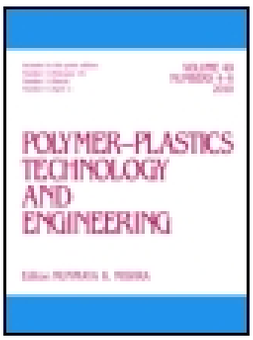

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Poly(propylene)-based Films Modified with a Tetracationic Phthalocyanine with Applications in Photodynamic Inactivation of *Candida albicans*

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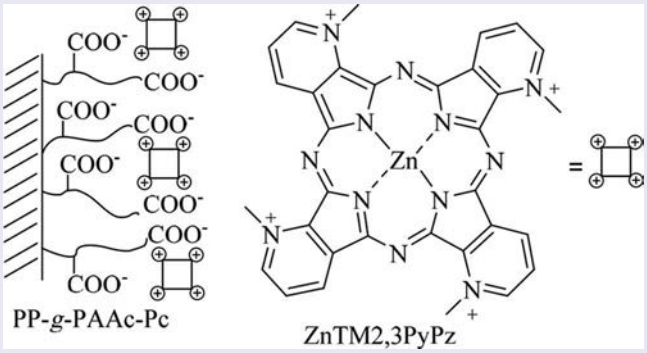
ABSTRACT

Photoactive films based on polymer-like poly(propylene) were generated and utilized as support of zinc(II)tetramethyltetrapyridino[2,3-*b*:2′,3′-*g*:2″,3″-*l*:2‴,3‴-*q*]porphyrazinium salt (ZnTM2,3PyPz). Using a photograft polymerization of acrylic acid, the poly(propylene) film was functionalized with carboxyl groups (PP-*g*-PAAc), which attached ZnTM2,3PyPz by electrostatic bond to form PP-*g*-PAAc-Pc films. In vitro investigations indicated that PP-*g*-PAAc-Pc films produced photodynamic inactivation of *Candida albicans* cells, mainly mediated by a contribution of type II process. According to the results, the photodynamic activity produced by the PP-*g*-PAAc-Pc film and visible light irradiation can successfully inactivate *C. albicans* deposited on the surface of the films.

KEYWORDS

Antimicrobial; photoactive surface; photodynamic inactivation; phthalocyanine; polymeric films

GRAPHICAL ABSTRACT



Introduction

Serious infections caused by opportunistic fungal pathogens are increasingly common, especially in immunocompromised patients. In particular, the incidence of candidiasis has increased fivefold in the past 10 years because of the widespread use of broad-spectrum antibiotics and the more expanded use of immunosuppressive agents, radiotherapy, and antitumoral drugs[1]. *Candida albicans* is the most virulent *Candida* species and represents an important public health challenge with a high economic and medical relevance due to the increased costs of care, time of hospitalization, and high morbidity and mortality rates[2]. *C. albicans* is a member of the healthy human microbiota, asymptomatically colonizing several niches in the body, including the gastrointestinal tract, female reproductive tract, oral cavity, and skin. In most individuals with a healthy immune system, *C. albicans* is a harmless commensal that exists in harmony with other members of the microbiota. However, disturbances to this delicate balance can enable *C. albicans* to rapidly proliferate and cause infection[3]. The current treatment for candidiasis heavily relies on triazoles, even though patient responses to these antifungal drugs tend to be slow with a high risk of reinfection[4]. Thus, the development of new effective antifungal therapies becomes critical[5]. An interesting alternative is represented by photodynamic inactivation (PDI) of microorganisms[6,7]. This approach involves a

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photosensitizer, light and oxygen to produce cytotoxic reactive oxygen species (ROS), which specifically generates a cascade of biochemical events that produce cell damages inactivating the microorganisms[8]. Thus, the photosensitizer excited state can react with molecules from its direct environment by electron or hydrogen transfer, leading to the production of radicals including hydroxyl radical, superoxide anion radical, and hydrogen peroxide (type I reaction). Also, photosensitizer can transfer its energy to oxygen, generating the highly reactive singlet molecular oxygen, $O_2(^1\Delta_g)$ (type II reaction)[9]. ROS rapidly react with a variety of substrates producing damages in the biomolecules and leading to cell inactivation.

In general, applications of PDI have been performed using a photosensitizer in solution, which is added to cell suspensions[9]. This approach can leave a remaining photosensitizer in the site of action, and the photodynamic activity induced by visible light can produce an undesired effect in the surrounding medium. In this sense, photosensitizers immobilized on polymeric supports have been proposed to eradicate micro-organisms without contaminating the environment[10]. Therefore, the development of polymeric surfaces with either surface bound or impregnated photosensitizer molecules is interesting materials to eradicate microbes in domestic and health care media. Also, the concept of using photosensitizers immobilized on a surface for this purpose is intended to address a range of economic, ecological, and public health issues[11].

