J Ind Microbiol Biotechnol (2006) 33: 343–348
DOI 10.1007/s10295-005-0066-z

ORIGINAL PAPER

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Antimicrobial activity on glass materials subject to disinfectant xerogel coating

Received: 6 September 2005 / Accepted: 20 November 2005 / Published online: 9 December 2005

Abstract The antimicrobial compound dodecyl-di(aminoethyl)-glycine was immobilized in a silicon oxide xerogel matrix and used for glass surface coating. Coated glasses were tested for surface antimicrobial activity. The utilization of tetraethoxysilane (TEOS) as a silicon oxide polymer precursor, using the dip-coating process, allowed for the generation of transparent thin films over glass surfaces. Different concentrations of the antimicrobial compound were used to generate the coatings. The presence of dodecyl-di(aminoethyl)-glycine on coated and uncoated slides was analyzed by FT-IR spectra. Coated glass slides were exposed to suspensions of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* for 24 h. Surface contamination was evaluated by the microbial plate count technique. When antimicrobial-coated glasses were compared with antimicrobial-free coated glasses, the former showed greater than 99% reduction of colony-forming units (cfu) for *E. coli* and *P. aeruginosa*, when 1% of antimicrobial was present in the coating solution. The same percentage of reduction for *S. aureus* was achieved when 1.5% of the antimicrobial was present in the coating solution. In a direct inhibition test on agar plates, no inhibitory zone was observed, indicating that the antimicrobial did not diffuse into the media.

G.J. Copello and S. Teves are equally responsible for this work.

In memorian of Prof. Benjamin Frydman.

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Keywords Antibacterial surface · Bioactive glass · Disinfectant · *Escherichia coli* · *Pseudomonas aeruginosa* · Sol-gel · *Staphylococcus aureus*

Introduction

Microbial contamination on hospital surfaces has always been a serious problem that could lead to infectious processes in hospitalized patients. Usually, these problems are minimized by cleaning and disinfecting programs, which use several chemical compounds such as sodium hypochlorite, ethanol, quaternary ammonium salts, and amphoteric surfactants, among others [5, 7, 13, 24]. The efficiency of these programs is influenced by different factors, such as chemical concentration, type and frequency of application, operator training and evaluation program design.

Over the last years, new strategies have been added to the challenge of control and prevention of microbial contamination. One was the development of materials with antimicrobial action that could be applied to critical surfaces to limit microbial colonization.

The immobilization of an antimicrobial agent in a matrix capable of binding to different surfaces is an interesting way to achieve the objective mentioned above. There are several methods to immobilize proteins or other molecules on surfaces, such as ion exchange, adsorption, covalent binding, etc [3, 15].

The sol-gel method could be an effective procedure to entrap organic and inorganic compounds with antimicrobial activity on different surfaces. Among the techniques that could be employed to generate thin films by the sol-gel method are electrophoresis, spin coating or dip coating [8]. The dip coating process is suitable for different surfaces (e.g. windows, surgical instruments) producing almost transparent homogeneous films when silicon oxide precursors are used.

Several compounds have been included in matrices that were obtained by using the sol-gel process. These

matrices have been used with different applications in biotechnology, including hybrid material synthesis and biosensors design among others [4, 8, 22]. Sol-gel chemistry has been used to prepare antibacterial glass surfaces. There are literature reports on the use of silver as the immobilized bacteriostatic [6, 17–19]. Recently, sol-gel chemistry has also been extended to generate antibacterial coatings for orthopedic implants by a nitric oxide releasing matrix [20].

The aim of this work was to generate an antimicrobial coating for glass surfaces by the dip coating process, using an organic disinfectant immobilized in a silicon oxide matrix. The polymeric precursor chosen for this study was tetraethoxysilane (TEOS). The antimicrobial agent immobilized was dodecyl-di(aminoethyl)-glycine (Tego 51®). This is an amphoteric surfactant capable of producing plasmatic membrane disorganization interfering with its functions and can lead to loss of several metabolites [10]. Amphoteric surfactants are known as surface-active agents. Tego 51® has been used for a long time in hospitals for instruments and room disinfection, and as a sanitizer in industries [7, 10, 11].

