Environ Monit Assess (2017) 189: 342
DOI 10.1007/s10661-017-6049-5

The bactericidal effect of simultaneous titanium oxide on common hospital bacteria

Ziaeddin Bonyadi · Maisam Mirzaee · Mohammad Mehdi Ejtehadi · Mehdi Mokhtari

Received: 2 January 2017 / Accepted: 6 June 2017 / Published online: 16 June 2017

Abstract Bacteria prevalence has increased in health centers and hospitals. Infection control can decrease the number of bacteria. Bacteria cause 90% of nosocomial infections and fungi, viruses, or protozoa are less involved. Bacteria control can decrease the number of diseases. The Simultaneous titanium oxide (SMTiO) is a new method of disinfection. This process degrades bacteria by producing hydroxyl radical. The present study was conducted with the aim of analyzing the bactericidal effect of simultaneous titanium oxide on common hospital bacteria. In this study, SMTiO system was placed under a laminar hood. *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *and Citrobacter freundii* were selected as common hospital bacteria. Treatment operations were conducted at the bacteria concentration 10^6 cells/mL and the exposure times of 1 to 48 h. Then, the bacteria colonies were counted and removal rate calculated separately each of the bacteria. Finally, data analysis was conducted by using one-way ANOVA. Results showed that *E. coli* and *C. freundii* had the highest removal rate (40%) and the lowest removal (14%), respectively. Results confirmed that SMTiO can degrade Gram-positive and Gram-negative bacteria, but its effects were lower on Gram-positive bacteria. Results showed that removal efficiency increased gradually during exposure periods. We recommend that future studies should analyze the structure of bacteria after the treatment with SMTiO.

Keywords Bactericide · SMTiO · OH radical · Nosocomial infection

Z. Bonyadi · M. Mokhtari (✉)
Environmental Science and Technology Research Center, Department of Environmental Health Engineering, Shahid Sadoughi University of Medical Science, Yazd, Iran
e-mail: Mokhtari@ssu.ac.ir

Z. Bonyadi
MSc, Department of Environmental Health, Faculty of Health, Mashhad University of Medical Science, Mashhad, Iran

M. Mirzaee
Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

M. M. Ejtehadi
Mashhad University of Medical Science, Mashhad, Iran

Introduction

Microorganisms grow in different environments. Their prevalence has increased in health centers and hospitals (Pedrosa and Cardoso 2011). They can cause interference in individuals' immune system and increase their treatment cost. The rate of hospital infections ranging from 5 to 10% has been reported in developed countries (Haley 1995). Bacteria cause 90% of nosocomial infections, while fungi, viruses or protozoa are less involved in this regard (Jain and Singh 2007). *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Citrobacter freundii* are the pathogens commonly found in hospitals (Simon and Jack 2015). Pastuszka et al. (2005) reported that 57 to 78% of hospitals in Poland are contaminated by

Staphylococcus and *Micrococcus* (Pastuszka et al. 2005). Karlowsky et al. (2004) confirmed that *S. aureus*, *Klebsiella*, and *E. coli* are the most common bacteria in the blood samples of the patients of hospital (Karlowsky et al. 2004). Bacteria control can decrease the number of diseases. Owing to bacteria resistance against chemicals, usual chemicals methods of disinfection cannot be effective in the long term. Using various chemicals can have harmful effects. In the past centuries, researchers used a variety of physical and chemical methods against microorganisms. These methods include oven, autoclave, dry heat, ethylene oxide, and gamma rays. Using these methods has a number of disadvantages such as increasing power consumption and remaining toxic substances (Mirzanejhad et al. 2008). SMTiO is a new method of disinfection. The electrical discharge is conducted on the plate of poly silicate titanium, coated by poly hydroxyl alkanoates (PHA). Due to passing an electrical current through the plate of poly silicate titanium, electrons are transported from valence band to conduction band. The result of electron hole pair and molecules occurs on the surface of the semiconductor and a variety of hydroxide radicals are produced in the air (Cai et al. 1992). Depending on laboratory conditions, degrading microorganisms conducted by a variety of hydroxyl radicals (Mills and Le 1997). As a result, water and carbon dioxide gas are produced by the reaction between microorganisms and hydroxyl radicals (Jacoby et al. 1998). Studies indicated that hydroxyl radical plays a major role in the degradation of microorganism (Wamer et al. 1997; Jaeger and Bard 1979; Harbour and Hair 1986; Ireland and Valinieks 1992; Saito et al. 1992; Ireland et al. 1993). Silva et al. (2010) used the mist of hydrogen peroxide for the disinfection of hospital environment (Silva et al. 2010). The present study was conducted with the aim of analyzing the bactericidal effect of simultaneous titanium oxide on common hospital bacteria.

