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ORIGINAL PAPER

Proving the antimicrobial spectrum of an amphoteric surfactant-sol-gel coating: a food-borne pathogen study

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Abstract An antimicrobial coating was evaluated in this work for its antimicrobial efficacy against common food-borne pathogens. Dodecyl-di(aminoethyl)-glycine, an organic disinfectant, was immobilized in a silicon oxide matrix to generate thin films over surfaces by means of the sol–gel process. Tetraethoxysilane was used as the polymeric precursor. No alteration of optical transparency on the covered surfaces was observed. Topographic images obtained with atomic force microscopy showed a homogeneous film with no additional roughness added by the polymer to the surface. The attenuated total reflectance-Fourier transform infrared spectral data showed the presence of dodecyl-di(aminoethyl)-glycine in the silicon oxide network after a normal cleaning procedure. The antimicrobial efficacy test was performed by exposing coated slides to suspensions of common food-borne pathogens: *Escherichia coli*, *Staphyloccocus aureus*, *E. coli* O157:H7, *Salmonella typhi*, *S. cholerasuiss*, *Listeria innocua* and *L. monocytogenes*. The coating activity was not only bacteriostatic but also bactericidal. The percent reduction of viable microorganism exposure over 24 h to the coated surface ranged between 99.5%, for the more resistant gram-positive bacteria, and over 99.999%, for most gram-negative bacteria. The silicon matrix itself did not account for any reduction of viable microbial, even more an increase was observed.

Keywords Antimicrobial coating · Sol–gel · Food-borne pathogens · Bactericidal surfaces · Amphoteric disinfectant

Introduction

The latest development in microbial surface contamination control involves novel strategies along with disinfection procedures which utilizes the traditional chemical compound solutions, e.g., sodium hypochlorite, ethanol and iodine [13]. Among these contamination control strategies, in the recent past, new materials or coatings with antimicrobial properties such as bactericide metal ions such as silver, copper or zinc in alloy materials or coatings could be found [9, 15, 18, 25]. Within the same scope, organic compounds with proven antimicrobial activity have also been immobilized [12, 21] or covalently attached in order to generate antimicrobial coatings [20, 22].

Many of these developments were achieved with the aid of sol–gel chemistry. Inorganic ions and organic molecules were trapped into metal or silicon alkoxide polymeric network [4, 6, 16, 17]. Immobilization can be carried out by many methods, e.g., entrapment, electrostatic interaction, adsorption, covalent binding, and vapor deposition [14]. Sol–gel method has several advantages such as high purity precursors, homogeneity of the obtained material, low processing temperatures and, especially, the possibility

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of making composite materials with new chemical and mechanical properties.

Several techniques such as electrophoresis spin coating or dip coating [7], could be employed in the generation of thin films by the sol–gel method. . The dip-coating process is used on surfaces of a wide range of material composition and allows the obtaining of thin and homogeneous films.

In a previous work we immobilized in a xerogel coating the organic disinfectant dodecyl-di(aminoethyl)-glycine (Tego 51®) [8], which has been used as sanitizer in food industry [5, 11] for a long time. This is an amphoteric surfactant capable of producing plasmatic membrane disorganization, interfering with its functions and of leading to metabolites loss [10]. Different bacteria have different membrane properties associated with its composition. Thus, it is necessary to perform further studies to ensure a wide spectrum antimicrobial effect of the coatings, prior to its application in a wide variety of industrial, medical, community and private settings.

The aim of this work is to evaluate the antimicrobial efficacy against common food-borne pathogens of an antimicrobial coating generated by the sol–gel process, for which we immobilized the organic disinfectant dodecyl-di(aminoethyl)-glycine (Tego 51®) over glass surfaces. The microorganism employed to test the antimicrobial spectrum includes both gram-negative *Escherichia coli*, *E. coli* O157:H7, *Salmonella typhi* and *S. cholerasuiss*, and gram-positive bacteria, *Staphyloccocus aureus*, *Listeria innocua* and *L. monocytogenes*. They were chosen as model bacteria because of their involvement in the food industry: they are common contamination indicators and food-borne pathogens. Herein we develop a coating procedure which was physically and biologically characterized suggesting its potential application in food industry.