Photoinactivation of yeast strains, in particular, *C. albicans*, using photoactive polymeric films is scarce. A bioadhesive patch-based delivery of 5-aminolevulinic acid (ALA) was proposed for photokilling of onychomycosis in nails[12]. Likewise, a mucoadhesive patch containing toluidine blue O (TBO) was investigated as a delivery system for photodynamic antimicrobial of oropharyngeal candidiasis[13]. Moreover, electrochemically generated porphyrin polymeric films on optically transparent indium tin oxide electrodes were used to inactivate *C. albicans* cells[14]. Also, photoactive bridged polysilsesquioxane films were prepared by modification with a porphyrin derivative to photoinactivate *C. albicans* on the surfaces[15]. Therefore, the development of antimicrobial photoactive surfaces bearing photosensitizers that absorb in the phototherapeutic window, such as phthalocyanines, plays an important role in decreasing the incidence of nosocomial infection.

In this work, novel photoactive films based on polymer-like poly(propylene) (PP) were generated and utilized as support of zinc(II)tetramethyltetrapyridino[2,3-*b*:2′,3′-*g*:2″,3″-*l*:2‴,3‴-*q*]porphyrazinium salt (ZnTM2,3PyPz). Using a photograft polymerization of acrylic acid (AAc), the PP film was functionalized with carboxyl groups (PP-*g*-PAAc), which attached ZnTM2,3PyPz by electrostatic interaction to form PP-*g*-PAAc-Pc. It was previously established that ZnTM2,3PyPz is an active photosensitizer for the photo-oxidation of biological substrates in a biomimetic system formed by reverse micelles[16]. Moreover, polymeric fibers modified with phthalocyanines were applied for the fabrication of photoantimicrobial surfaces using electrospinning technique to inactivate bacteria[17,18]. Therefore, PP-*g*-PAAc-Pc films were evaluated as potential photodynamic surfaces to inactivate *C. albicans* cells.

Materials and methods

General

Absorption spectra were recorded on a Shimadzu UV-2401PC spectrometer (Shimadzu Corporation, Tokyo, Japan). Scanning electron microscopy (SEM) was performed on a Phillips SEM501 B instrument (Amsterdam, The Netherlands). Fluence rates were obtained with a Radiometer Laser Mate-Q (Coherent, Santa Clara, CA, USA). Cell suspensions were irradiated with a Novamat 130 AF (Braun Photo Technik, Nürnberg, Germany) slide projector containing a 150-W lamp. The light was filtered through a 2.5-cm glass cuvette filled with water to absorb heat. A wavelength range between 350 and 800 nm was selected by optical filters. Experiments were performed at room temperature with a fluence rate of 30 mW cm^{-2}. Chemicals from Aldrich (Milwaukee, WI, USA) were used without further purification. ZnTM2,3PyPz was synthesized as previously described[16]. Stock solutions (0.5 mM) of photosensitizers were prepared by dissolution in 1 mL of water. Isotactic PP film with a thickness of 20 µm was supplied by Converflex (Buenos Aires, Argentina). Acrylic acid (Merck, Darmstadt, Germany) was purified by distillation under reduced pressure. Benzophenone (BP, Mallinckrodt, MO, USA) was recrystallized by decreasing the temperature from a methanolic solution. Ultrapure water was obtained from Labconco (Kansas, MO, USA) equipment model 90901-01.

Preparation of films modified with ZnTM2,3PyPz

The PP-*g*-PAAc film was prepared as previously described[19]. Briefly, the surface of the PP film was initially modified with AAc using photograft polymerization at room temperature, BP as a radical initiator, and different reaction times. A grafting degree (*G*) of 23% was used to obtain the film with ZnTM2,3PyPz.

The molecular structure of ZnTM2,3PyPz encloses four positive charges, which allows its fixation to the film grafted with PAAc through electrostatic interaction with the carboxylate groups of the PAAc polymeric chain. The film grafted with PAAc (8 mg, $4 \times 4\ cm^2$) was left in contact with 50 mL of 0.5 mM aqueous solution of ZnTM2,3PyPz for 8 h at room temperature. The film (PP-*g*-PAAc-Ps) was then exhaustively washed with distilled water to eliminate the unattached phthalocyanine. The degree of ZnTM2,3PyPz immobilization (I) was calculated according to I (wt%) = [1 − (weight of PP-*g*-AAc/weight of PP-*g*-PAAc-Ps)] × 100 given 22% (0.14 mg cm^{-2})[19].

Steady-state photolysis

Solutions of 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA, 35 µM) in water (2 mL) containing ZnTM2,3PyPz or PP-*g*-PAAc-Ps film were irradiated with visible light[20].The samples were irradiated as described above, but using a GG455 cutoff filter. The kinetics of ABDA photo-oxidation were studied following the decrease in the absorbance (A) at $\lambda_{max} = 379$ nm. The observed rate constants (k_{obs}) were obtained by a linear least-square fit of the semilogarithmic plot of Ln A_0/A *vs* time.

