

DOI 10.2478/v10181-011-0006-y

Original article

Survival of *Staphylococcus aureus* exposed to UV radiation on the surface of ceramic tiles coated with TiO₂

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Abstract

The aim of this study was to determine and compare the antimicrobial activity of UV radiation of wavelength 253.7 nm (used in typical germicidal lamps) against *Staphylococcus aureus* on the surfaces of conventionally produced white ceramic wall tiles (matt and shiny) and the same tiles coated with TiO₂ using three different methods: RF diode sputtering, atmospheric pressure chemical vapour deposition (APCVD) and spray pyrolysis deposition (SPD). Results clearly indicate that the bactericidal action of UV radiation is much stronger on the surfaces of tiles coated with TiO₂ than on the tiles uncovered. The strongest bactericidal effect of UV radiation was found for film prepared by APCVD. Results of experiments for shiny and matt tiles did not differ statistically. The use of ceramic wall tiles coated with TiO₂ films in hospitals, veterinary clinics, laboratories, food processing plants and other places where UV radiation is applied for disinfection should greatly improve the efficiency of this treatment.

Key words: TiO₂, photocatalysis, UV radiation, *Staphylococcus aureus*, ceramic wall tiles

Introduction

In the legislation defining the health and veterinary requirements for food production (e.g. Regulation No. 853/2004 of the European Parliament and the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin) it is repeatedly stressed that the floors, walls and ceilings, window sills and surfaces, doors, tables, worktops, machinery and equipment of production and storage facilities should be smooth, non-absorbable and easy to clean and disinfect. The regulations of this kind are totally justified because the effectiveness of cleaning and disinfection

largely depends on the surface properties, especially its water absorption, and smoothness. This phenomenon was often observed in research on disinfectants for veterinary medicine and food industry conducted previously in the Department of Food Hygiene and Public Health, University of Life Sciences, Warsaw (Poland).

Anyone interested in problems of disinfection follows with special interest the progress of nanotechnology, which enables the production of photocatalytic films. They are not only smooth, non-absorbable and easy to clean and disinfect, but also exhibit hydrophilic, antistatic, deodorant and antibacterial properties

(Lopez and Jacoby 2002, Kim et al. 2003, Marciano et al. 2009a,b). Research on production technology and properties of ceramic materials coated with titanium dioxide fragmented into nanoparticles is conducted at the Institute of Electronic Materials Technology (ITME) in Warsaw (Poland). This study was performed under a contract No. 26/H/10/ITME/D/G ITME.

The aim of this study was to determine and compare the antimicrobial activity of UV radiation of wavelength 253.7 nm (used in typical germicidal lamps) against *Staphylococcus aureus* on the surfaces of conventionally produced white ceramic wall tiles (matt and shiny) and tiles coated with TiO₂ using three different methods: RF diode sputtering, atmospheric pressure chemical vapour deposition (APCVD) and spray pyrolysis deposition (SPD).

Materials and Methods

In this work three different methods were used to prepare TiO₂ films: RF diode sputtering, atmospheric pressure chemical vapour deposition (APCVD) and spray pyrolysis deposition (SPD). The first one, sputtering, was realized by RF diode sputtering in professional equipment SBR-2306E made by Ulvac (Japan) at 350 W. Target-to-substrate distance was kept at 1.9 cm. Sputtering was performed in pure argon at pressure of 4.4 Pa. TiO₂ targets of 100 mm diameter were used. The deposition temperature did not exceed 52°C. A deposition time was chosen to achieve a film thickness close to 400 nm.

The second method for preparation of TiO₂ films was atmospheric pressure chemical vapour deposition (APCVD). Titanium tetraisopropoxide (TTIP), obtained from Aldrich, was used as precursor and stored in a glass Dreschler bubbler and maintained at 210°C. Argon carrier gas was used to transport the TTIP through silicon and quartz lines towards the vertical tube furnace. The flow rate through the bubbler was 2.8 l/min. The temperature of the tube furnace was maintained at 600°C. A deposition time of 35 min was used.

In case of spray pyrolysis deposition (SPD) the same technique was used as for APCVD. There was only one difference. Instead of Dreschler bubbler for efficient aerosolization of precursor a six jet Collison nebulizer from BGI Inc., USA, working at room temperature was applied. The start precursor was solution of TTIP in a pure propanol in 1:3 ratio. The substrate temperature was 460°C. Filtered air was used as carrier and director gas. The air flow rate was 3.8 l/min. Deposition time was 55 min.

