. Liao, C. H. Yan, Control of ZnO morphology via a simple solution route, *Chem. Mater.* 14 (2002) 4172-4177.
19. P. Amornpitoksuk, S. Suwanboon, S. Sangkanu, A. Sukhoom, N. Muensit, J. Baltrusaitis, Synthesis, characterization, photocatalytic and antibacterial activities of Ag-doped ZnO powders modified with a diblock copolymer, *Powder Technol.* 219 (2012) 158-164.
20. M. Ashokkumar, S. Muthukumaran, Microstructure, optical and FTIR studies of Ni, Cu co-doped ZnO nanoparticles by co-precipitation method, *Opt Mater.* 37 (2014) 671-678.
21. S. Ameen, M. S. Akhtar, H. K. Seo, Y. S. Kim, H. S. Shin, Influence of Sn doping on ZnO nanostructures from nanoparticles to spindle shape and their photoelectrochemical properties for dye sensitized solar cells, *Chem. Eng. J.* 187 (2012) 351-356.
22. K. R. Raghupathi, R.T. Koodali, A. C. Manna, Size-Dependent Bacterial Growth Inhibition and Mechanism of Antibacterial Activity of Zinc Oxide Nanoparticles, *Langmuir* 27 (2011) 4020-4028.
23. F. Paladini, M. Pollini, A. Sannino, L. Ambrosio, Metal-Based Antibacterial Substrates for Biomedical Applications, *Biomacromolecules* 16 (2015) 1873-1885.
24. J. M. Pearce, Make nanotechnology research open-source. *Nature*, 491(7425) (2012) 519-521.
25. C. Abinaya, M. Marikkannan, M. Manikandan, J. Mayandi, P. Suresh, V. Shanmugaiah, C. Ekstrum, J. M. Pearce, Structural and optical characterization and efficacy of hydrothermal synthesized Cu and Ag doped zinc oxide nanoplate bactericides, *Materials Chemistry and Physics* 184 (2016) 172 -182.
26. O. Akhavan, M. Mehrabian, K. Mirabbaszadeh, and R. Azimirad, Hydrothermal synthesis of ZnO nanorod arrays for photocatalytic inactivation of bacteria, *J. Phys. D: Appl. Phys.* 42 (2009) 225305
27. M. J. Leboffe, B.E. Pierce, *A Photographic Atlas for the Microbiology Laboratory*. 2011: Morton Publishing Company.
28. G. W. Charville, E.M. Hetrick, C. B. Geer, and M. H. Schoenfisch, Reduced bacterial adhesion to fibrinogen-coated substrates via nitric oxide release. *Biomaterials*, 29 (2008) 4039-4044.
29. E. Mosquera, C. Rojas-Michea, M. Morel, F. Gracia, V. Fuenzalida, R. A. Zárat, Zinc oxide nanoparticles with incorporated silver: Structural, morphological, optical and vibrational properties, *Applied Surface Science* 347 (2015) 561–568
30. S. M. Hosseini, I. Abdolhosseini Sarsari, P. Kameli, and H. Salamati, Effect of Ag doping on structural, optical, and photocatalytic properties of ZnO nanoparticles, *Journal of Alloys and Compounds* Volume. 640 (2015) 408–415

