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Synergistic Antibacterial Effect of Metal Oxid Nanoparticles and Ultrasound Stimulation

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ABSTRACT

Recently, use of different nanoparticle metal oxides for preventing the spread of microorganisms has reached to the expanding field of nanomaterial research. The objective of this study is to validate combined ultrasound and CuO or MgO noparticle treatments for inactivating *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Results showed that nanoparticles of different materials vary in their effectiveness. Ultrasound increased the antibacterial effect of CuO nanoparticles more than the increased antibacterial effect of MgO. These results indicated that CuO or MgO nanoparticles exhibited antibacterial properties that could be additionally enhanced in the presence of ultrasound and, thus, should be further studied for a wide range of medical device anti-infection applications.

Key words: CuO nanoparticles, combined effect, MgO nanoparticles, *Staphylococcus aureus, Pseudomonas aeruginosa,* ultrasound

1. INTRODUCTION

The recent rise of antibiotic-resistant microorganisms has led to serious health problems. There are an increasing number of patients with bacterial infection that are resistant to at least one of the antibiotics which are generally used to eradicate the disease-causing bacteria**.** This problem encourages researchers to study the new advanced methods for characterizing antimicrobial agents which can effectively prevent bacterial growth (1). The inorganic antimicrobial agents have attracted great interest in recent years for the control of microbs. The key advantages of inorganic antimicrobial agents compared with the organic ones are improved safety and stability (2-9). Applications of nanotechnology in pharmaceuticals and microbiology have been promising to overcome resistance in infectious diseases. Various antibacterial agents, particularly nanoparticles such as metal and metal oxide, have been applied by researchers against various bacteria (1). Nanoparticles are much more active than larger-sized particles because of their much higher surface area. They also exhibit unique physical and chemical properties (6, 10, 11).

Several types of metal and metal oxide nanoparticles such as silver (Ag), silver oxide (Ag_2O), titanium dioxide (TiO_2), zinc oxide (ZnO), gold (Au), calcium oxide (CaO), silica (Si), copper oxide (CuO), and magnesium oxide (MgO) have been known to show antimicrobial activity (1). Metal or metal ions are also essential elements for human body and play a role in over 300 enzyme reactions in the body (12-14). The effect of ultrasound on the bacteria with and without conventional antibiotics has been previously specified. A multitude of studies have clearly demonstrated that ultrasound can increase the effectiveness of antibiotics. It has been demonstrated that low-intensity ultrasound could increase the bacterial proliferation due to enhanced diffusion of nutrients, oxygen, and metabolic waste products, as expected, the enhanced transport of antibiotic molecules has been shown to have a net inhibitory effect on bacteria (15, 16). However, there are few reports on the antimicrobial activity of MgO or CuO nanoparticles in the literature, which describe the use of MgO or CuO nanoparticles in combination with ultrasound to kill or inhibit the growth of pathogens. Thus, the objective of the present study is to determine, for the first time, the combined influence of ultrasound and MgO or CuO nanoparticles on inhibiting the functions of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Clearly, identifying techniques to enhance the antibacterial effect of

nanoparticles may promote the adoption of the clinical use of these novel materials.

2. MATERIALS AND METHODS

2.1. Materials

Standard strains of Staphylococcus aureus (PTCC: 1431) and Pseudomonas aeruginosa (PTCC: 1074) used in this research were collected from the Iran as lyophilized microorganisms. This strain of bacteria was cultured in trypticase agar medium (TSA, Merck, Germany) and was stored at 0-2°C for use in the subsequent steps. CuO nanoparticles used in this study was obtained from USA Aldrich company and had the purity of %98/99. Also, MgO nanoparticles of US Nano Company with the purity of %98/99 were prepared.