In case of all methods applied, TiO₂ films were deposited on white ceramic wall tiles prepared by

Opoczno S.A., Poland. In conducted studies 24 control tiles (12 matt and 12 shiny) and 72 tiles coated with photocatalytic films (36 mat and 36 shiny) were used.

Before each experiment the test tiles, both control and coated with TiO₂, were disinfected by immersion for 15 minutes in 70% ethanol, then rinsed with sterile distilled water and stored in darkness at approximately 20°C for 18-20 hours.

In the study, *Staphylococcus aureus* ATCC 25923 was used. The reason for selection of *Staphylococcus aureus* was its significance in the etiology of food poisoning and infections, frequency of occurrence in foods of animal origin and pathogenicity for humans and animals (Czarkowski et al. 2009).

Bacterial colonies taken from nutrient agar plates (BTL®) were suspended in a nutrient broth (BTL®) and incubated for 24 h at 37°C. The density of the bacterial suspension (2.5×10^8 cfu/ml) was determined by surface plating on nutrient and Baird-Parker Agar Base (Oxoid®), according to ISO 7218:1998, ISO 7218:1998 / A1: 2004.

From test tubes containing bacterial culture in nutrient broth 0.1 cm³ was taken and placed in the geometric center of sterile tiles. Then the tiles contaminated with *Staphylococcus aureus* were exposed to UV radiation. Device for eggs decontamination (NB-2 Mesko), equipped with 4 Philips TUV lamps T5 16W/G16 emitting radiation of UV-C of 253.7 nm wavelength was used in the experiments. Tiles were always placed in the same position, directly under the top, the front lamp unit. The distance between the plates and the lamp was 57 mm. Tiles were exposed to UV radiation for 0, 60, 90 and 120 seconds.

Immediately after exposure tiles to UV rays bacterial suspension was collected from the surface of each tile using sterile swabs. The tips of swabs were cut off with sterile scissors and placed in test tubes containing 4.9 cm³ of diluent (Maximum Recovery Diluent, Oxoid®). After thorough mixing from each test tube 1 cm³ of suspension was collected to prepare a series of decimal dilutions followed by plating onto Baird-Parker Agar (Oxoid®).

Plates were incubated at 37°C for 48 hours under the aerobic conditions. After incubation the colonies were counted, bacterial counts were multiplied by the appropriate dilutions and numbers of bacteria (colony-forming units) on the entire surface of each tiles were calculated.

The bacterial counts were transformed into logarithms and statistically analysed using the General Linear Models supplied by PASW Statistics 18 Edition 18.0.0 (2009-07-30). All experiments were performed in three replications.

Results

Results showing changes in the average *Staphylococcus aureus* counts on the surface of control tiles and tiles coated with TiO₂ films caused by UV radiation are presented in Table 1.

with TiO₂ than on the surfaces conventionally produced ceramic tiles.

The overall analysis of variance (Table 2) shows that the type of surface and time of exposure to UV radiation have significant effects on the logarithm number of *Staphylococcus aureus*. Interaction of both

Table 1. Effect of UV radiation on logarithm number of *Staphylococcus aureus* ATCC 25923 on the surfaces of test tiles (n=3).

Type of tiles	Time of exposition on UV radiation (s)	C – Control tiles	Tiles coated with photocatalytic (TiO ₂) films prepared by various methods		
			P1-Sputtering	P2- APCVD	P3- SPD
Shiny	0	7.44 ^{ab}	7.35 ^{ab}	7.46 ^{ab}	7.44 ^{ab}
	60	6.59 ^{cb}	1.79 ^{aA}	1.72 ^{aA}	2.65 ^{bA}
	90	6.15 ^{bAB}	1.69 ^{aA}	1.25 ^{aA}	1.50 ^{aA}
	120	5.13 ^{bA}	0.85 ^{aA}	0.37 ^{aA}	1.11 ^{aA}
Matt	0	7.50 ^{aA}	7.40 ^{aC}	7.46 ^{ab}	7.45 ^{ab}
	60	6.33 ^{cA}	3.61 ^{abB}	1.38 ^{aA}	3.85 ^{bA}
	90	6.40 ^{bA}	1.70 ^{aAB}	0.77 ^{aA}	2.62 ^{aA}
	120	6.30 ^{bA}	0.57 ^{aA}	0.29 ^{aA}	1.97 ^{aA}

^{a, b, c} Means for the same item within the same row bearing different superscripts are different at P < 0.05 (Student-Newman-Keuls test)

^{A, B, C} Means for the same item within the same column bearing different superscripts are different at P < 0.05 (Student-Newman-Keuls test)

Table 2. Probability of significance of the F values in the overall analysis of variance for *Staphylococcus aureus* counts.

