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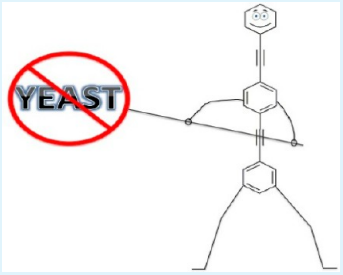
Antimicrobial Activity of Cationic Conjugated Polyelectrolytes and Oligomers against *Saccharomyces cerevisiae* Vegetative Cells and Ascospores

Ying Wang,[†,‡] Eva Y. Chi,[†] Donald O. Natvig,[§] Kirk S. Schanze,[⊥] and David G. Whitten*[,†]

[†]Department of Chemical and Nuclear Engineering, Center for Biomedical Engineering, [‡]Department of Chemistry and Chemical Biology, [§]Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131-1341, United States

[⊥]Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200, United States

ABSTRACT: The antifungal activities of poly(phenylene ethynylene) (PPE)-based cationic conjugated polyelectrolytes (CPEs) and oligo-phenylene ethynylenes (OPEs) were investigated using *Saccharomyces cerevisiae* (*S. cerevisiae*) as a model pathogen. The effect of the CPE and OPE materials on the vegetative cells and ascospores were tested in the dark or with UV-irradiation. A number of the tested polymers and oligomers significantly reduced the viability of the vegetative yeast cells in the dark, with activities exceeding the commonly used antibiotic Amphotericin B. With UV-irradiation, all of the tested CPEs and OPEs exhibited potent antifungal activities and completely inactivated the yeast cells. In particular, the oligomeric EO-OPE-1(Th, C2) strongly inactivates ascospores with UV-light at a dose level lower than sporicidal agents reported in the literature. Under conditions that promote spore germination, the CPEs and OPEs show efficient activities against the germinated spores. The protein-enriched outer envelope of yeast cells and germinated ascospores appears to serve as a main target for the CPE and OPE antimicrobial materials.



KEYWORDS: *antifungal, sporicidal, cationic conjugated polyelectrolytes, cationic oligo-phenylene ethynylenes, antimicrobial*

INTRODUCTION

Fungal infection is one of the most pressing public healthcare concerns worldwide.[1] The increased emergence of fungal infections especially associated with immunocompromised patients and medical devices and the shortage of efficient treatments has prompted the discovery and development of new antifungal agents.[2] A series of poly(p-phenylene ethynylene) (PPE)-based conjugated polyelectrolytes (CPEs) and oligo-(p-phenylene ethynylene)s (OPEs) with controlled chain lengths and functional groups have been demonstrated to exhibit significant light-activated biocidal activities against a broad range of clinically relevant pathogens, including Gram-positive and Gram-negative bacteria, viruses and biofilms.[3−5] Cationic CPEs and OPEs are amphiphilic. The photophysical and related antimicrobial properties of the CPEs and OPEs have been reported in our previous work.[6−10] Briefly, these materials have been demonstrated to bind to and inactivate pathogens both in the dark and under ultraviolet (UV)/visible light irradiation. In the dark, CPEs and OPEs can exert cytotoxicity by binding to and disrupting biomolecular self-assembled entities, such as lipid membranes, proteins and nucleic acids. Generally, the bacterial outer envelope and viral capsid serves as important targets for these materials. Under UV/visible light irradiation, CPEs and OPEs can generate corrosive singlet oxygen species ($^1O_2$) and subsequent secondary reactive oxygen species (ROS) after exposure to UV/visible light, which have been demonstrated to rapidly and efficiently inactivate microorganisms.[10−12] The cytoplasm of Gram-negative bacteria has been shown to be destroyed by the corrosive radicals.[10]

Because of the general mechanism(s) by which CPEs and OPEs inactivate bacteria and viruses, we investigate in this study the utility of the compounds as antifungal agents. The antifungal activities of a set of antimicrobial CPEs and OPEs against the vegetative eukaryotic *Saccharomyces cerevisiae* (*S. cerevisiae*) cells and ascospores/asci were measured. *S. cerevisiae* is a common opportunistic human pathogen and has long been used as a model fungal organism.[13] Because the cell envelope serves as the first point-of-contact for biocidal agents, the structure of the *S. cerevisiae* cell envelope has been the focus of many antibiotic development studies.[2] Ultrastructural and biochemical analyses reveal that the *S. cerevisiae* cell wall has a thick (100−200 nm) and layered structure and is largely composed of polysaccharides and proteins, with chitin being a minor component (Scheme 1).[14−16] The outer layer of the cell wall comprising primarily glycosylated mannoproteins represent and serve as an impermeable barrier to macromolecules due to the presence of the branched carbohydrate side chains of the mannoproteins. In addition, these carbohydrate side chains contain many phosphodiester bridges, which give rise to a negatively charged cell surface at physiological pH.[15] The inner