2.2. Antibacterial effect of ultrasound

Preliminary experiments which were performed in the absence of nanoparticles tested the effect ultrasound devices on the viability of bacteria. Bacterial suspensions were prepared as described above to produce cell populations with the density of 1x107 in 5 ml. S. aureus and P. aeruginosa suspensions were transferred to glass vials for experimentation. The ultrasound device, a water bath sonicator (Elma sonic, Germany), was operated setting for 1, 5, and 10 min. Immediately after the ultrasound stimulation, the samples were processed to determine the viable colony-forming unit density (15, 16).

2.3. Antibacterial activity of CuO and MgO nanoparticles under static conditions

A second group of experiments evaluated the antibacterial effect of nanoparticles in the absence of ultrasound stimulation. *S. aureus* and *P. aeruginosa* suspensions were prepared as described above to produce the cell populations at the density of 1 x 10^7 CFU ml^{-1} in 5 ml of TSB media. Nanoparticles of CuO with the diameter of about <50 nm or MgO with the diameter of 20-30 nm were then added to 1x 10^7 CFU ml^{-1} *S. aureus* or *P. aeruginosa* bacterial suspensions at the concentrations of 100; 250, or 500 µg ml^{-1}.The samples were placed in an incubator at 37°C for 24 h. After 24 h, the samples were serially diluted and processed to determine the viable colony-forming unit density (15-18).

2.4. Antibacterial activity of the combination of CuO or MgO nanoparticles and ultrasound

Finally, the experiments were conducted to evaluate the effect of the combination of CuO or MgO nanoparticles and ultrasound stimulation. CuO or MgO nanoparticles were added at the concentration of 250 and 500 µg ml^{-1} to 5 ml of *S. aureus* or *P. aeruginosa* cultures in glass vials at the cell density of 1 x 10^7 CFU ml^{-1}. Cell suspensions were ultrasonically stimulated by the ultrasound device, as described above, setting for 5 and 10 min. The samples were then placed in an incubator at 37°C. At 6 h and 24 h, they were serially diluted and plated, as previously described. After an overnight incubation of the plated samples, the visible CFU was counted (15, 16).

2.6. Statistical analysis

Numerical data were analyzed for significance using analysis of variance. Experiments were repeated for three times. Values were reported as mean ± SEM. The threshold for significance was set at $p < 0:05$.

3. RESULTS AND DISCUSSION

3.1. Results of bacterial activity with ultrasound stimulus

Ultrasound stimulation with the water bath sonicator (Figure 1) devices did not result in the reduced viability of the bacterial species tested in any of the considered exposure periods. The mechanism of action of ultrasound energy on bacteria is complex and parameter-dependent. However, ultrasound has the ability or capacity for bactericidal activity either alone or in association with an additional antimicrobial agent. While low intensity ultrasound may prompt cell proliferation, high intensity ultrasound has the ability or capacity for killing cells (15, 16). Johnson et al. investigated the range of time necessary to completely destroy a biofilm with low frequency ultrasound (19). As determined by total population counts, a bacteria biofilm grown for 14 h was completely destroyed after 6 h of ultrasound exposure. Low frequency ultrasound (26 kHz) was shown to kill a wide range of microorganisms (including *S. aureus, P. aeruginosa, B. subtilis, and E. coli*) in a time-dependent manner (20). With the exception of *E. coli*, all of the microorganisms were killed in a dose-dependent manner as well.