Materials and methods

Materials and microorganisms

Tetraethoxysilane (TEOS) was purchased from Fluka (Buchs, Switzerland). Dodecyl-di-(aminoethyl)-glycine (Tego 51®) was purchased from T.H. Goldsmidt (A.G. Essen, Germany). Trypticase soy Agar (TSA) and trypticase soy Broth (TSB) were from Britania Labs (Buenos Aires, Argentina). Tween 80 was from Riedel-de-Haën (A.G. Seelze, Hannover, Germany). All other reagents were of analytical grade. *Escherichia coli* (ATCC #8739), *Staphylococcus aureus* (ATCC #6538), *S. typhi* (CCM A29-68), *L. innocua* (CIP 8011) and *L. monocytogenes* (NCTC 7973) were kindly provided by the Microbial Culture Collection of Facultad de Farmacia y Bioquímica (CCM A29)—Universidad de Buenos Aires; *Salmonella cholerasuiss* Co 34 (ANLIS Malbrán, Buenos Aires, Argentina). *Escherichia coli* O157:H7 was isolated from contaminated ground meat. All microorganisms were grown at 35 °C for 24 h on TSA slants.

Film formation

The sol was prepared by sonicating (Transonic 540 sonicator, 35 kHz) a mixture of 7.6 ml TEOS and 2.4 ml 0.04 mol l^{-1} HCl for 30 min at 20 °C. Then, 5 ml of the sol (hydrolyzed TEOS) was added to 15 ml ethanol solutions containing 3.75 ml 0.04 mol l^{-1} HCl with 1.5% w/v and without Tego in order to prepare the coating solution. This antimicrobial concentration was determined in a previous work where all samples with 1.5% w/v Tego–TEOS sol coatings proved to be efficient antimicrobial coatings. Higher concentrations of the disinfectant interfere with the polymerization of hydrolyzed TEOS monomers [8]. All the following Tego concentrations are expressed as weight percent unless specified otherwise.

The film was made using standard microscope slides (75 mm × 25 mm) by the dip-coating process. The slides were cleaned up with acetone and then with ethanol, before the coating process. They were immersed vertically in the corresponding coating solution and left in it for 15 s. Then, they were air-dried generating the xerogel coating and aged at 60 °C overnight. All the slides were polished with absorbent paper until optical transparency to emulate ordinary cleaning procedures and remove the overall extra residue.

All experiments were carried out over two types of surfaces: (1) slides coated with TEOS sols with 1.5% Tego (1.5% Tego–TEOS sol); (2) slides coated with TEOS sols with 0% Tego (0% Tego–TEOS sol).

Film characterization

UV–Vis spectra

UV–Vis spectra of uncoated, 0 and 1.5% Tego–TEOS sol coated slides were acquired in the range 200–800 nm interposing the slides in the optic path of the spectrophotometer (Cecil CE 3021, Cambridge, England).

Infrared spectra

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) transmission spectra were acquired in the range of 4,000–800 cm^{-1} using an ATR-FTIR spectrometer (Perkin Elmer, Spectrum One IR). All slides were previously dried at 60 °C for 24 h to avoid water-related band interference.

Atomic force microscopy

Atomic force microscopy (AFM) measurements were performed using a NanoScope IIIa Digital Instruments microscope (Santa Barbara, USA). All images were captured in the tapping mode using silicon cantilevers under nitrogen conditioning. Image processing was carried out with WSxM 4.0 Develop 8.5 Scanning Probe Microscopy Software (Nanotec Electronics, España). WSxM is a free software downloadable at http://www.nanotec.es. No slide surface polishing was performed for AFM imaging samples in order to avoid coating scratching or artifact generation.

Surface activity test

The antibacterial activity test was performed using a methodology similar to those described in literature to characterize antibacterial coatings [1–3, 17, 20]. In the antimicrobial efficacy test, slide surfaces were exposed to 0.4 ml of a microorganism suspension with a concentration between 2.5×10^5 and 1.5×10^7 cfu ml^{-1}. Suspensions were prepared from 24-h TSA slants in TSB (diluted 1:500 with sterile water).

Inoculated surfaces were covered with Parafilm® to spread the solution in a thin layer over the entire slide for avoiding evaporation and for preventing the adverse effects that the drying process h as on some bacteria, which could lead to false positive results [19, 27]. They were then kept for 24 h at 35 °C and at a relative humidity over 90%. The above-mentioned conditions ensure maximal surface-microorganism interaction.

Each step of this assay was performed in triplicate, as follows: two slides were coated with the sol–gel polymer without the antimicrobial agent (0% Tego–TEOS sol). Both were inoculated with the microorganism suspension. One of them was used to count viable cells right after inoculation; the other was utilized to count viable cells after 24 h incubation.