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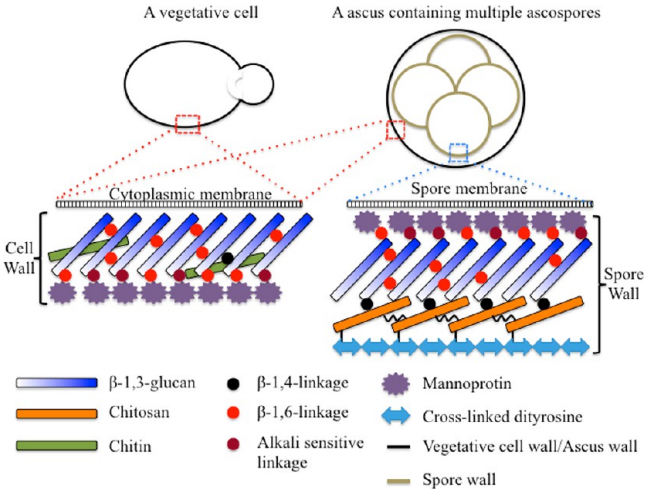
Scheme 1. Models of Yeast Vegetative Cell Wall and Spore Wall Organization[a]

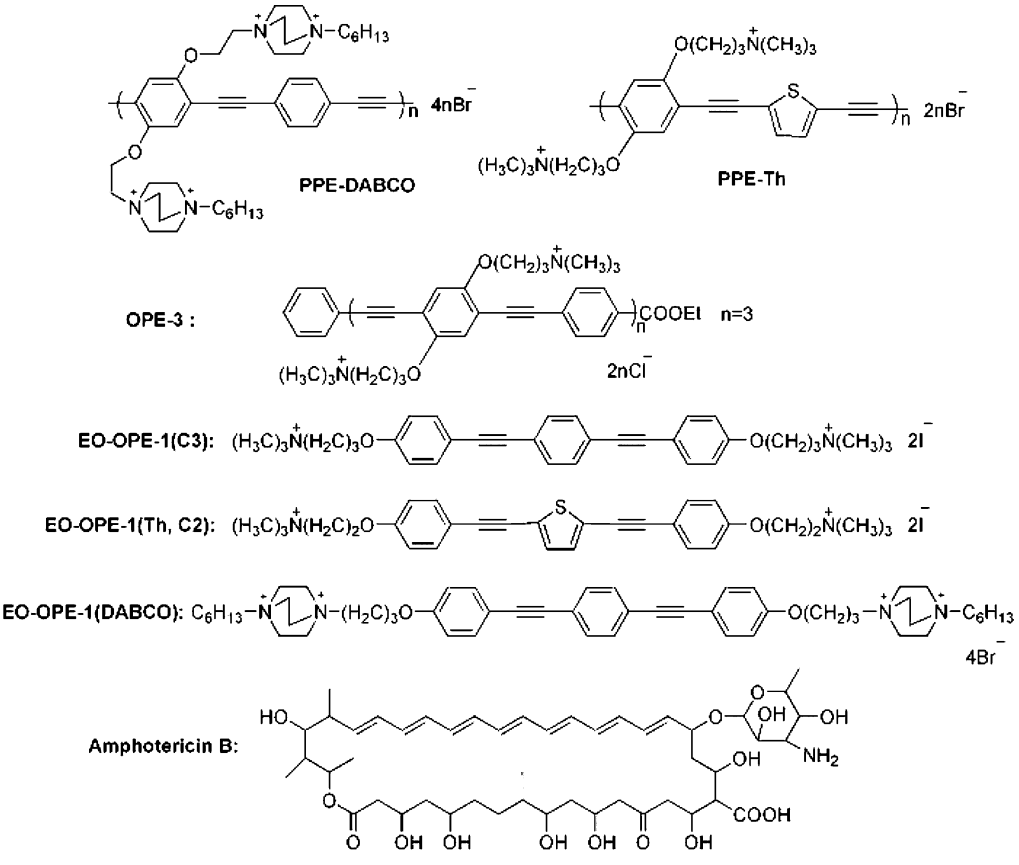


[a]Modified with permission from ref 21. Copyright Genetics Society of American 2011.

layer of the cell wall is permeable and comprised of glucans and chitin; this fibrillar layer provides mechanical strength to the cell wall. Beneath the cell wall is the cytoplasmic membrane, which is about 7.5 nm thick and contains polar lipids and proteins.[17] The lipids are distributed asymmetrically in the membrane, where the inner leaflet is largely composed of anionic phosphatidylserine (PS) and phosphatidylinositol (PI), zwitterionic phosphatidylethanolamine (PE), whereas the outer leaflet is enriched in zwitterionic phosphatidylcholine (PC) and sphingolipids of varying charges.[17]

Some yeast cells produce ascospores when they encounter certain environmental stresses, such as a lack of nutrients.[18] The spores are in a dormant state, which enables them to survive for long periods in unfavorable environments.[19] Ascospores are resistant to ambient stresses, such as antibiotics, alcohols, and moderate heat. Extreme conditions, such as strong oxidants, high heat, and γ-radiation, can efficiently inactivate bacterial and fungal spores. However, these treatments are neither environmentally friendly nor practical to use in the treatment of patients. Once spores are exposed to suitable conditions, they can germinate and become pathogenic. Unlike bacterial endospores, yeast ascospores form through a meiotic process.[18] Ascospores have a unique multilayered wall, which enables them to be more resistant to environmental stresses and damages compared to vegetative cells (Scheme 1).[18] The two inner layers of the ascospore wall are composed of polysaccharides mannan and glucan.[18] On top of the glucan layer is a layer of chitosan and a layer of cross-linked dityrosine.[20,21] Multiple ascospores are enclosed by the ascal coat, which is derived from the cytoplasmic membrane and cell wall of the vegetative mother cell,[18] to form an ascus. These thick protective structures make the inert ascospores highly resistant to the antibiotics. A 2% glucose solution has been shown to be an excellent germinating agent for the yeast ascospores.[22] And, applying antimicrobial agents under

Scheme 2. Structures of the Antimicrobial Oligomers and Polymers Used in This Study[a]



[a]*n* denotes the number of repeat units.

conditions that induce germination has been proven to be an efficient strategy to inactivate bacterial spores.[23]

Because of the cationic nature of the PPE-based polymers and oligomers, the materials are expected to readily associate with anionic groups on the surfaces of vegetative yeast cells and asci. After exposure to UV/visible light, singlet oxygen and secondary ROS species generated by the bound CPEs and OPEs could cause severe damages to the outer envelopes of these cells.