Materials and methods

This study was conducted in the basic medical diagnosis laboratory of Doctor Ejtehadi in Mashhad. In this study, SMTiO system (model of SM3427, Iran) was placed under a laminar hood. *E. coli (*PTCC 1270), *K. pneumonia* (PTCC 1290), *S. aureus (*PTCC 1431), *P. aeruginosa (*PTCC 1181), *and C. freundii* (PTCC 1600) were selected as the common hospital bacteria. The bacterial cells, including *E. coli*, *K. pneumonia*, and *C. bacteria* were cultured on nutrient agar medium. *S. aureus* was grown on Trypticase soy agar. 0.1 mL of the bacterial cells with the concentration of 10^6 cells/mL was cultured on the surface of plastic plates containing sterile solid medium at 35.5 °C for 24 h. Then, the bacterial cultures on solid media were treated with SMTiO for 1, 5, 10, 24, and 48 h (Karlowsky et al. 2004). The concentration of hydroxyl radical produced by SMTiO was 1.84 m^3/h, being constant in all experimental procedures. Treatment operations were conducted at room temperature (26–28 °C). Control samples were separately placed in a laboratory hood, but no SMTiO injection was used. To ensure the regrowth of bacteria, the treated plates were incubated for 48 h. Then, the bacteria colonies were counted and removal rate was separately calculated for each of the bacteria. Finally, data analysis was conducted by using one-way ANOVA.

Results

In this study, the effect of parameters such as bacteria genera and exposure time was studied on the removal efficiency of bacteria. Figure 1 shows the effect of exposure time (1–48 h.) on the removal efficiency of *E. coli*, *K. pneumonia*, *S. aureus*, *P. aeruginosa*, and *C. freundii*. The highest removal rate of bacteria was 40% at the exposure time of 48 h. Figure 2 demonstrates the bactericidal effect of SMTiO on *E. coli*, *K. pneumonia*, *P. aeruginosa*, C. *freundii*, and *S. aureus* in 48 h. *E. coli*, and *C. freundii* had the highest removal rate (40%) and the lowest removal (14%), respectively.

Discussion

SMTiO technology produces the short circuit and spark using corona discharge. Then, the voltage with constant current was produced and titanium plates coated with PHB were charged. This is caused by changing the surrounding air of plate, increasing the adsorption of double bonds. Finally, free radicals chiefly produced OH radicals. The attack of hydroxide radicals to the cell wall leads to degrade bacteria (Tortora et al. 2010). SMTiO with degrading protein and lipid bonds in the membrane of bacteria can cause the death of cells.

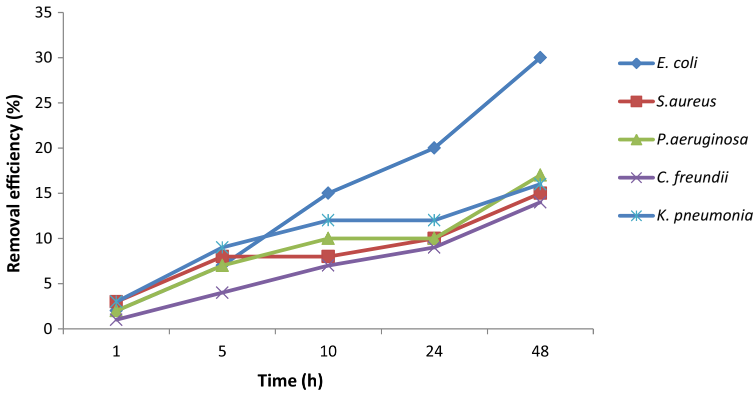

Fig. 1 Effect of exposure time on the removal efficiency of *E. coli*, *S. aureus*, *P. aeruginosa*, *C. freundii*, and *K. pneumonia*

Dobrynin et al. (2011) showed that oxidative methods such as natural active species, ozone, H_2O_2, and hydroxyl radicals as well as atomic oxygen destroy the bacteria (Dobrynin et al. 2011). Similar studies indicated that using corona discharge can produce oxygen radicals and hydrogen peroxide, and OH radical is considered the most effective agent (Cai et al. 1992; Dunford et al. 1997).