Micro-organisms and growth conditions

The strain of *C. albicans* PC31 was previously characterized and identified[21]. Yeast was grown aerobically overnight in Sabouraud (Britania, Buenos Aires, Argentina) broth (3 mL) at 37°C to stationary phase. An aliquot of this culture (1 mL) was dissolved in 3 mL Sabouraud broth. Then, cells were harvested by centrifugation of broth cultures (3,000 rpm for 15 min) and resuspended in 4 mL of 10 mM phosphate-buffered saline (PBS, pH 7.0), corresponding to ~10^7 colony-forming units (CFU) mL^{-1}. The *C. albicans* cells were appropriately diluted to obtain ~10^5 or ~10^6 CFU mL^{-1} in PBS. After the irradiation period, cellular suspensions were serially diluted with PBS and each solution was quantified using the spread plate technique in triplicate. Viable *C. albicans* cells were monitored and the number of CFU was determined on Sabouraud agar plates after ~48 h incubation at 37°C.

Photosensitized inactivation of C. albicans in PBS suspension

The films were positioned in a Teflon holder (15 × 15 mm) as shown in Figure 2. The dose of photosensitizer in the plastic PP-*g*-PAAc-Ps film was 0.14 mg cm^{-2}. Cell suspensions of micro-organisms (100 µL) of different cell densities (~10^5 or ~10^6 CFU mL^{-1}) in PBS were deposited on the films. Each holder was placed into a sterilized Petri dish. After that, the cultures were exposed to visible light using the irradiation system described above (30 mW cm^{-2}) for 0, 30, and 60 min. These periods of irradiation represent a light fluence of 0, 54 and 108 J cm^{-2}, respectively. The drop with the micro-organisms remained on the surface until the end of the experiments. Studies in deuterated water (D_2O) were performed using cell suspensions (2 mL) in PBS, which were centrifuged (3,000 rpm for 15 min) and resuspended in D_2O (2 mL). Then, 100 µL cell suspensions (10^6 CFU mL^{-1}) in D_2O were deposited on the films and irradiated for 30 min (54 J cm^{-2}) with visible light. Stock solutions (2 M) of sodium azide and D-mannitol were prepared in water. Cells (2 mL, 10^6 CFU mL^{-1}) in PBS were treated with 100 mM sodium azide or D-mannitol in dark for 10 min at 37°C, and then 100 µL cell suspensions were placed on the films and irradiated for 30 min (54 J cm^{-2}) with visible light. In all cases, the cell suspensions were serially diluted with PBS and the number of colonies formed was counted as described above.

Controls and statistical analysis

Control experiments were performed in presence and absence of polymer in the dark and in the absence of polymer with cells irradiated. Three values were obtained per each condition and each experiment was repeated separately thrice. Differences between means were tested for significance by one-way ANOVA. Results were considered statistically significant with a confidence level of 95% ($p < 0.05$). Data were represented as the mean ±standard deviation of each group.

Results and discussion

Synthesis of the films

The photograft polymerization of PAAc onto PP films was performed using a batch system, where the radical initiator BP incorporated into the reaction mixture is activated under the UV light effect (Figure 1)[19]. The activated BP was incorporated into the PP chain, which was subsequently substituted by addition of AAc. The immobilization reaction of ZnTM2,3PyPz was performed through electrostatic interactions between the ammonium group of phthalocyanine and carboxylic groups of the film grafted with PAAc, $G = 23\%$ (Figure 1). The film was then exhaustively washed with distilled water to eliminate the unattached phthalocyanine. This procedure produced a PP-*g*-PAAc-Ps film with

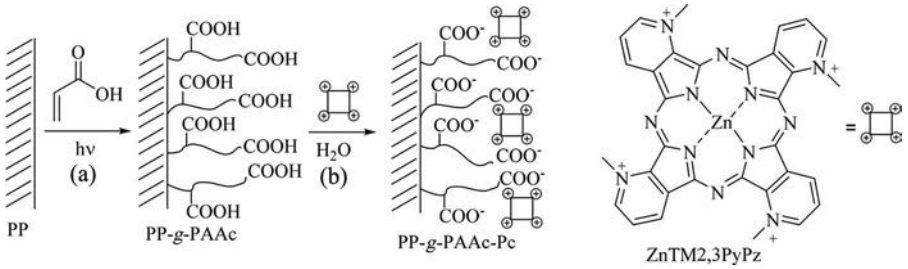

Figure 1. (a) Photograft polymerization of AAc onto the PP film surface, (b) attaching the ZnTM2,3PyPz onto carboxylic groups through electrostatic bond. *Note*: AAc, acrylic acid.