In the current study, we investigated the antifungal activities of a series of CPEs and OPEs (Scheme 2) in the dark and under UV-irradiation against *S. cerevisiae* vegetative cells, germinated ascospores and asci. The exact molecular weights of PPE-DABCO and PPE-Th are currently unavailable, but the number average molar mass (Mn) values are estimated to be within the range of 20-30 kD. As a comparison, the Food and Drug Administration (FDA)-approved, broad-spectrum antifungal agent Amphotericin B (AmB), was used as a benchmark antibacterial agent. It has been proposed that AmB exerts its toxicity by penetrating the fungal cell wall and binding to ergosterols, thereby perturbing the function of the fungal cytoplasmic membrane.[2] In this study, the viability of cells exposed to CPEs and OPEs under different conditions were determined. Additionally, cellular damages induced by the CPEs and OPEs on the morphological level were visualized by scanning electron microscopy (SEM).

EXPERIMENTAL METHODS

Materials. The antimicrobial materials (Scheme 2) used in this study were synthesized as reported previously.[5,24,25] All chemicals, antibiotics and yeast culture media were purchased from Sigma-Aldrich (St. Louis, MO) or BD Biosciences (Franklin Lakes, NJ). *S. cerevisiae* strains ATCC 9763 and 204722 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Ultrapure water was used throughout the study (Milli-Q, 18.2 MΩ cm^{-1} resistivity).

Yeast Strains and Culture Conditions. *S. cerevisiae* (ATCC 9763) vegetative cells were grown in YPD medium (1% Yeast extract, 2% Peptone and 2% Glucose). A fresh yeast culture was inoculated from an overnight culture and incubated for varying periods at 30°C to obtain different growth phases. Yeast cells were collected by centrifugation and washed twice with PBS buffer (10 mM sodium phosphate, 138 mM NaCl, and 2.7 mM KCl at pH 7.4). The cell pellet was resuspended with PBS buffer to $O.D._{600} \sim 1.0$. A highly sporulated yeast strain (ATCC 204722) was used to prepare ascospores/asci. The yeast cells were first grown in the YPAD medium (1% yeast extract, 2% peptone, 0.04% adenine sulfate, and 2% glucose) overnight at room temperature. The recovered cells at exponential phase were pelleted, washed with PBS and then prepared for sporulation on an acetate agar (0.1% glucose, 0.18% KCl, 0.25% yeast extract, 0.82% sodium acetate·$3H_2O$ and 1.5% agar) for 1 week. The harvested asci were washed, briefly sonicated, and resuspended in PBS to $O.D._{600} \approx 1.2$. The germinated asci or spores were prepared via incubating with the germination solution (2% glucose and 0.37% NH_4Cl) for 20 h at room temperature.

Antifungal Activity. A sample consisting of 10 μg/mL of the CPEs, OPEs, or AmB was used for the antifungal test against *S. cerevisiae* vegetative cells (ATCC 9763). The yeast cell solutions were incubated with the biocidal agents at 30°C in the dark with continuous shaking for 60 min. The UV-light irradiation experiments were carried out in a photoreactor (4 UV-lamps, LZC-ORG, Luzchem Research Inc., Ottawa, Canada) at room temperature for 30 min. Two irradiation sources were employed based on the different light-absorbing properties of the CPEs and OPEs. LZC-420 (centered at ~420 nm) and UVA (centered at ~350 nm) were used to irradiate CPEs and OPEs, respectively.[4] The sporicidal activity of the CPEs and OPEs (30 μg/mL) were evaluated against the asci (ATCC 204722) with or without germination in the dark or under UV-irradiation (10 lamps in the photoreactor) for 60 min. The ability of a CPE or an OPE to inactivate vegetative cells and spores was determined by the plate counting method and was calculated as log (N_o/N), where N is the number of colony forming units (CFU) of the yeast solution after exposure to a CPE or an OPE and N_o is the CFU of a control (without biocidal material or UV-irradiation). The Log CFU reductions caused by the biocidal treatments relative to the control are reported. The reported values and errors were averages and standard deviations of duplicate measurements, respectively.

Electron Microscopy. Samples for ultrastructural examination were prepared as described above. For SEM imaging, cell pellets were resuspended in 2.5% glutaraldehyde and incubated at room temperature overnight to preserve the interface between the cells and antimicrobial agents, followed by washing with PBS buffer. Fixed cells were dehydrated by sequential treatment with increasing concentrations of ethanol for 30 minutes. The dehydrated samples were dried at room temperature and sputtercoated with ~12 nm thick gold/palladium. Samples were observed by SEM (Quanta 3D, Dual beam FEGSEM/FIB, FEI, Hillsboro, OR).