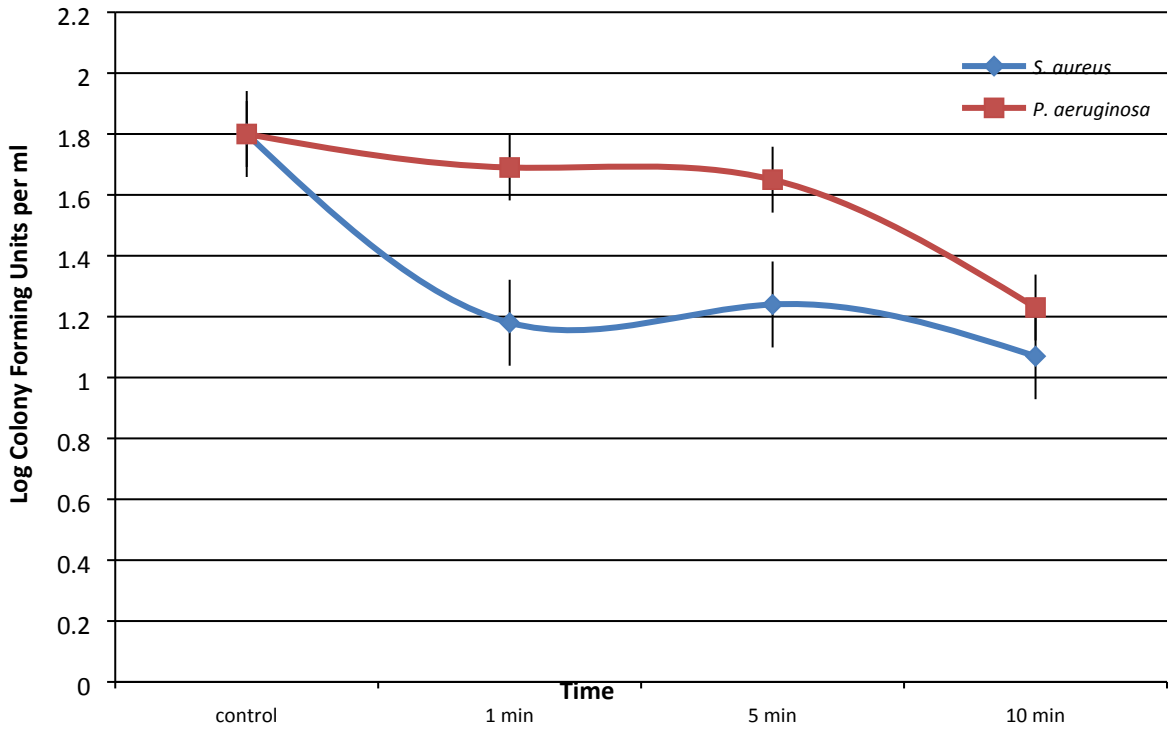

Figure 1. Ultrasound stimulation of bacteria in a waterbath sonicator for 1-10 min did not significantly reduce viability compared to unstimulated control groups for either *S. aureus* or *P. aeruginosa*. Values are mean ± SEM; N = 3.

3.2. Results of bacterial activity in the presence of MgO or CuO nanoparticles

MgO nanoparticles did not significantly reduce the growth of *S. aureus*. *P. aeruginosa*; however, they were significantly reduced (8.55% and 9.01% reduction compared to the control, respectively) in the presence of the 500 µg/ml concentration of MgO nanoparticles (Figure 2). Makhluf et al. (2005) studied the antibacterial activities of MgO and demonstrated the following antibacterial mechanisms:

Active oxygen production due to the presence of MgO, attractive interaction between MgO nanoparticles and cell wall, diffusion of MgO nanoparticles into cells, and reformation of MgO within the cell (21). Stoimenov et al. (2002), on the other hand, indicated that electrostatic interactions between the bacterial surface and MgO nanoparticles killed the bacteria (22). CuO nanoparticles significantly reduced the growth of both *S. aureus* and *P. aeruginosa* at the concentration of 500 µg/ml and also reduced the growth of *S. aureus* and *P. aeruginosa* by 24% and 7.9%, respectively (Figure 2). Copper, like silver, showed antimicrobial properties. Copper nanoparticles reduced *E. coli* and *B. subtilis* by 90% at the concentrations of 33.40 µg/ml and 28.20 µg/ml, respectively (23). The mechanism which the copper nanoparticles reduced the number of viable bacteria was related to protein inactivation, specifically cysteine-containing enzymes, via thiol interactions (15).