A slide with 1.5% Tego–TEOS sol coating was inoculated. After 24 h incubation it was used to count surviving cells.

The test was performed in triplicate with seven different microorganisms, *E. coli*, *Staphylococcus aureus*, *S. typhi*, *S. cholerasuiss*, *L. innocua*, *L. monocytogenes* and *E. coli* O157:H7.

The microbial counts were performed by washing the slides with 10 ml of an antagonist solution (3% Tween 80; 0.85% NaCl) in a sterile container. In order to enhance detachment of potentially attached microbial cells, the slides were thoroughly rubbed in a sterilized pouch. This solution was subjected to tenfold dilution to optimize plate counting in TSA, and incubated during 48 h at 35 °C.

Antimicrobial activity was calculated using two different parameters: percent reduction (D) and value of antimicrobial activity (R).

D was calculated from 0% Tego–TEOS sol coated slide-1.5% Tego–TEOS sol coated slide microbial count percentage ratio.

R was calculated according to JIS Z 2801:2000 [3]:

$$R = \log(B/C)$$

where B is the average number of viable cells of bacteria on the 0% Tego–TEOS sol coated slide after 24 h and C is the average number of viable cells of bacteria on the 1.5% Tego–TEOS sol coated slide after 24 h.

Results

Film characterization

UV–Vis spectra

The three types of surfaces, uncoated, 0 and 1.5% Tego–TEOS sol coated slides, showed the same absorbance spectrum. No absorbance was detected between 400 and 800 nm (visible spectrum). In the range of 200–400 nm (UV spectrum) the three different slides showed interference due to typical absorbance of glass materials with saturation of the signal.

Infrared spectra

Attenuated total reflectance-Fourier transform infrared spectra of coated slides with 0 and 1.5% Tego–TEOS sol coating solutions are shown in Figs. 1 and 2. Both coated slides showed broad bands between 850 and 900 cm^{-1} and

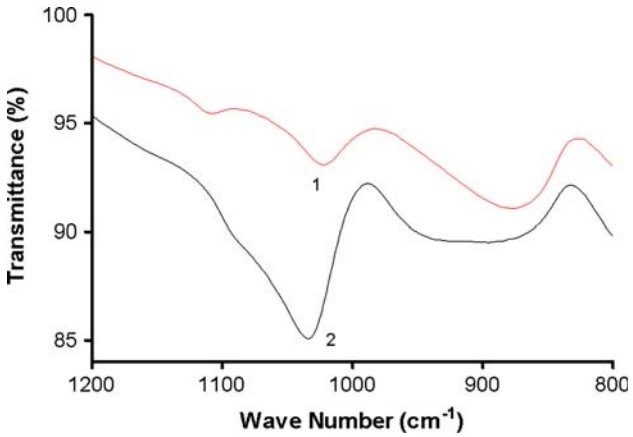

Fig. 1 Attenuated total reflectance-Fourier transform infrared spectra between 800 and 1,200 cm^{-1} of 0% Tego–TEOS sol coated slide (*1*) and 1.5% Tego–TEOS sol coated slide (*2*). Bands at 850–900 and 1,000–1,050 cm^{-1} corresponding to Si–O–Si bonds of the silicon oxide matrix can be observed

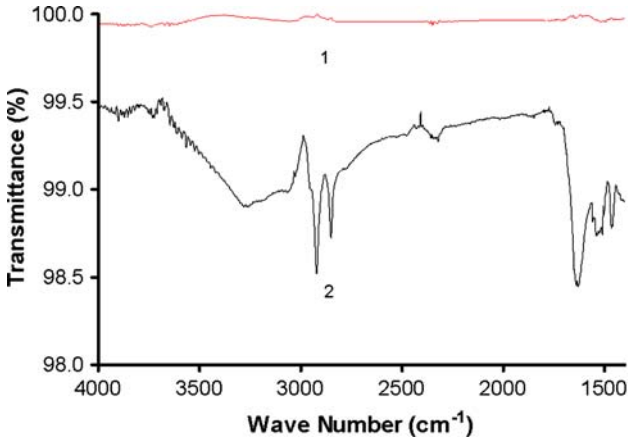

Fig. 2 Attenuated total reflectance-Fourier transform infrared spectra between 1,400 and 4,000 cm^{-1} of 0% Tego–TEOS sol coated slide (*1*) and 1.5% Tego–TEOS sol coated slide (*2*). Bands at 2,920, 2,850, 1,630, 1,460 and 3,250–3,300 cm^{-1} corresponding to the organic disinfectant structure can be observed in (*2*)

1,000–1,050 cm^{-1} corresponding to symmetric and asymmetric Si–O–Si bond stretching, respectively, because of the presence of the SiO_2 polymer coating (Fig. 1) [23, 24].