Different concentrations of the immobilized antimicrobial were evaluated for effectiveness against gram-negative and gram-positive bacteria and the development of the optimum matrix formulation. *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were chosen as model systems to evaluate antimicrobial action because these microorganisms are commonly found in the hospital environment and they are widely used in antimicrobial evaluation tests [1, 2, 10, 16].

Materials and methods

Chemicals and microorganisms

Tetraethoxysilane was purchased from Fluka (Buchs, Switzerland). Tego 51®: dodecyl-di-(aminoethyl)-glycine (Tego) 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) and 2,3,5-triphenyltetrazoliumchloride was from Merck (Darmstadt, Germany). All other reagents were of analytical grade. *P. aeruginosa* (ATCC #9027), *E. coli* (ATCC #8739) and *S. aureus* (ATCC #6538) were gently provided by the Microbial Culture Collection of Facultad de Farmacia y Bioquímica (CCM 29), University of Buenos Aires.

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. The sol (hydrolyzed TEOS) was added to 15 ml ethanol solutions containing 3.75 ml 0.04 mol l^{-1} HCl and different Tego concentrations, ranging from 0 to 2% final concentration, in order to make the coating solution. An additional coating solution was prepared mixing 3.75 ml 0.04 mol l^{-1} HCl, 0.3 ml Tego (for 1.5% Tego final concentration) in 15.95 ml ethanol. This was used to evaluate the adsorption of Tego to glass surfaces.

The film was made using standard microscope slides by the dip coating process. The slides were cleaned with ethanol before the coating process took place. They were vertically immersed in the corresponding coating solution and left in it for 15 s. The slides were aged at 60°C overnight. All the slides were cleaned with absorbent paper to emulate ordinary cleaning procedures and to remove the overall extra matrix residue.

All experiments were carried out on three types of surfaces: (1) Slides coated with TEOS sols with different Tego percentages (X% Tego–TEOS sol); (2) Slides coated with TEOS sols with 0% Tego (0% Tego–TEOS sol); and (3) Slides treated with 1.5% Tego–ethanol solution.

Instrumentation

Infra red transmission spectra were acquired in the range 4,000–2,000 cm^{-1}, interposing the coated slides in the optic path of a Fourier transform infrared spectrometer (Bruker, IFS 25). All slides were previously dried 24 h at 60°C to avoid water-related band interference.

UV–Vis spectrum of 2% Tego–TEOS sol coated slide and glass slide were acquired in the range 200–800 nm, interposing the slides in the optic path of the spectrophotometer (Cecil, CE 3021).

Antimicrobial efficacy assays

Surface activity test

The antibacterial activity test was performed using methodology similar to those described in the literature to characterize antibacterial coatings [1, 2, 12, 16, 21, 23]. In the antimicrobial efficacy test, slide surfaces were exposed to 0.4 ml of a microorganism suspension with a concentration between 1×10^5 and 1.5×10^6 cfu/ml. Suspensions were prepared from 24 h TSA slants in TSB (diluted 1:500 with sterile water).

The inoculated surfaces were covered with Parafilm® for test inoculums, spreading a thin layer over the entire slide and avoiding evaporation. They were kept for 24 h at 35°C and a relative humidity of no less than 90%.

Six slides were coated with the silicon oxide polymer without the antimicrobial agent (0% Tego–TEOS sol). All of them were inoculated with the microorganism suspension. Three of them were used to count viable cells immediately after inoculation; the other three were used to count viable cells after a 24-h incubation.

In every determination with each X% Tego–TEOS sol coating, three slides with the antimicrobial coating were inoculated and used to count surviving cells after a 24-h incubation.

The test was run with three different microorganisms: *E. coli*, *S. aureus* and *P. aeruginosa*.

The test was also carried out with slides treated with 1.5% Tego–ethanol solution to evaluate the adsorption of the disinfectant to the slide material.

All slides were cleaned with absorbent paper to emulate ordinary cleaning procedures and to remove the overall extra matrix residue before inoculation.

Microbial counts were performed after washing the slides with 10 ml of an antagonist solution (3% Tween 80; 0.85% NaCl) in a sterile container. This solution was subjected to tenfold dilution to optimize plate counting in TSA, and incubated 48 h at 35°C.