RESULTS AND DISCUSSION

The vast majority of antibiotics that have been developed to control bacterial infections are not effective against fungi or spores.[26] In addition, growing attention is being paid to the need to decontaminate environments contaminated by spores. Here, we report the antifungal and sporicidal activities of a class of synthetic arylene-ethynylene-based polymers and oligomers.

CPEs and OPEs Exhibit Efficient Dark and Light-Enhanced Antifungal Activities. Figure 1 summarizes the biocidal effects of different CPEs and OPEs in the dark (blue bars) or with UV/visible light irradiation (red bars) against fresh *S. cerevisiae* vegetative cells (ATCC 9763) prepared in the

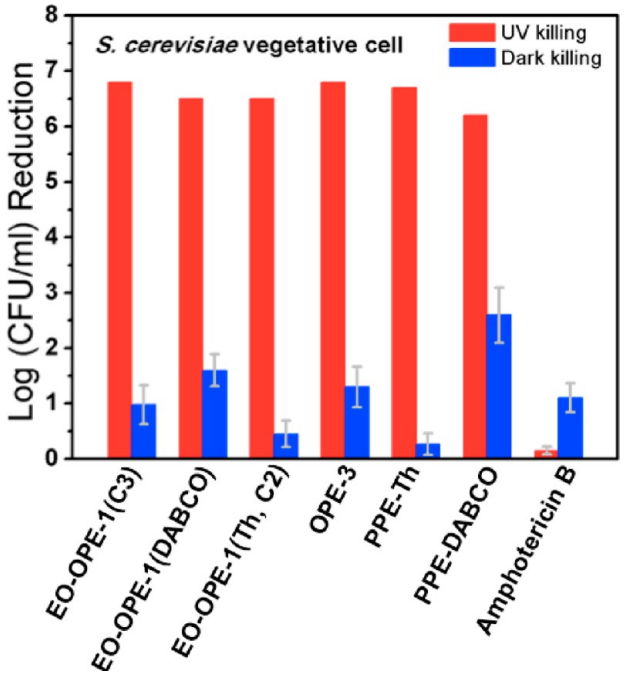


Figure 1. Inactivation of the *S. cerevisiae* vegetative cells (ATCC 9763) (~2×10^6 CFU/mL) at exponential phase by CPEs or OPEs (10 μg/mL) in the dark (blue bars, 60 min incubation) or with UV-light irradiation (red bars, 4 UV-lamps and 30 min incubation). The detection limit for the assay is 6–7 logs of CFU/mL. UVA and LZC-420 irradiation alone causes about 0.16 and 0.17 log of inactivation, respectively.

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YPD medium for 4 h. As shown, in the absence of irradiation, the polymeric PPE-DABCO exhibited significant antifungal activity against the vegetative cells in 60 min, reducing the number of CFU by more than 2 orders of magnitude. By comparison, the oligomeric EO-OPE-1(DABCO), OPE-3 and EO-OPE-1(C3) induced approximately a 10-fold decrease in CFU in the dark, on the same order as AmB. However, limited dark inactivation activities were observed for EO-OPE-1(Th, C2) and PPE-Th. UV irradiation significantly enhanced the inactivation activities of all CPEs and OPEs against the yeast vegetative cells relative to the activities obtained in the dark. After just 30 min of irradiation, no living cells were detected. Interestingly, AmB's biocidal activity decreased with UV-irradiation compared to its activity in the dark and is comparable to the inactivation caused by the UV-light alone (Figure 1). This is likely due to: 1) damages caused by the UV-light to the polyene rings of the AmB molecules, thus compromising its antifungal activity and/or 2) the shorter incubation time (30 min) employed with the UV-light irradiation experiment as compared to the dark incubation experiment (60 min).

It has been shown previously that CPEs and OPEs can bind to and denature anionic protein assemblies in the dark[27] and covalently modify cytoplasmic proteins under UV/visible light irradiation.[10] PPE-DABCO and EO-OPE-1(DABCO), which are both functionalized with DABCO-based quaternary ammonium groups (Scheme 2), exhibited the highest dark fungal inactivation activities among the tested agents. The high activities are likely in part due to the unique structural features of the compounds. The DABCO-based quaternary ammonium groups possess the highest positive charge density on its side chains among the CPEs and OPEs used in this study, which may enable these materials to most strongly associate with the negatively charged cell surface and induce the greatest degree of disruption of the self-assembled protein structures on the cell wall. This could cause the integrity of the cell envelope to be compromised. Moreover, the proteins on the cell surface could readily react with singlet oxygen and secondary ROS species generated by the CPEs and OPEs under UV-irradiation,[10] leading to a higher extent of inactivation.

Microorganisms always exhibit various biological characters during their life cycle,[28] such as viability and metabolic activity, thus the cells at different growth phases may show different susceptibilities to the biocidal agents. Figure 2 shows that the susceptibility of vegetative *S. cerevisiae* cells to the dark antifungal activity of EO-OPE-1(Th, C2) varies with growth phase. Yeast cells grow exponentially for the first 12 h in the YPD medium, after which the cells shift to diauxic and postdiauxic phases.[28] After continuous growth for about 1 week in the same medium, the cells enter the stationary phase.[28] The level of nutrients is one of the main factors controlling the cell cycle and the stationary phase has been recognized as a dormancy state in response to nutrient starvation.[28] As shown in Figure 2, EO-OPE-1(Th, C2) exhibited growth phase-dependent dark antifungal activities, where the inactivation activity increased during the first 24 h of incubation, during which the yeast cells had undergone rapid growth with high metabolism and are highly susceptible to EO-OPE-1 (Th, C2)-induced toxicity. When glucose becomes exhausted, cells switch from fermentative growth to respiratory metabolism and grow at a much lower rate.[28] As shown, the metabolically inactive yeast cells were more resistant to the biocidal activity of EO-OPE-1(Th, C2) after 24 h in YPD medium (Figure 2). These