$I = 22\%$ ($0.14\ \mathrm{mg\ cm^{-2}}$) (Figure 2). Similar procedure was previously used to form biocomposite films based on PP to obtain materials as support of substrates, such as chitosan by electrostatic interaction[19].

SEM of the films

The morphological characterization of the PP films was previously performed by SEM[19]. It corroborated the existence of a marked difference between the PP and PP-*g*-PAAc films. As is observed in Figure 3, the surface of PP-*g*-PAAc forms granules as deriving from its chain arrangement. After immobilization, reaction of ZnTM2,3PyPz has taken place, the surface of the PP-*g*-PAAc-Ps product exhibited granules with small aggregates. Similar results were previously observed for PP-*g*-PAAc films used to bind antifungal agents, such as natamycin and crystal violet[22].

UV–visible absorption spectroscopy of the films

The absorption spectra of PP-*g*-PAAc-Ps film, PP-*g*-PAAc film, and ZnTM2,3PyPz are shown in Figure 4.

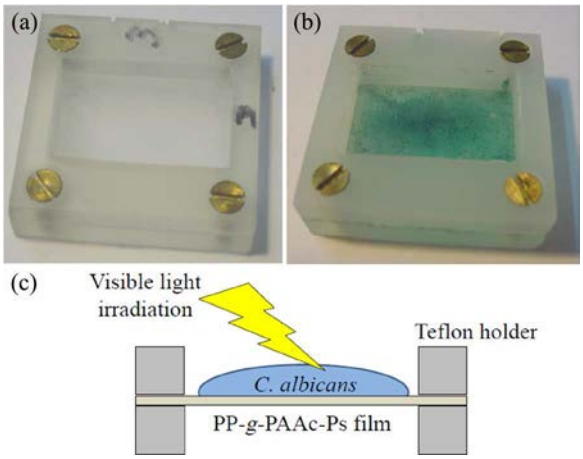

Figure 2. Photograph of the polymeric film (a) PP-*g*-PAAc and (b) PP-*g*-PAAc-Pc and (c) schematic of the photoinactivation process.

The PP-*g*-PAAc-Ps films showed the typical *Q*-bands in the region of ~660 nm, which are characteristics of Zn(II) phthalocyanine[23,24]. In the films, the *Q* bands show similar electronic transitions to those observed for the corresponding phthalocyanine in solution. For PP-*g*-PAAc-Ps films, broader and shifted electronic transition was observed in comparison with that of monomeric ZnTM2,3PyPz in solution. These facts indicate that the phthalocyanine in the film keeps the spectroscopic characteristics observed for ZnTM2,3PyPz dissolved in solution as monomer[16]. The spectrum of PP-*g*-PAAc-Ps is essentially a linear combination of the spectra of the corresponding ZnTM2,3PyPz and PP-*g*-PAAc film, with only minor differences in wavelength maxima and band shapes (Figure 4). Thus, the absorption spectra are consistent with only a weak interaction between the polymer and the phthalocyanine in the ground state, and the tetrapyrrolic macrocycle retains its individual identities. The absorption of the PP-*g*-PAAc-Ps film was about 0.9 at the maximum of the band *Q*. Taking into account a molar absorption coefficient of $9.1 \times 10^4\ \mathrm{M^{-1}\ cm^{-1}}$ for ZnTM2,3PyPz in water[16], a concentration of about 10 µM of phthalocyanine can be estimate attached on the film.

Singlet molecular oxygen detection

The detection of $O_2(^1\Delta_g)$ in water was performed using ABDA as a molecular probe[20]. A value of $k_{obs} = (1.6 \pm 0.1) \times 10^{-5}\ \mathrm{s^{-1}}$ was obtained for the photo-oxidation of

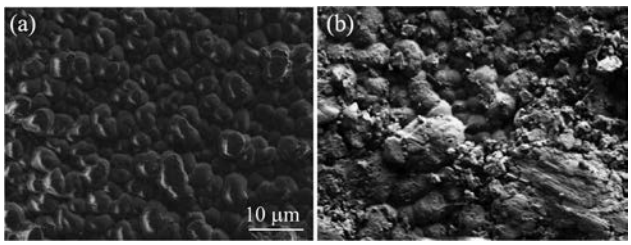

Figure 3. SEM of (a) PP-*g*-PAAc c and (b) PP-*g*-AAc-Pc films with $G = 23\%$ (scale bar 10 µm). *Note*: SEM, Scanning electron microscopy.

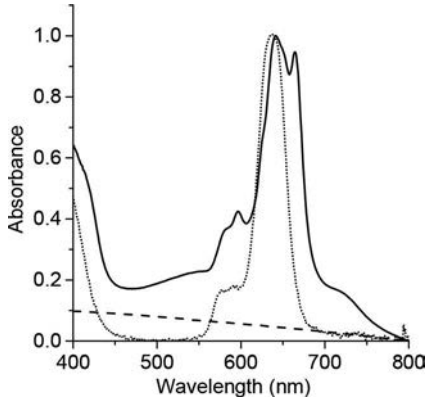

Figure 4. Absorption spectra of PP-*g*-PAAc-Pc (solid line) film, PP-*g*-PAAc (dashed line), and ZnTM2,3PyPz (dotted line) in water.