Direct inhibition on agar plate

The antimicrobial activity was also determined by placing slides treated with 1.5% Tego–ethanol solution, coated with 1.5% Tego–TEOS sol as well as 0% Tego–TEOS sol over TSA plates inoculated with a 1×10^6 cfu *E. coli* suspension and triphenyltetrazolium chloride (70 mg/l) as a biological activity indicator. The plates were incubated for 24 h at 35°C. Growth inhibition was evaluated visually. Slides were cleaned before being placed on agar plates to emulate ordinary cleaning procedures.

Results

Coating characteristics

The coating process led to homogeneous films on the surface of the slides. No cracking sign, due to material aging, was observed. Only the ones doped with 2% Tego–TEOS sol coating solutions showed opalescent surfaces. When higher concentrations of the ampholyte in the coating solution were used (> 2%) the gelification took place immediately, avoiding the coating process and generating a heterogeneous gel. Glasses treated with 1.5% Tego–ethanol solution (without the silicon oxide matrix) also had opalescent surfaces. In these, Tego adsorption led to greasy films easy to remove by absorbent paper cleaning.

The absorbance spectrum between 400 and 800 nm (visible spectrum) showed no absorbance for the 2% Tego–TEOS sol coated glass slide. The UV spectrum (200–400 nm) showed the normal interference of glass with no extra peaks. The coating was shown to be transparent in the visible region (data not shown).

FT-IR analysis

In Fig. 1 the FT-IR spectra of coated slides with 0 and 1% Tego–TEOS sol coating solutions are shown. The bands at 2,980 and 2,880 cm^{-1} correspond to saturated C–H bond and unspecific C–H bond stretching [14]. These bands were present for all surfaces coated with Tego–TEOS sol coating solution. In slides with 0% Tego–TEOS sol coatings, these bands were absent. During TEOS hydrolysis, ethanol and silicic acid monomers are generated. In the evaporation step of the dip-coating process all of the ethanol is volatilized. Thus, 2,980 and 2,880 cm^{-1} bands, present only in molecules with alkyl residues in their structures, indicated the presence of dodecyl-di(aminoethyl)-glycine (Tego). The inclusion of the disinfectant in the xerogel matrix was demonstrated by the presence of these bands in all coated glasses; Tego concentration in the coating solution ranged from 0.1 to 2%. In IR spectra of 1.5% Tego–ethanol solution treated slides, the bands at 2,980 and 2,880 cm^{-1} are only present in IR spectra before cleaning. These bands are absent in IR spectra after cleaning the surface with absorbent paper, as shown in Fig. 2. The broad band at 3,400 cm^{-1} indicates SiO–H [15] or HO–H bond stretching or N–H bond stretching [14] in those slides coated with the suspension containing the disinfectant.

Surface activity test

All samples with 1.5% Tego–TEOS sol coatings proved to be efficient for inhibiting *E. coli*, *S. aureus* and *P. aeruginosa* (Table 1). The antimicrobial efficacy for *E. coli* and *P. aeruginosa* was achieved at lower concentrations of Tego (1%). The 1.5% Tego–ethanol treated slide did not demonstrate antimicrobial efficacy after absorbent paper cleaning, indicating low interaction processes.

Direct inhibition on agar plates

Figure 3 shows the bacteriostatic activity of the coating. There was no bacterial growth under the area of the slide treated with 1.5% Tego–TEOS sol coating, confirming the surface activity results. Besides, no inhibitory zone was observed, indicating that over the time period of the experiment, the activity of the antimicrobial coating is focused on the slide surface and the leach of the ampholyte, if present, did not reach a high enough level to show antimicrobial activity.

The slide treated with 1.5% Tego–ethanol solution after the cleaning procedure showed microbial growth under and out of its surface. The same was observed for the slide treated with 0% Tego–TEOS sol coating. In the first case, the concentration was not enough to produce an inhibitory effect, and the second shows that the matrix did not have significant antimicrobial activity.