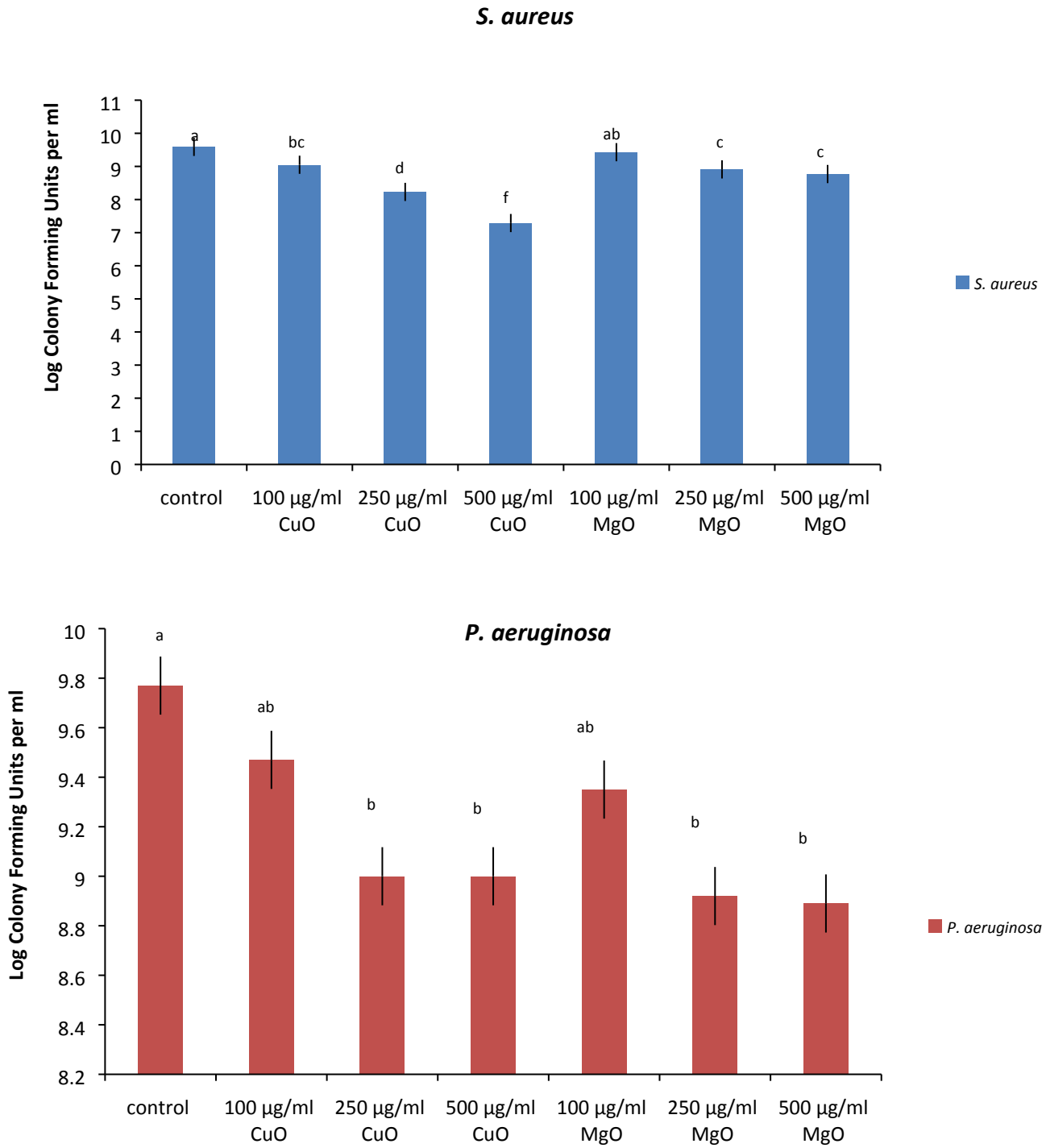

Figure 2. Growth of *S. aureus* (top) and *P. aeruginosa* (bottom) in the presence of CuO or MgO at 100 µg/ml, 250 µg/ml or 500 µg/ml for 24 h. Values are mean ± SEM; N = 3; **p* < 0.05 (compared to control).