ABDA sensitized by PP-*g*-PAAc-Ps film (Figure 5). This result was compared with that induced by ZnTM2,3PyPz, which produced a $k_{obs} = (9.4 \pm 0.3) \times 10^{-3}\ s^{-1}$. It is known that the ZnTM2,3PyPz can produce $O_2(^1\Delta_g)$ with a high quantum yield of 0.65[16]. The k_{obs} for the PP-*g*-PAAc-Ps film is more than two orders of magnitude lower than for the reference ZnTM2,3PyPz. This result is expected since in the PP-*g*-PAAc-Ps film, the $O_2(^1\Delta_g)$ is formed only on the surface, while using ZnTM2,3PyPz, the photodynamic effect takes place in the solution bulk.

Photoinactivation of C. albicans *cells*

The antifungal activity photoinduced by the poly(propylene)-based films modified with ZnTM2,3PyPz was evaluated against *C. albicans* cells. This yeast represents a significant human and animal pathogen, which has the ability to colonize and cause disease within a diverse range of mammalian host sites[2]. Therefore, it is necessary to find different alternatives to prevent and eradicate infections caused by this yeast. Photoinactivation of *C. albicans* was investigated depositing a drop with the cells on poly(propylene)-based films. A schematic of the photoinactivation process is shown in Figure 2c. Figure 6 shows typical microscopic images of PP-*g*-PAAc-Pc film and PP-*g*-PAAc-Pc film bearing *C. albicans* cells on the surface. This antifungal drop-test can be used to inactivate *C. albicans* cells growing on surfaces. Thus, samples of 100 µL of PBS containing different cell densities ($\sim 1 \times 10^5$ and $\sim 1 \times 10^6$ cell mL^{-1}) were located on film and the plates were irradiated with visible light for different times. Typical results of cell survival of *C. albicans* cells are shown in Figure 7. Control experiments showed that the viability of *C. albicans* was unaffected by illumination alone or by dark incubation with the PP-*g*-PAAc-Pc films for 60 min (results not shown). Also, the cell viability was not changed by irradiation of the cultures on PP-*g*-PAAc films. This indicates that the cell mortality obtained after irradiation of the cultures on PP-*g*-PAAc-Pc films was due to the photosensitization effect of the immobilized ZnTM2,3PyPz, produced by visible light. The PP-*g*-PAAc films without ZnTM2,3PyPz present a high visible light transmission (>90%) at the wavelength of maximum absorption of the photosensitizer. Therefore, irradiation is mainly absorbed by ZnTM2,3PyPz embedded in the films, triggering the production of ROS that inactivate the cells. After treatment, spectroscopic analyses showed that the cultures were not contaminated with ZnTM2,3PyPz. Moreover, the absorbance spectra of the films indicated a 16% photobleaching of ZnTM2,3PyPz after 60 min irradiation with visible light.

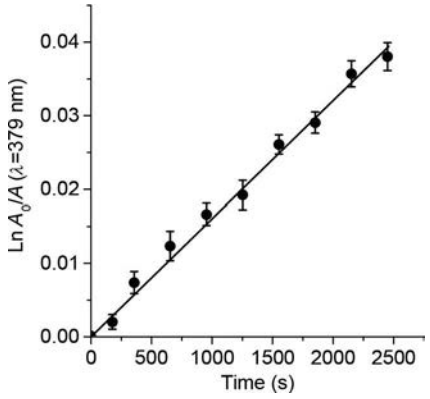

Figure 5. First-order plots for the photo-oxidation of ABDA (35 µM) in water, photosensitized by PP-*g*-AAc-Pc film. *Note*: ABDA, 9,10-anthracenediyl-bis(methylene) dimalonic acid.

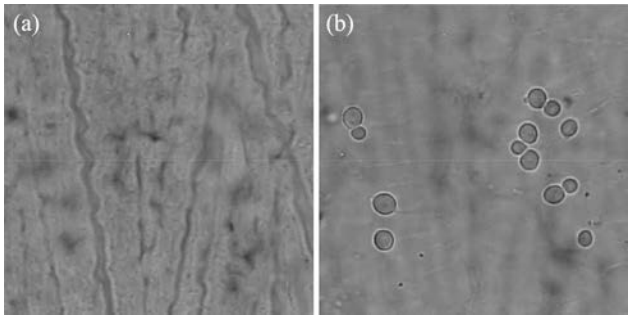

Figure 6. Microscope photograph of (a) PP-*g*-PAAc-Pc film and (b) PP-*g*-PAAc-Pc film containing *C. albicans* cells; 100 × microscope objective.