Main effect	Type III sum of squares	df	Mean square	F	Probability of significance
Tiles type (shiny, matt)	2.454	1	2.454	3.646	0.061
Surface type (C, P1, P2, P3)	217.803	3	72.601	107.837	0.000*
Exposition time	415.224	3	138.408	205.582	0.000*
Tile type x Surface type	3.193	3	1.064	1.581	0.203
Tile type x Exposition time	1.090	3	0.363	.540	0.657
Surface type x Exposition time	74.526	9	8.281	12.300	0.000*
Tile type x Surface type x Exposition time	6.292	9	0.699	1.038	0.420
Error	43.088	64	0.673		
Overall	2253.652	96			

* significance at P < 0.01

In a detailed comparison of mean log values for all tiles exposed to UV radiation for 60, 90 and 120 seconds (Table 1) it can be concluded that the average number of *Staphylococcus aureus* on surfaces of control tiles is much higher than that on the tiles coated with photocatalyst films. In all cases the differences observed were statistically significant (Table 1). These results clearly indicate that the bactericidal action of UV radiation is much stronger on the surfaces coated

of these factors proved to be also statistically significant. The effect of tiles type (shiny or matt) was not strong enough to be statistically important.

Comparing in detail the average results given in Table 1 it is difficult to determine on which photocatalytic surface the bactericidal effects of UV radiation are the strongest. The mean values for homogeneous groups included in Table 3 give unambiguous answer to this question. Compared means for each

Table 3. Logarithm number of *Staphylococcus aureus* ATCC 25923 on the surfaces of test tiles exposed on UV radiation – mean values for homogeneous groups containing results for matt and shiny tiles which did not differ statistically.

Type of surface	Number of samples (n)	Log no. of <i>S. aureus</i> (log)
C – control tiles	24	6.48 ^C
P1 – tiles coated with TiO ₂ film by sputtering	24	3.12 ^B
P2 – tiles coated with TiO ₂ film by APCVD	24	2.59 ^A
P3 – tiles coated with TiO ₂ film by SPD	24	3.57 ^B

^{A, B, C, D} Means for the same item within the same column bearing different superscripts are different at $P < 0.05$ (Student-Newman-Keuls test)

Table 4. The relationship between the logarithm number of *Staphylococcus aureus* on the surface of ceramic tiles and time of exposure to UV radiation.

Type of tiles	Type of surface	The linear regression equation	Correlation (R)	D-value* (s)
Shiny	C – Control	$y = -0.018x + 7.558$	0.844	54.8
	P1 – Sputtering	$y = -0.054x + 6.584$	0.917	18.4
	P2 – APCVD	$y = -0.059x + 6.716$	0.928	16.8
	P3 – SPD	$y = -0.055x + 6.851$	0.934	18.3
Matt	C – Control	$y = -0.010x + 7.306$	0.751	100.0
	P1 – Sputtering	$y = -0.058x + 7.254$	0.933	17.2
	P2 – APCVD	$y = -0.061x + 6.605$	0.924	16.4
	P3 – SPD	$y = -0.047x + 7.134$	0.867	21.3

* The time needed to reduce the number of bacteria by 1 log unit (90%)

type of surface (C, P1, P2, P3) include all individual results obtained in the experiment, i.e. results related to shiny and matt tiles as well as results for all exposure times. A comparison of the mean values for homogeneous groups (Table 3) shows that the strongest bactericidal effect of UV radiation was observed on the surface of tiles coated with TiO₂ by APCVD (P2). The effects observed on the surfaces coated by sputtering (P1) and spray pyrolysis deposition (P3) were weaker. Differences between the average numbers of bacteria on these last two surfaces were statistically insignificant (Table 3).