3.3 Results of bacterial activity with CuO or MgO nanoparticles and ultrasound stimulus

CuO nanoparticles and a 5 or 10 min ultrasound stimulus reduced *S. aureus* or *P. aeruginosa* viability so effectively that was comparable with CuO nanoparticles. At 24 h, 500 µg/ml of CuO nanoparticles and 10 min ultrasound stimulus reduced *S. aureus* or *P. aeruginosa* viability by about 2.63 log and 2.88 compared to the control, respectively (Figure 3). The nanoparticles, due to their increased functional surface area and potential to penetrate into cell membranes, were more effective antimicrobial agents (6, 10, 11).

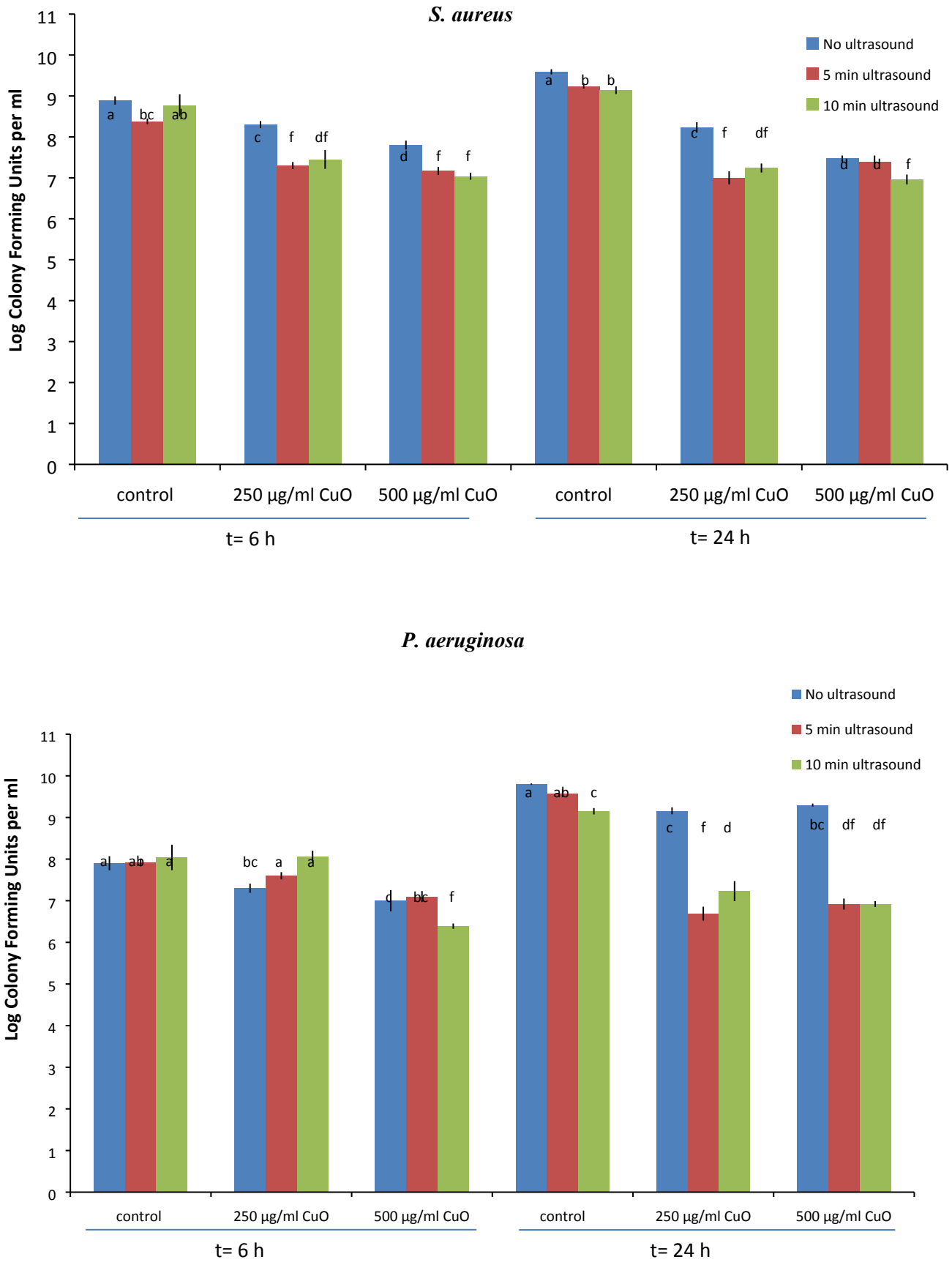

Figure 3. Reduced *S. aureus or P. aeruginosa* in the presence of CuO nanoparticles and ultrasound stimulus. The addition of ultrasound enhanced the antibacterial effect of CuO nanoparticles. Values are mean ± SEM; N = 3; #$p < 0.05$ (compared to control at the same time point).

At the concentrations of 500 µg/ml, MgO nanoparticles reduced *S. aureus* viability under static conditions by approximately about 0.82 log at 24 h, respectively (Figure 4). The addition of 5 or 10 min ultrasound stimulus to the *S. aureus* suspension with 500 µg/ml of MgO nanoparticles had no significant inhibition in *S. aureus* density. MgO nanoparticles and 5 or 10 min ultrasound stimulus reduced *P. aeruginosa* viability so effectively that was comparable with MgO nanoparticle alone. However, ultrasound did not significantly increase the antibacterial

effect of MgO nanoparticles (Figure 4).