As is observed in Figure 7, the viability of *C. albicans* cells in presence of PP-*g*-PAAc-Pc films was depend on the visible light exposure. Treatments of a cell suspension of 10^5 cells mL^{-1} with PP-*g*-PAAc-Pc film produced a photosensitizing activity causing a 3 log decrease of

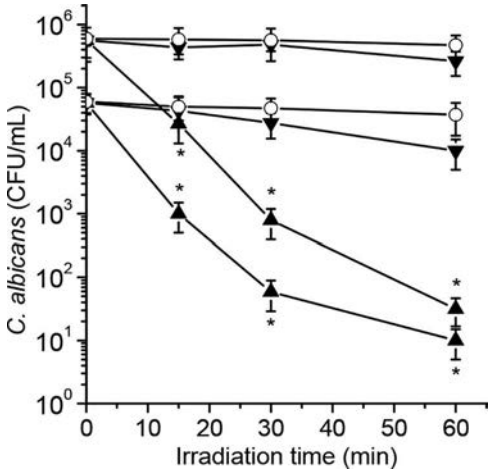

Figure 7. (a) Survival curves of *C. albicans* cells (~10^5 and ~10^6 CFU mL^{-1}) on PP-*g*-PAAc film (▼) or PP-*g*-PAAc-Pc film (▲) after different irradiation periods with visible light. Control cultures of irradiated cells (○); *$p < 0.05$ compared with control.

C. albicans survival after 30 min irradiation. These results represent a value greater than 99.9% of cellular inactivation. Also, the photodynamic action of PP-*g*-PAAc-Pc film produced a 4 log (99.99%) decrease in the cell viability of yeast, when the cultures were irradiated for 60 min. Similar PDI results were obtained with a cell density of 10^6 cells mL^{-1} using PP-*g*-PAAc-Pc films. After 30 min irradiation, a decrease in 3 log (99.9%) was found in the *C. albicans* survival. Also, photoinactivation experiments were compared with that using only photosensitizer. Thus, cell suspensions (10^6 cells mL^{-1}) were treated with 10 μM ZnTM2,3PyPz in dark for 30 min at 37°C and irradiated with visible light. This procedure produced a decrease in 0.3 log and 0.6 log in *C. albicans* survival after 15 and 30 min, respectively. Thus, ZnTM2,3PyPz alone shows lower PDI effect than P$\widetilde{P}$*g*-PAAc-Pc films, in contrast to their $O_2(^1\Delta_g)$ production efficiency. However, the binding of photosensitizer to the micro-organisms is required to obtain a higher inactivation[21]. This photosensitizer is soluble in aqueous media as a monomer and possibly it has a low interaction with *C. albicans* cells in suspensions, which decreases its photoinactivation capacity[16]. In contrast, photokilling efficacy increased in the PP-*g*-PAAc-Pc films because the cells were directly placed on the surfaces. On the other hand, when the irradiation time was extended to 60 min in presence of the films, a higher photosensitizing activity was achieved for PP-*g*-PAAc-Pc film. This photoactive film produced a 4.5 log decrease in cell survival, generating a 99.99% of cellular inactivation.

Experimental conditions difficult a direct comparison between different surfaces containing immobilized photosensitizers. Moreover, there are very few studies with photoactive surfaces bearing immobilized photosensitizers to inactivate yeast. The in vitro penetration of ALA was evaluated across human nail and into neonate porcine hoof when released from a bioadhesive patch containing 50 mg cm^{-2} ALA[12]. Patch application for 24 h allowed an ALA concentration of 2.8 mM to be achieved on the ventral side of excised human nail. Incubation of *C. albicans* and *Trichophyton interdigitale* with ALA concentrations of 10.0 mM for 30 min and 6 h, respectively, caused reductions in viability of 87 and 42%, respectively, following irradiation with red light. Reports on a mucoadhesive patch containing TBO was proposed as a potential delivery system for oropharyngeal candidiasis[13]. When releasing directly into an aqueous sink, patches containing 50 and 100 mg TBO cm^{-2} both generated receiver compartment concentrations exceeding the concentration (2.0–5.0 mg mL^{-1}) required to produce high levels of kill (>90%) of both planktonic and biofilm-growth of *C. albicans* upon illumination. In previous investigations, electrochemically generated polymeric films bearing porphyrin units on optically transparent indium tin oxide electrodes were studied as potential photodynamic surfaces to inactivate *C. albicans* cells[14]. These films exhibited a photosensitizing activity causing a ~2 log decrease of *C. albicans* cellular survival after 60 min of irradiation with visible light. Also, a bridged polysilsesquioxane film modified with porphyrin was formed by polymerization in presence of porphyrin[15]. In vitro investigations showed that these films produce photosensitized inactivation of *C. albicans* in aqueous suspensions. After 60 min of irradiation, the films exhibit a photosensitizing activity causing a ~2.5 log (99.7%) decrease of *C. albicans* cellular survival. On the other hand, polymeric fibers containing phthalocyanines were applied for the fabrication of photoantimicrobial surfaces by electrospinning technique[17,18,25,26]. Polymeric polyurethane nanofabrics modified with zinc tetraphenylporphyrin and/or zinc phthalocyanine (ZnPc) photosensitizers were prepared by the electrospinning method[25]. These nanofabrics have bactericidal surfaces and photo-oxidize inorganic and organic substrates. Also, two different phthalocyanine compounds, [2,9,16,23-tetra(4-terbutyl)phthalocyaninato] zinc(II) (TBZnPc) and (2,9,16,23-tetrasuphoxyphthalocyaninato) zinc(II) (ZnPcTS), were entrapped into a silicate matrix prepared from tetraethyl orthosilicate by the sol–gel method[27]. The photobactericidal results confirmed that the tetracationic ZnPcTS was more effective than the neutral TBZnPc in killing *Escherichia coli* in polluted waters. Therefore in comparison with these reported materials, PP-*g*-PAAc-Pc films represent an interesting photoactive material with high photoinactivation capacity of *C. albicans*.