Figure 2 displays the bands at 2,920 and 2,850 cm^{-1} corresponding to saturated C–H asymmetric and symmetric stretchings, respectively [26]. These bands were present in the surfaces coated with 1.5% Tego–TEOS sol coating solution. In slides with 0% Tego–TEOS sol coatings, these bands were less intense, thus indicating that TEOS hydrolysis was not fully complete and alkoxy moieties were being detected. During TEOS hydrolysis, ethanol and silicic acid monomers were generated. All ethanol was volatilized in the evaporation step of the dip-coating process. Thus, the increase of the 2,920 and 2,850 cm^{-1} bands, present in molecules with alkyl rest in their structures as in Tego IR spectra (data not shown) indicated the presence of dodecyl-di(aminoethyl)-glycine (Tego). In addition, the antimicrobial coating spectra showed bands at 1,630 cm^{-1} (C=O symmetric stretching of carboxylic acids), 1,460 cm^{-1} (ionized carboxyl, zwitter ions) and between 3,250 and 3,300 cm^{-1} (N–H stretching vibration) also present in Tego IR spectra (data not shown) and absent in the 0% Tego–TEOS sol coating spectra [26].

Atomic force microscopy

Atomic force microscopy was applied to the slides in order to analyze the coated surface. The topography of 0% Tego–TEOS sol coatings is shown in Fig. 3. The surface did not present important height differences. A similar image was obtained for the naked glass sample (data not shown). In the AFM image of the 1.5% Tego–TEOS sol coated surfaces a similar topography was observed (Fig. 4). In a cross-section of both coating surfaces the difference

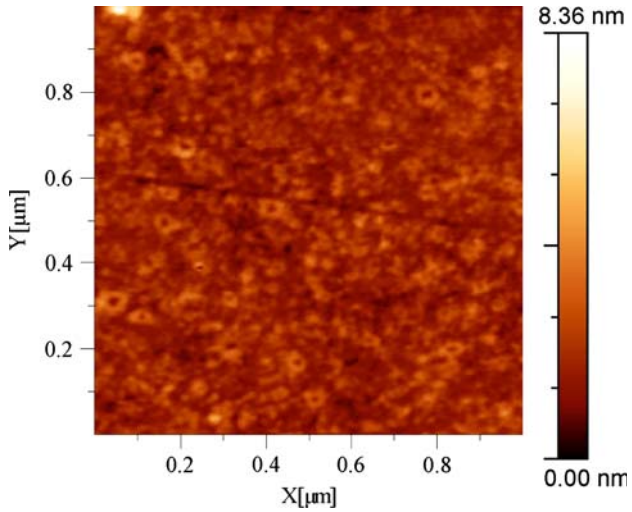

Fig. 3 Atomic force microscopy topography image of a non-polished 0% Tego–TEOS sol coated slide. Image size 1 µm × 1 µm

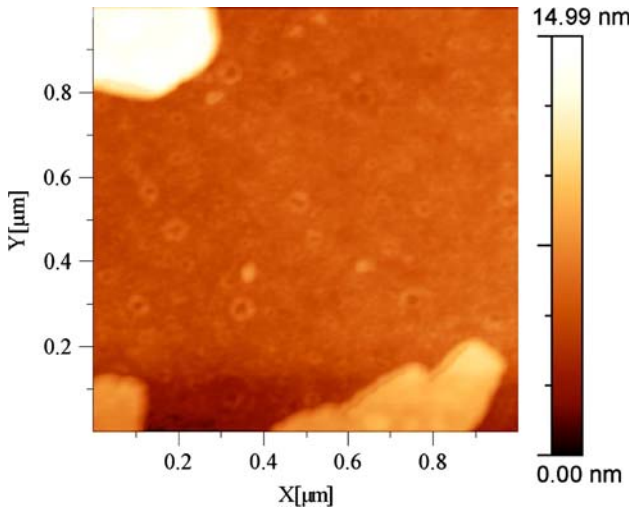

Fig. 4 Atomic force microscopy topography image of a non-polished 1.5% Tego–TEOS sol coated slide. Image size 1 µm × 1 µm

between the lowest and highest spots was under 2 nm indicating a flat surface. Over the non-polished coating disinfectant residues, probably due to an excess of surfactant (Fig. 4), could be observed. After the coating process, homogeneous films were observed over the slide surfaces. No cracking sign due to material aging was observed.