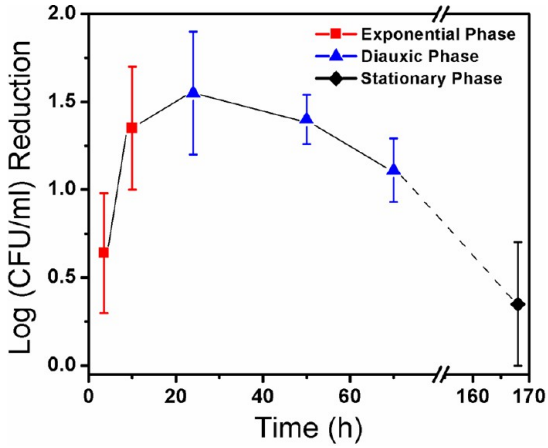

Figure 2. Inactivation of vegetative *S. cerevisiae* cells (ATCC 9763) (~1 × 10^6 CFU/mL) at different growth phases by 10 μg/mL EO-OPE-1(Th, C2) in the dark for 60 min. Only ~1 × 10^5 CFU/mL of the yeast cells were alive after 168 h continuous incubation. The growth phases are determined on the basis of ref 28.

results imply that the dark antifungal activities of the CPEs and OPEs are dependent on the metabolism of the yeast cells, and the cells are more susceptible when they are metabolically active. It is also important to note that the light-enhanced activity of EO-OPE-1(Th, C2) were growth phase-independent under the conditions tested. After exposure to the UV-light (4 lamps, 30 min), 10 μg/mL EO-OPE-1(Th, C2) completely inactivated the vegetative cells (~1 × 10^6 CFU/mL) at all of the tested growth phases. Thus in addition to damage caused to the cell envelope, it is possible that the CPEs and OPEs may be taken up by the yeast cells and interfere with metabolic pathways, thereby contributing to cell death. This seems particularly plausible in the case of damage caused in the dark, given that killing in the dark was dependent on the metabolic state of the cells.

CPEs and OPEs Exhibit Limited Sporicidal Activities. Inactivation of bacterial and fungal spores is widely recognized as being more difficult than the killing of vegetative cells, and therefore spores present a special challenge to human health. As described, ascospores have a multilayered protective coat and moreover, ascospores are covered by an additional layer of ascal membrane and wall (Scheme 1).[18] These structures enable ascospores to be highly resistant to environmental stresses and damages and the inactivation of these organism requires damages to and penetration through both the ascal layer as well as the spore coat.

As shown in Figure 3, in the absence of UV-light, none of the tested oligomers or polymers was effective at inactivating ascospores after 60 minutes of incubation. The viability of ascospores did not decrease after treatment with OPE-3 (30 μg/mL) for 5 h in the dark (data not shown). In contrast, upon treatment with EO-OPE-1(Th, C2) and strong UV-irradiation (10 lamps), ascospore viability decreased about 95% within 1 h (Figure 3). No increased sporicidal efficiency was observed for EO-OPE-1(Th, C2) after extended UV-light exposure (3 h). All other tested oligomers and polymers were essentially inactive with UV-light irradiation under the current experimental conditions. Our results indicate that, except EO-OPE-1(Th, C2), other tested biocidal agents may not be able to disrupt or penetrate through the ascal coat or spore coat.

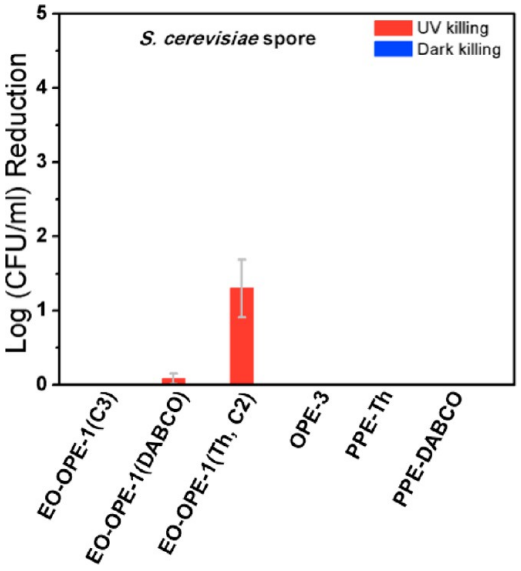

Figure 3. Inactivation of *S. cerevisiae* ascospores (ATCC 204722) (~2×10^6 CFU/mL) by CPEs or OPEs (30 μg/mL) in the dark (blue bars) or with UV-light irradiation (red bars, 10 UV-lamps) for 60 min. UVA and LZC-420 irradiation alone did not cause spore inactivation under the experimental conditions.