Mechanistic insight of the C. albicans *photoinactivation*

The effect of D_2O and two suppressors of ROS, sodium azide and D-mannitol, was investigated to obtain insight about the photodynamic mechanism involved in the photosensitized inactivation of *C. albicans* cells mediated by PP-*g*-PAAc-Pc films. A cell density of 10^6 cells mL^{-1} and an irradiation time of 30 min were chosen to not produce a complete eradication of *C. albicans*, which allow observing a photoprotective effect or an increase in the photokilling under different experimental conditions used to inactivate this yeast.

Photoinactivation of *C. albicans* induced by PP-*g*-PAAc-Pc film was performed in cells suspended in D_2O to evaluate the $O_2(^1\Delta_g)$-mediated damage of microbial cells. A medium with D_2O was used to increase the lifetime of $O_2(^1\Delta_g)$ in the biological system[28]. No toxicity was detected in the presence of D_2O under irradiation without PP-*g*-PAAc film (Figure 8, line 5). Also, PP-*g*-PAAc-Pc film was not toxic for cells in dark (result not shown). However, a high cell photoinactivation of 4 log decrease was observed for *C. albicans* cells incubated with PP-*g*-PAAc-Pc film in D_2O (Figure 8, line 6), which was 1 log more effective than that found in PBS cell suspension after 30 min of irradiation.

Also, the photoinactivation of *C. albicans* was studied in presence of 100 mM sodium azide. Azide anions can be used as a quencher of intracellular $O_2(^1\Delta_g)$[29]. However, it also can deactivate photosensitizers in their triplet excited state preventing both type I and type II photoprocesses. No toxicity was detected using this concentration of azide ions under irradiation on PP-*g*-PAAc film for 30 min with visible light (Figure 8, line 7) or in the dark on PP-*g*-PAAc-Pc film (result not shown). Under this condition, no more than a 0.5 log decrease was observed in the survival of the yeast treated with PP-*g*-PAAc-Pc film after 30 min of irradiation. Therefore, the azide ion quenched the photocytotoxic species, producing a protective effect on *C. albicans*.

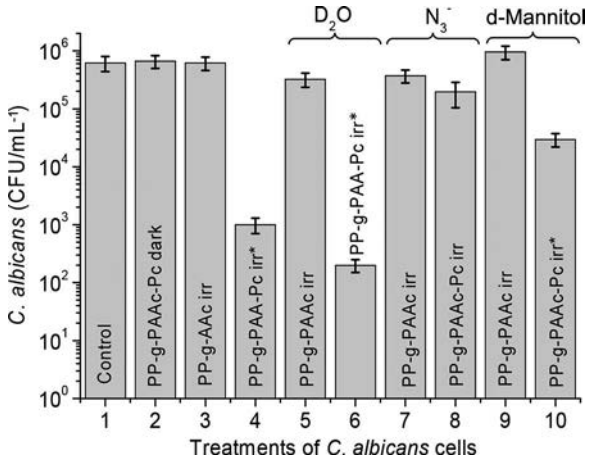

Figure 8. Survival of *C. albicans* cells (~10^6 CFU mL^{-1}) under different experimental conditions keeping in dark or exposed to visible light for 30 min; 1, cells in dark; 2, cells on PP-*g*-PAAc-Pc in dark; 3, irradiated cells on PP-*g*-PAAc; 4, irradiated cells on PP-*g*-PAAc-Pc; 5, irradiated cells on PP-*g*-PAAc in D_2O; 6, irradiated cells on PP-*g*-PAAc-Pc in D_2O; 7, irradiated cells on PP-*g*-PAAc treated with 100 mM azide; 8, irradiated cells on PP-*g*-PAAc-Pc treated with 100 mM azide; 9, irradiated cells on PP-*g*-PAAc treated with 100 mM D-mannitol; 10, irradiated cells on PP-*g*-PAAc-Pc treated with 100 mM D-mannitol; *$p < 0.05$, compared with control.