The linear regression equations describing the relationship between the logarithm number of *Staphylococcus aureus* on the test tiles and time of exposure to UV radiation are presented in Table 4. On the basis of linear regression coefficients, D-values ($-1/b$) were calculated, i.e. times needed to reduce the number of bacteria by 1 log unit or by 90%.

A comparison of the D-values shows that *Staphylococcus aureus* can be reduced on the surfaces coated with photocatalytic films to the same degree by UV radiation in much shorter time than on surface of control tiles (Table 4). The smallest D-values were found for the test bacteria subjected to UV radiation

on the surfaces of both shiny and matt tiles coated with TiO₂ by APCVD (Table 4).

Discussion

The results obtained showed very similar regularities to those found in our previously conducted studies in which *Salmonella* Enteritidis was subjected to UV radiation on photocatalytic surfaces (Szczawiński et al. 2010).

The comparison of the present results with the data given by other research workers is relatively difficult due to differences in the conditions of the experiments. The differences are related to the various model systems used in the photocatalytic reactions, the types and power of lamps emitting UV radiation, the distance from the lamps to irradiated objects and time of exposure to radiation. One of the most important factor is the wavelength of radiation emitted by the lamp. In general, it is believed that the photocatalytic reactions in titanium dioxide are raised by the UV radiation of wavelength 388 nm or less (Anon 2004). For this reason, in the most of the studies aqueous solutions of TiO₂ powder were mixed with bacter-

ial suspensions and irradiated with UV light with a wavelength of 365 nm, which is considered harmless to humans, or visible light was used for excitation of photocatalysis (Maness et al. 1999, Kim et al. 2003, Tsuang et al. 2008, Cheng et al. 2009).

Bactericidal effects of UV radiation of wavelength 365 nm on photocatalytic surfaces were observed in many works. Ohko et al. (2001) have found that silicone catheters coated with a thin layer of TiO₂ can be decontaminated using UV radiation of low intensity. Similar bactericidal effects were also observed in experiments on the sterilization of contaminated dental implants coated with titanium dioxide (Suketa et al. 2005). Bactericidal properties of photocatalytic coatings have been used in filters cleaning the air (Lopez and Jacoby 2002) and in installations for water decontamination (Ireland et al. 1993, Alrousan et al. 2009, Chong et al. 2010).

In the most above-mentioned studies, relatively weak reductions of the number of bacteria were observed, mostly at the level of several to tens percent. Specialists dealing with problems of disinfection would consider such range of bacterial reduction as inadequate. It should be noted that testing of the effectiveness of disinfectants is carried out on the basis of European standards and guidelines. A combination of time and disinfectant concentration is regarded as effective as soon as 5 log₁₀ units of the microorganisms on the test surface have been inactivated (Fraise 2008).

It is considered that a major cause of bacteria inactivation in the processes of photocatalysis is peroxidation of polyunsaturated phospholipids constituting the cell membrane. As a result of this, the functions of bacterial cell membrane are disrupted, especially the processes of breathing, what is usually the direct cause of cell death (Maness et al. 1999). UV radiation with a wavelength around 254 nm, applied in this study and typically used in germicidal lamps, has a much higher energy and therefore the ability of bacterial DNA damage as a result of production of pyrimidine dimers (Anon 2004).

It seems that combination of both these mechanisms of bacteria inactivation was the reason for exceptionally high reductions of *Staphylococcus aureus* on the photocatalytic surfaces observed in the studies. Number of staphylococci placed on tiles coated with TiO₂ films and subjected to UV radiation for 120 seconds decreased by 5.48 to 7.17 log units, while reduction of staphylococci on control tiles amounted only to 1.20-2.31 log units (Table 1). The strongest bactericidal effect of UV radiation was observed on the surfaces of tiles coated with TiO₂ by APCVD (Tables 1 and 3).

In conclusion, the use of ceramic wall tiles coated with TiO₂ films in hospitals, clinics, outpatient clinics, doctors' surgeries and veterinary clinics as well as in

food processing industry, catering outlets, laboratories, and wherever UV radiation is applied to disinfect the surface should greatly improve the efficiency of disinfection and help to radically improve the hygienic conditions prevailing in these areas.

Acknowledgments

The study was supported by the State Committee for Scientific Research, Grant No. R08 031 02.

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