Working with *Bacillus* spores, Kane and coworkers showed that upon germination, spores become more susceptible to biocidal agents.[23] In the presence of germinant, that can trigger the spore germination program, the ascal and spore coats could be removed and the spore re-entries into the vegetative cell cycle.[18,22] Herein, we tested the effect of germination on the susceptibility of yeast ascospores to the CPEs and OPEs. The ascospores were first treated with 2% glucose and 0.37% NH_4Cl for 20 h to promote germination. Then the germinated spores were incubated with a CPE and an OPE in the dark or under UV-irradiation for 1 h. As shown in Figure 4, in the dark, 30

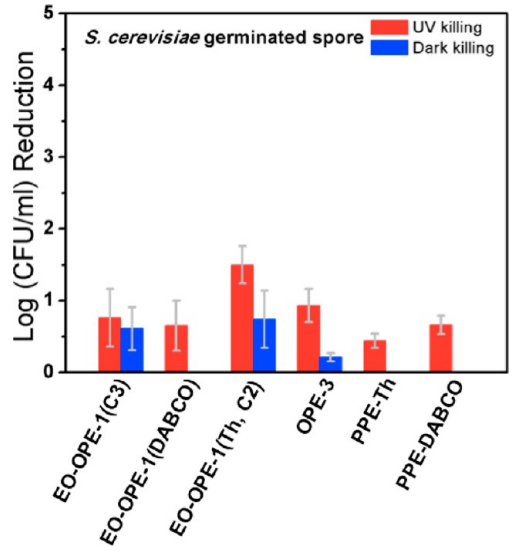

Figure 4. Inactivation of the germinated *S. cerevisiae* ascospores (ATCC 204722) (~2 × 10^6 CFU/mL) by CPEs or OPEs (30 μg/mL) in the dark (blue bars) or with UV-light irradiation (red bars, 10 UV lamps) for 60 min. UVA and LZC-420 irradiation alone did not cause obvious spore inactivation under these current experimental conditions.

μg/mL of EO-OPE-1(C3), EO-OPE-1(Th, C2), and OPE-3 resulted in more than 50% reduction in the viability of germinated spores. Moreover, all of the tested agents became effective at reducing spore viability with UV light irradiation. EO-OPE-1(Th, C2) inactivated more than 95% of the germinated spores and showed the highest light-enhanced biocidal efficacy compared to other polymers or oligomers. It is interesting to note that PPE-DABCO and EO-OPE-1-(DABCO) did not exhibit any biocidal effect agains the germinated ascospores in the dark, whereas they were fairly active against the vegetative cells (Figure 1). This could be due to the existence of the extra ascus coat outside the ascospores. The ascus coat is derived from the envelope of the vegetative mother cell, and thereby has similar characteristics, such as chemical components and net charge, to those of the mother cell envelope. Since the DABCO functionalized oligomers and polymers readily associate to the vegetative cell envelope, they are expected to bind to the ascus coat with high affinity. Once bound to the coat, the polymer or oligomer molecules can become hindered from binding to and damaging the germinated spores underneath the coat.

The EO-OPE-1(Th, C2) oligomer exhibited efficient biocidal activity against both dormant and germinated ascospores under UV-light irradiation, probably due to its ability to sensitize singlet oxygen species with a high quantum yield in addition to its high solubility in water.[5,29] However, none of the tested agents reduced the number of CFU by more than 2 orders of magnitude against the germinated ascospores. Even when the germinated spores were treated with 30 μg/mL OPE-3 for extended periods (2 to 3 h) with UV-light exposure, no improvement in biocidal activity was observed. Rine and co-workers have reported that 2% glucose can germinate 95% of the yeast spores (~1 × 10^7/mL) within 12 h.[22] Although the ascospores samples employed in our study were incubated for 20 h in 2% glucose to promote complete germination, viewing the ascospores under a light microscope showed that a number of the spores did not undergo germination (data not shown). Incomplete germination of the spores can account the low inactivation levels shown in Figure 4. Additionally, although YPD is an excellent germination medium, bio-macromolecules in this rich medium, such as proteins and nucleic acids, may bind to the CPEs and OPEs, attenuating their biocidal activities.

CEPs and OPEs Induced Morphological Damages to *S. cerevisiae* Vegetative Cells and Asci. To gain some insights to the antifungal and sporicidal activities of the CPEs and OPEs, morphological changes of yeast cells and ascospores in response to exposure to the different agents were examined by SEM imaging. As shown in Figure 5A, the vegetative cells alone in PBS buffer maintained their integrity with smooth cell surfaces. Yeast cells treated with EO-OPE-1(C3) in the dark remained intact, but cell surfaces appeared rough and wrinkled (Figures 5B). Some of the PPE-Th treated cells exhibited obvious morphological damages (Figure 5C) compared to the untreated samples, and significant cell envelope defects were observed. PPE-Th has been demonstrated to associate strongly with and denature anionic proteins,[27] and it may bind to the anionic groups on the yeast cell surface and induce lethal defects. The sample preparation process for SEM imaging may amplify these defects and result in large holes on the cell surface.[30] Similar to the untreated cells (Figure 5A), control yeast cells irradiated by the UV-light for 30 min without the addition of biocidal agents appeared intact with smooth surfaces (Figure 6A), consistent with our findings that UV-