Surface activity test

The coating showed antimicrobial activity against the seven food-borne pathogens under study. The percent reduction of viable microorganism exposed over 24 h to the coated surface were 99.5%, for the more resistant gram-positive bacteria, and over 99.999%, for most gram-negative bacteria, as shown in Table 1.

Table 1 Surface activity test

Microorganism	cfu[a] (0 h)	cfu (24 h)		D^b (%)	R^c
		Tego (%)			
		0	1.5		
Escherichia coli	1.3×10^6	3.1×10^6	<10	>99.999	>5.49
E. coli O157:H7	2.8×10^5	2.0×10^7	1.5×10^3	99.992	4.12
Salmonella cholerasuiss	1.3×10^6	3.0×10^7	55	>99.999	5.74
S. typhi	9.7×10^5	4.3×10^6	5.6×10^2	99.987	3.88
Listeria monocytogenes	1.3×10^7	9.9×10^6	1.9×10^3	99.981	3.72
L. innocua	1.1×10^6	3.4×10^6	2.8×10^3	99.917	3.08
Staphyloccocus aureus	1.4×10^6	2.2×10^6	1.1×10^4	99.500	2.30

[a] cfu was calculated from the mean of three cfu counts

[b] Percent reduction

[c] Value of antimicrobial activity

Discussion

The coating has demonstrated its antimicrobial efficacy against common food contamination indicators and food-borne pathogens. After the coating and aging process, no optical transparency modification due to the film was observed. With the aid of AFM it was demonstrated that antimicrobial presence in the coating did not increase the roughness of the surface. It was found to be flat and homogeneous all over the surface and was found as an advantage in the prevention of bacterial adhesion to surface roughness.

Both the silicon oxide network and the antimicrobial compound have been detected by IR spectra over coated glass. The presence of disinfectant was also observed even after polishing the slides emulating an ordinary cleaning procedure. In a previous work, IR spectra showed that a Tego film deposited over the slide without any silicon oxide matrix protection is easily removed [8], emphasizing the importance of disinfectant immobilization within the matrix network in order to avoid disinfectant removal.

A high concentration of pathogen bacteria was used to simulate a "worst case scenario" based on the premise that survival of a lower concentration of bacteria would be inhibited in the same or lesser time. Pathogens remain viable on dry surfaces and represent a contamination hazard for several days. Results herein demonstrate that the microbial number diminishes by at least two logarithmic units (99%) when bacteria are exposed to the antimicrobial surface. In none of these tests a rise in microbial number was observed in comparison with initial inoculums, which implies that lower inoculums, such as the usual microbial residues after disinfection, could be eliminated or proliferation could be inhibited in a 24 h period of time. This is an important task, especially in cases where biofilm is a problem, since the coating could delay its formation.

It should be noted that the drop in the microbial number is due to the activity of trapped antimicrobial agent, and that the silicon oxide matrix and/or incubation conditions does not have any effect on microbial viability. In fact after 24 h, in 0% Tego–TEOS sol coated slides an increase in the number of bacteria was observed in most cases.

Since the coating methodology does not need a surface activation step no toxic or caustic reagents need to be used for its in situ application. Glass was chosen as a model surface even though this technique allows the generation of stable coatings over a wide range of materials such as steel, ceramics, etc. The coating immobilizing Tego showed to be more efficient against gram-negative than gram-positive bacteria. Since the antimicrobial immobilization method is an inclusion process within the polymeric network, it makes the coating system versatile for other antimicrobials when specific activity is required. Thus, coating procedure is proposed as a potential tool to prevent microbial food cross-contamination.