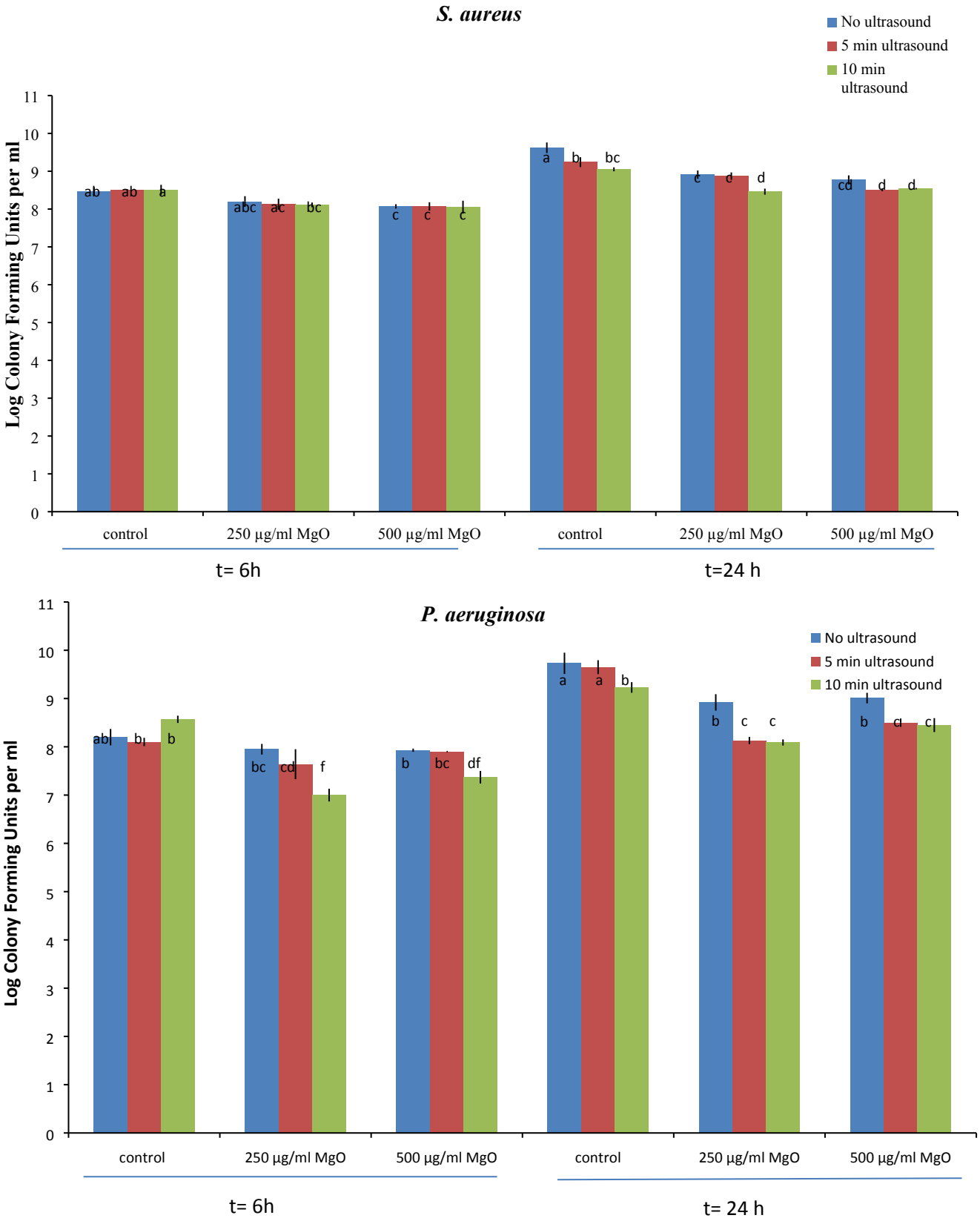

Figure 4. Reduced *S. aureusor* or *P. aeruginosa in* the presence of MgO nanoparticles and ultrasound stimulus. The addition of ultrasound enhanced the antibacterial effect of MgO nanoparticles. Values are mean ± SEM; N = 3; #$p < 0.05$ (compared to control at the same time point).

Justin and Thomas (2012) investigated the antimicrobial effect of ZnO nanoparticles combined with ultrasound. Results showed that addition of ultrasound increased the antimicrobial effect of ZnO nanoparticles (16). However, there are few reports on the antimicrobial activity of nanoparticles in the articles describing the use of nanoparticles in combination with ultrasound to kill or inhibit the growth of bacteria. The antibacterial and antibiofilm mechanisms of nanoparticles may be definitely increased in combination with ultrasound. Specifically,

physical interactions between the nanoparticle and bacterial membrane may be enhanced due to nanoparticle disassociation; also, nanoparticle penetration into cell membranes may be enhanced through ultrasound stimulation. Furthermore, antimicrobial metal ions may be released from particle surfaces more rapidly in the presence of ultrasound stimulation (15, 16).

4. CONCLUSION

Due to the ever-increasing ineffectiveness of traditional antibiotics, nanoparticles have received greater attention for their potential antimicrobial effects and applications. In vitro studies have identified nanoparticle concentrations which inhibit a variety of bacteria species, including *S. aureus* and *P. aeruginosa*. Nanoparticles of different materials vary in their effectiveness. Ultrasound increased the antibacterial effect of CuO nanoparticles more than the increase in the antibacterial effect of MgO.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