Discussion

The silicon oxide matrix containing the ampholyte disinfectant demonstrated its antimicrobial surface activity

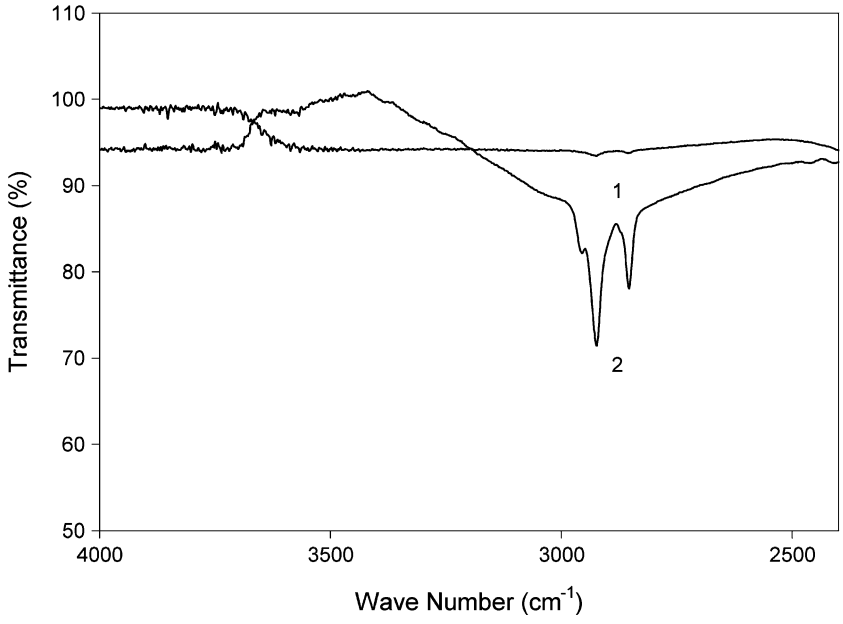

Fig. 1 FT-IR spectra of 0% Tego–TEOS sol coated slide (*1*) and 1% Tego–TEOS sol coated slide (*2*). The bands at 2,980 and 2,880 cm^{-1} corresponding to carbon skeleton of the antimicrobial can be observed. The slides were cleaned before tested

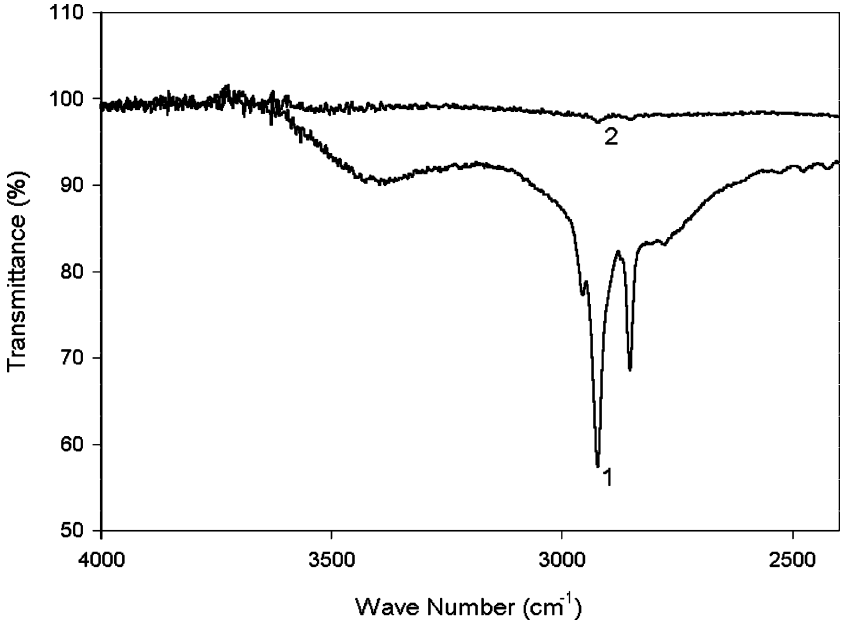

Fig. 2 FT-IR spectra of 1.5% Tego–ethanol solution treated slide (*1*) before, and (*2*) after cleaning with absorbent paper

Table 1 Surface activity test results

Coating solution		*Escherichia coli*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
Tego (%)	TEOS sol	cfu[a] (24 h)	*D*[b] (%)	cfu (24 h)	*D* (%)	cfu (24 h)	*D* (%)
0	+	3.1×10^6	–	5.0×10^6	–	2.2×10^6	–
0.1	+	2.1×10^6	32	NT	NT	2.1×10^6	4
0.5	+	3.6×10^4	99	1.3×10^5	97	4.0×10^5	82
1	+	< 10	> 99	50	> 99	1.2×10^5	94
1.5	+	< 10	> 99	< 10	> 99	1.1×10^4	> 99
2	+	< 10	> 99	< 10	> 99	70	> 99
1.5	–	2.2×10^6	29	NT	NT	NT	NT