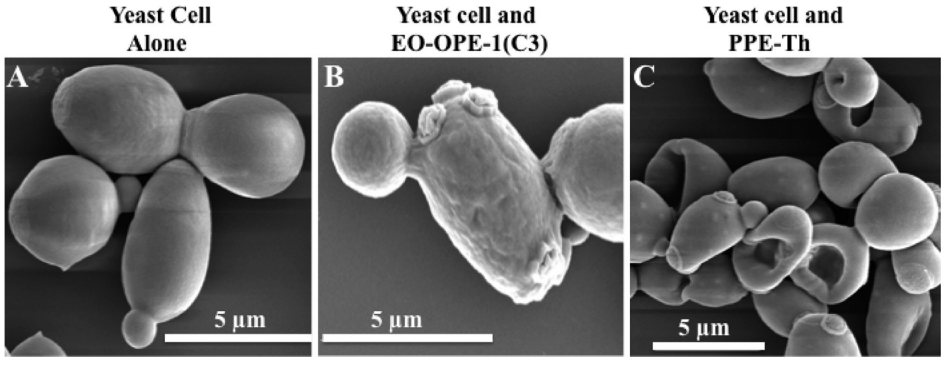

Figure 5. SEM images of (A) *S. cerevisiae* vegetative cells alone (ATCC 9763) (~2×10^6 CFU/mL) and incubated with 10 μg/mL (B) antimicrobial EO-OPE-1(C3) or (C) PPE-Th for 1 h in the dark.

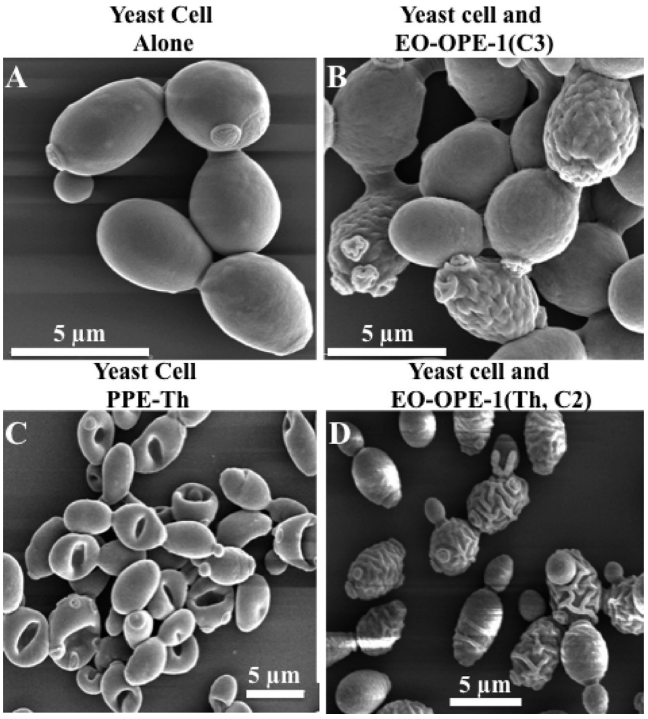

Figure 6. SEM images of *S. cerevisiae* vegetative cells (ATCC 9763) (~2×10^6 CFU/ml) alone (A) and incubated with 10 μg/mL EO-OPE-1(C3) (B) or PPE-Th (C) or EO-OPE-1(Th, C2) (D) for 30 min with UV-light irradiation (4 lamps).

irradiation alone caused low-levels of toxicity to the vegetative cells (Figure 1). However, the addition of oligomeric EO-OPE-1(C3) or EO-OPE-1(Th, C2) with UV-irradiation caused significant changes to the cell envelope (Figure 6B, D). Similar to the damage seen in the dark, PPE-Th disrupted the cell envelope and created large defects on the cell surface (Figure 6C). However, roughening and wrinkling of the cell surface were not observed. The polymeric PPE-Th and oligomeric EO-OPEs induced different types of damages to the yeast cells both in the dark and with UV-irradiation, which may be due to the permeability of the cell wall top layer. The glycosylated mannoproteins with branched carbohydrate side chains on the cell surface can render the cell wall impermeable to the polymeric PPE-Th. As a result, the relatively hydrophobic PPE-Th chains may aggregate[31] and act to create defects on the cell surface. However, the EO-OPEs with a nearly linear conformation may penetrate deeper into the cell envelope and reorganize the layered structure of the cell envelope. It is important to note that the yeast cells seem to be damaged to different extents by the biocidal materials (Figures 5 and 6), which is probably due to the nonuniform binding of these materials toward the yeast cells.

EO-OPE-1(Th, C2) was shown to inactivate more than 95% of dormant and germinated yeast ascospores (Figures 3 and 4). As shown in Figure 7A, the ascus exhibits a classic tetrahedral

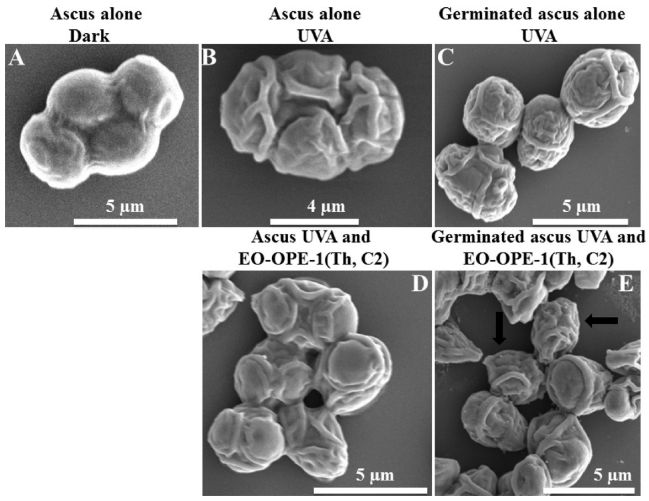

Figure 7. SEM images of (A) an *S. cerevisiae* ascus containing four ascospores (ATCC 204722) (~1 × 10^6 CFU/mL), (B) an ascus irradiated with UV-light, (C) germinated asci irradiated with UV-light, (D) asci incubated with 30 μg/mL EO-OPE-1(Th, C2) and irradiated with UV-light for 1 h, and (E) germinated asci incubated with 30 μg/mL EO-OPE-1(Th, C2) and irradiated with UV-light for 1 h. All UV-irradiation experiments were carried out with 10 lamps.