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References

1. Anonymous (1998) Antibacterial finishes on textile materials: an assessment: test method 100-1993. American Association of Textile Chemists and Colorist, AATCC. AATCC technical manual, USA, pp 143–144
2. Anonymous (1998) Antibacterial finishes on textile materials: parallel streak method: test method 147-1993. American Association of Textile Chemists and Colorist, AATCC. AATCC technical manual, USA, pp 253–254
3. Anonymous (2001) Antimicrobial products-test for antimicrobial activity and efficacy: JIS 2801:2000. Japanese Industrial Standard, JAP
4. Bellantone M, Coleman NJ, Hench LL (2000) Bacteriostatic action of a novel four-component bioactive glass. J Biomed Mater Res A 51(3):484–490

5. Block S (1983) Quaternary ammonium compounds in disinfectants and antiseptics. Surface active agents. Lea & Febiger, Philadelphia, pp 263–273
6. Bottcher H, Jagota C, Trepte J, Kallies KH, Haufe H (1999) Sol–gel composite films with controlled release of biocides. J Control Release 60(1):57–65
7. Brinker C, Scherer G (1990) Sol–gel science. Academic Press, San Diego
8. Copello GJ, Teves S, Degrossi J, D'Aquino M, Desimone MF, Diaz LE (2006) Antimicrobial activity on glass materials subject to disinfectant xerogel coating. J Ind Microbiol Biotechnol 33(5):343–348
9. Cowan MM, Abshire KZ, Houk SL, Evans SM (2003) Antimicrobial efficacy of a silver–zeolite matrix coating on stainless steel. J Microbiol Biotechnol 30(2):102–106
10. D'Aquino M, Rezk R (1995) Desinfección: Desinfectantes, Desinfestantes, Limpieza. Características de los agentes químicos desinfectantes.. E.U.DE.B.A, Buenos Aires, pp 67–76
11. Domagk G (1935) A new class of disinfectants. Dtsch Med Wochenschr 61:829–839
12. Etienne O, Gasnier C, Taddei C, Voegel JC, Aunis D, Schaaf P, Metz-Boutigue MH, Bolcato-Bellemin AL, Egles C (2005) Antifungal coating by biofunctionalized polyelectrolyte multilayered films. Biomaterials 26(33):6704–6712
13. Gardner J, Peel M (1986) Introduction to sterilization and disinfection. Churchill Livingstone, Melbourne
14. Iler R (1979) The chemistry of silica. Wiley, New York
15. Jain P, Pradeep T (2005) Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnol Bioeng 90(1):59–63
16. Jeon HJ, Yi SC, Oh SG (2003) Preparation and antibacterial effects of Ag-SiO_2 thin films by sol–gel method. Biomaterials 24(27):4921–4928
17. Kawashita M, Toda S, Kim HM, Kokubo T, Masuda N (2003) Preparation of antibacterial silver-doped silica glass microspheres. J Biomed Mater Res A 66(2):266–274
18. Klueh U, Wagner V, Kelly S, Johnson A, Bryers JD (2000) Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. J Biomed Mater Res A 53(6):621–631
19. Lee SB, Koepsel RR, Morley SW, Matyjaszewski K, Sun Y, Russell AJ (2004) Permanent, nonleaching antibacterial surfaces. 1. Synthesis by atom transfer radical polymerization. Biomacromolecules 5(3):877–882
20. Liang J, Wu R, Wang JW, Barnes K, Worley SD, Cho U, Lee J, Broughton RM, Huang TS (2007) *N*-halamine biocidal coatings. J Ind Microbiol Biotechnol 34(2):157–163
21. Milovic NM, Wang J, Lewis K, Klibanov AM (2005) Immobilized *N*-alkylated polyethylenimine avidly kills bacteria by rupturing cell membranes with no resistance developed. Biotechnol Bioeng 90(6):715–722
22. Murata H, Koepsel RR, Matyjaszewski K, Russell AJ (2007) Permanent, non-leaching antibacterial surface—2: How high density cationic surfaces kill bacterial cells. Biomaterials 28(32):4870–4879
23. Muroya M (1999) Correlation between the formation of silica skeleton structure and Fourier transform reflection infrared absorption spectroscopy spectra. Colloid Surf A 157:147–155
24. Nakagawa T, Soga M (1999) A new method for fabricating water repellent silica films having high heat-resistance using the sol–gel method. J Non-Cryst Solids 260(3):167–174
25. Rusin P, Bright K, Gerba C (2003) Rapid reduction of *Legionella pneumophila* on stainless steel with zeolite coatings containing silver and zinc ions. Lett Appl Microbiol 36(2):69–72
26. Weast R, Astle M (1981) CRC handbook of chemistry and physics. CRC Press, Florida
27. Xie X, Li Y, Zhang T, Fang HH (2006) Bacterial survival in evaporating deposited droplets on a Teflon-coated surface. Appl Microbiol Biotechnol 73(3):703–712