Photodynamic inactivation of *C. albicans* was investigated in the presence of 100 mM D-mannitol. This compound can be used as a scavenger of the superoxide anion radical (O_2^-) and hydroxyl radical (type I reaction)[30]. The addition of 100 mM D-mannitol was not cytotoxic for irradiated cells on PP-*g*-PAAc film (Figure 8, line 9). Also, it was not toxic for *C. albicans* cells treated with D-mannitol on PP-*g*-PAAc-Pc film in dark (result not shown). Cell inactivation mediated by PP-*g*-PAAc-Pc film exhibited a photoprotective effect of about 1 log in suspensions containing D-mannitol after 30 min of irradiation (Figure 8, line 10).

The participation of $O_2(^1\Delta_g)$ in several photosensitized processes is accepted by the observed D_2O enhancement and azide inhibition of diverse oxidative reaction rates[30–32]. Therefore, the photodamage produced to the *C. albicans* cells mediated by PP-*g*-PAAc-Pc film appears to be mainly facilitated by the intermediacy of $O_2(^1\Delta_g)$. Although in a minor contribution, the participation of other ROS could not be neglected. It was previously observed that ZnTM2,3PyPz can produce $O_2(^1\Delta_g)$ with a high quantum yield of 0.65 in sodium bis(2-ethylhexyl)sulfosuccinate (AOT) micelles[16]. Similar behavior was also obtained with a bridged polysilsesquioxane film modified with porphyrin[15]. The killing of *C. albicans* cells by this film and visible light irradiation seem to be mediated mainly by $O_2(^1\Delta_g)$. Also, PDI studies of *C. albicans* cell suspensions using a tetracationic phthalocyanine showed that this photosensitizer appears to act mainly through the intermediacy of $O_2(^1\Delta_g)$, although a minor contribution of other ROS cannot be absolutely ruled out in the photoinactivation of this yeast. It was observed that polyamide nanofiber membranes functionalized with a zinc phthalocyanines were capable of producing $O_2(^1\Delta_g)$[33].

Therefore, the presence of oxygen is essential for the generation of $O_2(^1\Delta_g)$ through the type II photosensitization mechanism that involves a triplet energy transfer reaction[28]. However, oxygen also plays a major role in the type I mechanism by adding to biochemical radicals.

In a type I process, the light-excited photosensitizers directly interact with substrate to yield radical ions in a hydrogen atom or electron transfer reaction. The majority of these radicals instantaneously reacts with oxygen and generates a complicated mixture of highly reactive oxygen intermediates, which can oxidize a wide variety of biomolecules. Oxygen is also necessary for the formation of superoxide anion radical that can occur as the result of the reaction of molecular oxygen with the radical anion of the photosensitizer.

Conclusion

Photoactive films based on polymer-like poly(propylene) modified with a tetracationic phthalocyanine, ZnTM2,3PyPz, were formed by electrostatic interaction. These plastic films represent an appropriated surface to obtain antifungal surfaces. In vitro investigations showed that these PP-*g*-PAAc-Pc films produce photosensitized inactivation of *C. albicans* in aqueous suspensions. After 60 min of irradiation, the films exhibit a photosensitizing activity causing a 4 log decrease of *C. albicans* cellular survival. The photodynamic effect induced by the photoactive films can be used to prevent the growth of *C. albicans* cells in an appropriated culture medium. To obtain inside insight about the oxidative processes that occur during the killing of yeast, first, the effect of the D_2O was analyzed on cell photoinactivation. Photo-oxidative cell killing was further enhanced in D_2O due to a prolonged lifetime of $O_2(^1\Delta_g)$. Moreover, photoprotection was found using sodium azide as type II scavengers. However, some contribution of type I pathway cannot be completely ruled out due to the protective effect of D-mannitol. Thus, the killing of *C. albicans* cells by PP-*g*-PAAc-Pc films and visible light irradiation seem to be mediated mainly by a contribution of both photodynamic processes. The results indicate that PP-*g*-PAAc-Pc films modified with ZnTM2,3PyPz are interesting photodynamic surfaces to inactivate *C. albicans*. Films can be easily manipulated with the shape of the surface to be covered, they could be used to form permanent antifungal surfaces activated by visible light. For example, to control fungal proliferation and maintain aseptic conditions on surfaces involved in the healthcare. Therefore, the photoinactivation activity of these films showed promising applications as antifungal surfaces for controlling *C. albicans*.

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