NT not tested

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

[b]Percent reduction was calculated from 0% Tego–TEOS sol coated slide-X% Tego–TEOS sol coated slide percent ratio. Initial inoculums were 1.3×10^6 cfu for *E. coli*, 4.6×10^5 cfu for *P. aeruginosa*, 1.4×10^6 cfu for *S. aureus*

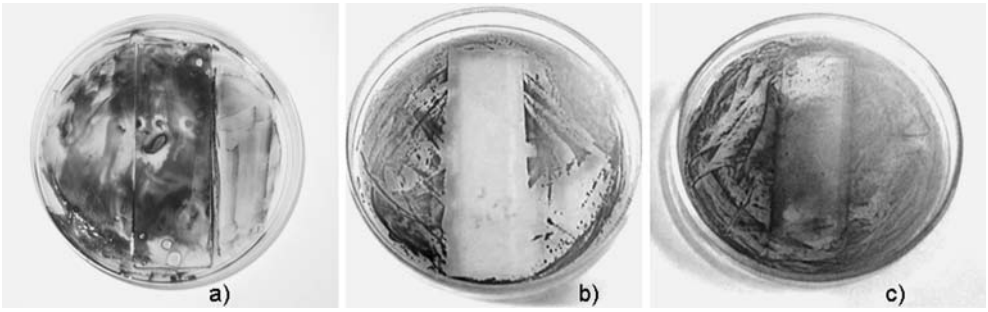

Fig. 3 *Escherichia coli*-inoculated agar plate with the addition of Triphenyltetrazolium as biological activity indicator: **a** 0% Tego–TEOS sol coated slide, **b** 1.5% Tego–TEOS sol coated slide and **c** 1.5% Tego–ethanol solution treated slide. All slides were cleaned before tested. Clear areas indicate no microbial growth

for two gram-negative bacteria, *E. coli* and *P. aeruginosa*, and one gram-positive bacterium, *S. aureus*. Although the Tego group disinfectants are not selective in their activity among gram-negative and gram-positive bacteria [7], lower concentrations of the antimicrobial were needed to achieve the same percentage of microbial reduction for the gram-negative bacteria than the gram-positive.

The absence of an inhibitory zone in the direct inhibition test indicated that there was no significant diffusion of the disinfectant to the agar in the time scale of the experiment. This could prevent human skin from disinfectant exposure. The absence diffusion of the agent, minimizes its release to the medium, increasing the efficacy period.

The 1.5% Tego–ethanol treated glass surfaces showed low antimicrobial activity after a standard cleaning procedure. A major percent of the disinfectant was removed during the process. This was demonstrated with FT-IR spectra where important reduction of characteristic bands occurs, after cleaning these surfaces, indicating that although Tego compounds have the ability to absorb onto solid surfaces [7], they were not capable of leaving a resistant film and could be removed by mechanical action. This emphasizes the advantage of immobilizing the antimicrobial in a protective matrix. The possibility of retaining an antimicrobial in a silicon oxide matrix could help to extend its action in time, due to its resistance to mechanical ablation of cleaning processes. It protects the agent from being removed from the surface where the finishing is applied. The mechanical properties of the encapsulation matrix allow the bactericidal effect to be protected from surface cleaning.

The use of TEOS as polymeric precursor generates a transparent coating. The transparent property of the film and its thickness make the coating attractive for windows, bottles, ceramics, and surgical instruments, among other glass surfaces. Nevertheless, when concentrations of Tego higher than 2% (in the coating solution) are required because of the microorganism susceptibility, the film obtained in these conditions is opalescent and inhomogeneous. This could be due to the ampholyte property of the disinfectant interfering with the polymerization of hydrolyzed TEOS monomers. The possibility to immobilize several antimicrobials in silicon matrices is being evaluated for particular cases where specificity of the antibiotic is required.

Acknowledgements G.J.C., M.F.D. and J.D are grateful for their doctoral fellowship granted by Universidad de Buenos Aires. L.E.D. is supported by grants from Universidad de Buenos Aires; Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). L.E.D. is member of CONICET research career.

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