This study confirmed that this method can remove Gram-negative and Gram-positive bacteria. The bactericidal effect of SMTiO was more significant against Gram-negative bacteria than Gram-positive bacteria. *E. coli* and *P. aeruginosa* had the highest removal rate and their resistance was less. The highest removal rate was 40, 19, 17, 16, and 10%, respectively, *for E. coli*, *P. aeruginosa*, *K. pneumonia*, *C. freundii*, and *S. aureus* in 48 h. The complexity and density of the cell wall are the main factors affecting the resistance of bacteria against environmental elements. The cell wall of Gram-negative bacteria such as *P. aeruginosa* and *E. coli* is thin and slack, but in Gram-positive bacteria such as *S. aureus*, it is thick and dense (Klaus et al. 2003). The thickness of cell capsule affects bacteria resistance against SMTiO (Pastuszka et al. 2005).

As Fig. 1 shows, *K. pneumonia* compared to *E. coli* is resistant to SMTiO, since the capsule of *E. coli* was extremely thin and less dense so that it was less than 1/10 as thick as that of *K. pneumonia* (Pastuszka et al. 2005). Laroussi et al. (2003) confirmed that the cell wall of Gram-negative bacteria was more vulnerable than Gram-positive bacteria, since Gram-positive bacteria had stable peptidoglycan structure (Laroussi et al. 2003). Kowalski et al. (1998) indicated that using ozone can reduce 99.99% of *E. coli* (Kowalski et al. 1998). According to Fig. 2, there is a direct relationship between the removal efficiency of bacteria and exposure time. Results showed that removal efficiency increased gradually during exposure periods ($P < 0.05$). This is because with increasing time, SMTiO has enough time to produce hydroxyl radicals and degrade the cell wall of bacteria.

Roth et al. concluded that using active species of plasma can prominently reduce both *E. coli* and *S. aureus* in 25 min (Roth et al. 2000). Boxhammer et al. (2012) indicated that using cold atmospheric plasma can reduce bacteria (with density of 10^5 cells per 20 μl) up to five logs in 5 min (Boxhammer et al. 2012).

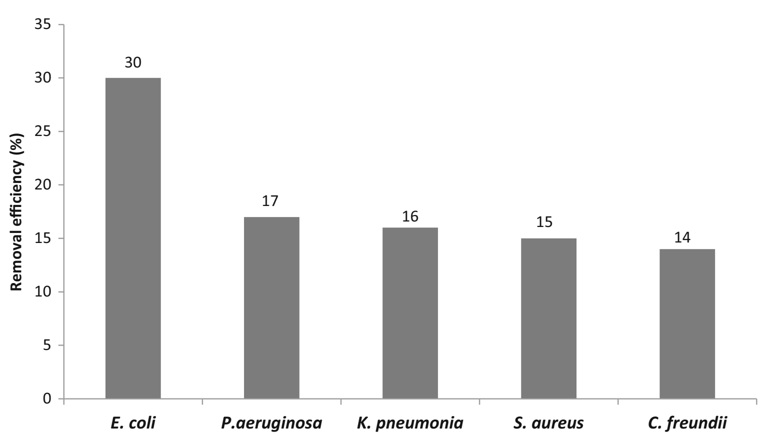

Fig. 2 Bactericidal effect of SMTiO on *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. aureus*, and *C. freundii* in 48 h

Conclusion

Results indicated that SMTiO can degrade Gram-positive and Gram-negative bacteria, but its effects were lower on Gram-positive bacteria. Results showed that removal efficiency increased gradually during exposure periods. We recommend that future studies should analyze the structure of bacteria after the treatment.

References

Boxhammer, V., Morfil, G. E., Jokipii, J. R., Shimizu, T., Klämpfl, T., Li, Y.-F., Köritzer, J., Schlegel, J., & Zimmermann, J. L. (2012). Bactericidal action of cold atmospheric plasma in solution. *New Journal of Physics, 14*, 113042 (18pp).

Cai, R., Kubota, Y., Shuin, T., Sakai, H., Hashimoto, K., & Fujishima, A. (1992). Induction of cytotoxicity by photoexcited TiO_2 particles. *Cancer Research, 52*, 2346–2348.

Dobrynin, D., Friedman, G., Fridman, A., & Starikovskiy, A. (2011). Inactivation of bacteria using dc corona discharge: role of ions and humidity. *New Journal of Physics, 13*, 1–14.

Dunford, R., Salinaro, A., Cai, L., Serpone, N., Horikoshi, S., Hidaka, H., & Knowland, J. (1997). Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients. *FEBS Letters, 418*, 87–90.

Haley, R. W. (1995). The scientific basis for using surveillance and risk factor data to reduce nosocomial infection rates. *Journal of Hospital Infecttion., 30*, 3–14.