REFERENCES

1. Dizaj SM, Mennati A, Jafari S, Khezri K, Adibkia K. Antimicrobial activity of carbon-based nanoparticles. Advanced pharmaceutical bulletin. 2015;5(1):19.
2. Sawai J, Yoshikawa T. Quantitative evaluation of antifungal activity of metallic oxide powders) MgO, CaO and ZnO) by an indirect conductimetric assay. Journal of applied microbiology. 2004;96(4):803-9.
3. Guo T, Cao S, Su R, Li Z, Hu P, Xu Z. Adsorptive property of Cu 2+-loaded montmorillonite clays for Escherichia coli K 88 in vitro. Journal of Environmental Sciences. 2011;23(11):1808-15.
4. Jin T, He Y. Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. Journal of Nanoparticle Research. 2011;13(12):6877-85.
5. Zhang L, Jiang Y, Ding Y, Povey M, York D. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). Journal of Nanoparticle Research. 2007;9(3):479-89.
6. Mirhosseini M, Barzegari Firouzabadi F. Preparation of ZnO-Polystyerne Composite Films and Investigation of Antibacterial Properties of ZnO-Polystyerne Composite Films. Iranian Journal of Pathology. 2014;9(2):99-106.
7. Seven O, Dindar B, Aydemir S, Metin D, Ozinel M, Icli S. Solar photocatalytic disinfection of a group of bacteria and fungi aqueous suspensions with TiO 2, ZnO and Sahara desert dust. Journal of Photochemistry and Photobiology A: Chemistry. 2004;165(1):103-7.
8. Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF, Fiévet F. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Letters. 2006;6(4):866-70.
9. Sawai J. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. Journal of Microbiological Methods. 2003;54(2):177-82.
10. El-Nahhal I, Zourab S, Kodeh F, Selmane M, Genois I, Babonneau F. Nanostructured copper oxide-cotton fibers: synthesis, characterization, and applications. International Nano Letters, 2012. 2012.
11. Jones N, Ray B, Ranjit KT ,Manna AC. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS microbiology letters. 2008;279(1):71-6.
12. Wilczynski M, editor Anti-Microbial Porcelain Enamels. 62nd Porcelain Enamel Institute Technical Forum: Ceramic Engineering and Science Proceedings, Volume 21, Issue 5; 2000: Wiley Online Library.
13. Roselli M, Finamore A, Garaguso I, Britti MS, Mengheri E. Zinc oxide protects cultured enterocytes from the damage induced by Escherichia coli. The Journal of nutrition. 2003;133(12):4077-82.
14. Sandstead HH. Understanding zinc: recent observations and interpretations. The Journal of laboratory and clinical medicine. 1994;124(3):322-7.
15. Seil JT, Webster TJ. Antibacterial effect of zinc oxide nanoparticles combined with ultrasound. Nanotechnology. 2012;23(49):495101.
16. Seil JT, Webster TJ. Antimicrobial applications of nanotechnology: methods and literature. International journal of nanomedicine. 2012;7:2767.
17. Firouzabadi FB, Noori M, Edalatpanah Y ,Mirhosseini M. ZnO nanoparticle suspensions containing citric acid as antimicrobial to control Listeria monocytogenes, Escherichia coli, Staphylococcus aureus and Bacillus cereus in mango juice. Food Control. 2014;42:310-4.
18. Mirhosseini M, Arjmand V .Reducing Pathogens by Using Zinc Oxide Nanoparticles and Acetic Acid in Sheep Meat. Journal of Food Protection®. 2014;77(9):1599-604.
19. Johnson LL, Vaughn Peterson R, Pitt WG. Treatment of bacterial biofilms on polymeric biomaterials using antibiotics and ultrasound. Journal of Biomaterials Science, Polymer Edition. 1998;9(11):1177-85.
20. Scherba G, Weigel R, O'brien W. Quantitative assessment of the germicidal efficacy of ultrasonic energy. Applied and Environmental Microbiology. 1991;57(7):2079-84.
21. Makhluf S, Dror R, Nitzan Y, Abramovich Y, Jelinek R, Gedanken A. Microwave-Assisted Synthesis of Nanocrystalline MgO and Its Use as a Bacteriocide. Advanced Functional Materials. 2005;15(10):1708-15.
22. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. Langmuir. 2002;18(17):6679-86.
23. Yoon K-Y, Byeon JH, Park J-H, Hwang J. Susceptibility constants of Escherichia coli and Bacillus subtilis to silver and copper nanoparticles. Science of the Total Environment. 2007;373(2):572-5.