31. L. Chow, O. Lupan, G. Chai, H. Khallaf, L.K. Ono, B. Roldan Cuenya, I.M. Tiginyanu, V.V. Ursaki, V. Sontea, A. Schulte, Synthesis and characterization of Cu-doped ZnO one-dimensional structures for miniaturized sensor applications with faster response, *Sensors and Actuators A* 189 (2013) 399–40
32. N. Padmavathy, R. Vijayaraghavan, Enhanced bioactivity of ZnO nanoparticles-an antimicrobial study, *Science and Technology of Adv. Mat.* 9 (2008) 03500466.
33. O. Akhavan and E. Ghaderi, Cu and CuO nanoparticles immobilized by silica thin films as antibacterial materials and photocatalysts, *Surface and Coatings Technology* 205 (2010) 219 - 223
34. O. Akhavan, R. Azimirad, S. Safa and E. Hasani, CuO/Cu(OH)₂ hierarchical nanostructures as bactericidal photocatalysts, *J. Mater. Chem.*, 2011, 21, 9634-9640
35. O. Akhavan and E. Ghaderi, Copper oxide nanoflakes as highly sensitive and fast response self-sterilizing biosensors, *J. Mater. Chem.*, 2011, 21, 12935-12941
36. O. Akhavan, Lasting antibacterial activities of Ag–TiO₂/Ag/a-TiO₂ nanocomposite thin film photocatalysts under solar light irradiation, *Journal of Colloid and Interface Science* 336 (2009) 117–124
37. <http://www.antimicrobe.org/b237tabrev.htm>, *Staphylococcus aureus* Tables.
38. Y. Liu, X. Wang, F. Yang, X. Yang, Excellent antimicrobial properties of mesoporous anatase TiO₂ and Ag/TiO₂ composite films, *Microporous and Mesoporous Materials* 114 (2008) 431–439;
39. O. Akhavan, M. Abdollahad, Y. Abdicd and S. Mohajerzadeh, Silver nanoparticles within vertically aligned multi-wall carbon nanotubes with open tips for antibacterial purposes, *J. Mater. Chem.*, 2011, 21, 387–393;
40. M. Kawashita, S. Toda, H. M. Kim, T. Kokubo, N. Masuda, Preparation of antibacterial silver-doped silica glass microspheres, *J. Biomed. Mater. Res.* 66 (2003) 266-274
41. M. Kawashita, S. Tsuneyama, F. Miyaji, T. Kokubo, H. Kozuk, K. Yamamoto, Antibacterial silver-containing silica glass prepared by sol gel method, *Biomaterials* 21 (2000) 393-398
42. A. Balamurugan, G. Balossier, D. Laurent-Maquin, S. Pina, A. H. S. Rebelo, J. Faure, J. M. F. Ferreira, An in vitro biological and anti-bacterial study on a sol–gel derived silver-incorporated bioglass system, *dental materials* 24 (2008) 1343–1351
43. O. Akhavan, E. Ghaderi, Bactericidal effects of Ag nanoparticles immobilized on surface of SiO₂ thin film with high concentration, *Current Applied Physics* 9 (2009) 1381–1385;
44. O. Akhavan, E. Ghaderi, Capping antibacterial Ag nanorods aligned on Ti interlayer by mesoporous TiO₂ layer, *Surface & Coatings Technology* 203 (2009) 3123–3128

Table Captions

Table 1: EDX results quantitative weight and atomic percentage of the as synthesized samples of pure ZnO, CuZnO and AgZnO. * The presence of carbon is due to the carbon grid.

Table 2: Quantitative streak plate results comparing bacterial density present in each vial for all six materials tests (ZnO #1, ZnO #2, CuZnO, CuCl₂, AgZnO, AgCl) and the control (TSB).

Figure Captions

Figure 1: Open source hydrothermal synthesis method flow chart for preparation of nanoscale ZnO

Figure 2: XRD spectra for ZnO#1, ZnO#2, CuZnO and AgZnO

Figure 3: SEM micrographs for ZnO#1, ZnO#2, CuZnO and AgZnO

Figure 4: XPS spectra for ZnO#1, ZnO#2, CuZnO and AgZnO

Figure 5: Streak plate images after 24 hours incubation for all six materials (ZnO #1, ZnO #2, CuZnO, CuCl₂, AgZnO and AgCl) tested and the controls.

Sample Name	Element	Weight %	Atomic %
ZnO	Zn	78.45	46.60
	O	20.18	48.98
	C*	1.37	4.41
CuZnO	Zn	66.70	33.81
	O	27.51	56.97
	Cu	3.02	1.58
	C*	2.77	7.64
AgZnO	Zn	68.47	38.42
	O	23.66	54.25
	Ag	6.15	2.09
	C*	1.72	5.24

Table 1

Material	Plate #	Bacterial Density, x 10 ⁶ CFU/ml	Material	Plate #	Bacterial Density, x 10 ⁶ CFU/ml
ZnO #1 (as prepared)	1	8.09	ZnO #2 (annealed)	1	0
	2	4.91		2	0
	3	10.3		3	0
	Average	7.75 ± 2.69		Average	0.00 ± 0.00
CuZnO	1	0	CuCl ₂	1	10.6
	2	0		2	7.65
	3	0		3	5.24
	Average	0.00 ± 0.00		Average	7.83 ± 2.68
AgZnO	1	0	AgCl	1	4.03
	2	0		2	7.77
	3	0		3	3.45
	Average	0.00 ± 0.00		Average	5.08 ± 2.34
TSB	1	3.87			
	2	3.09			
	3	5.69			
	Average	4.22 ± 1.33			

Table 2

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5