Harbour, J. R., & Hair, M. L. (1986). Transient radicals in heterogeneous systems: detection by spin trapping. *Advaces in Colloid and Interface Science, 24*, 103–141.

Ireland, J. C., & Valinieks, J. (1992). Rapid measurement of aqueous hydroxyl radical concentrations in steady-state HO flux systems. *Chemosphere, 25*, 383–396.

Ireland, J. C., Klostermann, P., Rice, E. W., & Clark, R. M. (1993). Inactivation of *Escherichia coli* by titanium dioxide photocatalytic oxidation. *Applied and Environmental Microbiology, 59*, 1668–1670.

Jacoby, W. A., Maness, P. C., Wolfrum, E. J., Blake, D. M., & Fennell, J. A. (1998). Mineralization of bacterial cell mass on a photocatalytic surface in air. *Environmental Science Technology, 32*, 2650–2653.

Jaeger, C. D., & Bard, A. J. (1979). Spin trapping and electron spin resonance detection of radical intermediates in the photodecomposition of water at TiO2 particulate systems. *Journal Physical Chemistry, 83*, 3146–3152.

Jain, A., & Singh, K. (2007). Recent advances in the management of nosocomial infections. *Journal of medical eduction and Science, 9*(1), 3–8.

Karlowsky, J. A., Jones, M. E., Draghi, D. C., Thornsberry, C., Shahm, D. F., & Volturo, G. A. (2004). Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States. *Annals of Clinical Microbiology and Antimicrobials, 10*(3), 1–8.

Klaus, P., Iris, F., Karl, M., Manfred, S., Volker, W., Hans-Gunther, S., & Lothar, E. (2003). *D*isinfection of surfaces by photocatalytic oxidation with titanium dioxide and UVA light. *Chemosphere, 53*, 71–77.

Kowalski, W., William, P. B., & Whittam, T. (1998). *B*actericidal effect of high airborne ozone concentrations on *Escherichia coli* and *Staphylococcus aureus*. *Ozone Science and Engineering, 1998*(20), 205–221.

Laroussi, M., Mendis, D. A., & Rosenberg, M. (2003). Plasma interaction with microbes. *Journal of Physics, 5*, 41.1–41.10.

Mills, A., & Le, H. S. (1997). An overview of semiconductor photocatalysis. *Journal of Photochemistry and Photobiology A: Chemistry, 108*(1), 1–35.

Mirzanejhad, S., Sohbatzadeh, F., Hosseinzadeh Colagar, A., & Asadian, N. (2008). Bactericide effect of atmospheric plasma of flat and comb electrodes on *Escherichia coli*. *Cell Journal, 9*(4), 248–253.

Pastuszka, J. S., Marchwińska-Wyrwał, E. and. Wlazło, A. (2005) Bacterial aerosol in Silesian hospitals: preliminary results. Polish Journal of Environmental Studies. 14(6), 883–890.

Pedrosa, P. B., & Cardoso, T. A. (2011). Viral infections in workers in hospital and research laboratory settings: a comparative review of infection modes and respective biosafety aspects. *International Journal of Infectious Diseases, 15*, 336–376.

Roth, J. R., Sherman, D., Ben Gadri, R., Karakaya, F., Chen, Z. Y., Montie, T. C., Kelly-Wintenberg, K., & Tsai, P. (2000). A remote exposure reactor (RER) for plasma processing and sterilization by plasma active species at one atmosphere. *IEEE Transactions on Plasma Science, 28*, 56–63.

Saito, T., Iwase, T., Horie, J., & Morioka, T. (1992). Mode of photocatalytic bactericidal action of powdered semiconductor TiO_2 on mutans streptococci. *Journal of Photochemocal Photobiology, 14*, 369–379.

Silva, A. P., Pina-Vaz, C., Rodrigues, A. G., & Carvalho, T. (2010). Efficacy of a hydrogen peroxide dry-mist disinfection systemfor hospital environment disinfection. *Journal of Hospital Infection, 76*(1), S23.

Simon, L., & Jack, A. (2015). Hospital-associated microbiota and implications for nosocomial infections. *Trends in Molecular Medicine., 21*(7), 427–432.

Tortora, J. G., Funke, B. R., & Case, C. L. (2010). *Microbiology an introduction* (Vol. 7, 10th edition 2010 ed.pp. 185–207). Pearson Education, Inc., publishing as Pearson Benjamin Cummings.

Wamer, W. G., Yin, J. J., & Wie, R. R. (1997). Oxidative damage to nucleic acids photosensitized by titanium dioxide. *Free Radical Biology and Medicine, 23*(6), 851–858.