shape with a smooth coat and each individual ascospore is clearly seen in the ascus. With UV-light irradiation alone, the ascus coat became ridged in appearance, while maintaining its structural integrity and continuing to enclose the ascospores (Figure 7B). After treatment with EO-OPE-1(Th, C2) in the presence of UV-light (Figure 7D), asci exhibited similar structural features as shown in Figure 7B and no further morphological damage could be observed to the asci, implying that the oligomer may use other mechanisms to inactivate the ascospores.

In contrast to the asci, germinated ascospores have a distinctive appearance and a smaller size (Figure 7C), indicating that the ascus coat may be partially removed during

the germination process. The addition of EO-OPE-1(Th, C2) with UV-irradiation caused obvious damages to the surface of the germinated spores with the vegetative cell wall (Figure 7E, see arrows), which appeared very similar to the vegetative cells treated by the oligomer with UV-light (Figure 6B, D).

CONCLUSIONS

This study explored the antifungal and sporicidal activities of the arylene-ethynylene-based CPEs and OPEs using *S. cerevisiae* as a model pathogen toward the goal of extending the utility of these polymers and oligomers as biocidal agents. In the dark, the CPEs and OPEs exhibited moderate inactivation of vegetative yeast cells. In particular, PPE-DABCO, EO-OPE-1(DABCO) and OPE-3 showed comparable or higher antifungal activities compared to the widely-used antibiotic AmB. With UV-irradiation, all of the tested agents induced more than 6-log reductions in yeast cell viability. Moreover, antifungal activities of the compounds were shown to be dependent on the growth phase of the yeast cells where cells in growth phases that correspond to higher metabolic activities were more susceptible to the biocidal activities of CPEs and OPEs. These materials showed limited inactivation activities towards ascospores. In the dark, all compounds tested were not effective at reducing spore viability and with UV irradiation, only EO-OPE-1(Th, C2) was active, inactivating more than 95% of the yeast ascospores. The materials were more effective at inactivating ascospores once they undergo germination, where the tested agents showed inactivation activity with UV-light irradiation. SEM imaging revealed that the envelopes of the vegetative cell and germinated ascospore are targets of the CPEs and OPEs.

AUTHOR INFORMATION

Corresponding Author

*E-mail: whitten@unm.edu.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Wisplinghoff, H.; Bischoff, T.; Tallent, M.; Seifert, H.; Wenzel, R. P.; Edmond, M. B. *Clin. Infect. Dis.* **2004**, *39*, 1093−1093.
(2) Odds, F. C.; Brown, A. J. P.; Gow, N. A. R. *Trends Microbiol.* **2003**, *11*, 272−279.
(3) Tang, Y. L.; Corbitt, T. S.; Parthasarathy, A.; Zhou, Z. J.; Schanze, K. S.; Whitten, D. G. *Langmuir* **2011**, *27*, 4956−4962.
(4) Wang, Y.; Canady, T. D.; Zhou, Z. J.; Tang, Y. L.; Price, D. N.; Bear, D. G.; Chi, E. Y.; Schanze, K. S.; Whitten, D. G. *ACS Appl. Mater. Interfaces* **2011**, *3*, 2209−2214.
(5) Dascier, D.; Ji, E.; Parthasarathy, A.; Schanze, K. S.; Whitten, D. G. *Langmuir* **2012**, *28*, 11286−11290.
(6) Ji, E.; Corbitt, T. S.; Parthasarathy, A.; Schanzes, K. S.; Whitten, D. G. *ACS Appl. Mater. Interfaces* **2011**, *3*, 2820−2829.
(7) Wang, Y.; Chi, E. Y.; Schanze, K. S.; Whitten, D. G. *Soft Matter* **2012**, *8*, 8547−8558.
(8) Jiang, H.; Taranekar, P.; Reynolds, J. R.; Schanze, K. S. *Angew. Chem., Int. Ed.* **2009**, *48*, 4300−4316.
(9) Wang, Y.; Corbitt, T. S.; Jett, S. D.; Tang, Y.; Schanze, K. S.; Chi, E. Y.; Whitten, D. G. *Langmuir* **2012**, *28*, 65−70.
(10) Wang, Y.; Jett, S. D.; Crum, J.; Schanze, K. S.; Chi, E. Y.; Whitten, D. G. *Langmuir* **2013**, *29*, 781−792.
(11) Xing, C. F.; Xu, Q. L.; Tang, H. W.; Liu, L. B.; Wang, S. *J. Am. Chem. Soc.* **2009**, *131*, 13117−13124.
(12) Dahl, T. A.; Midden, W. R.; Hartman, P. E. *J. Bacteriol.* **1989**, *171*, 2188−94.
(13) Goldstein, A. L.; McCusker, J. H. *Genetics* **2001**, *159*, 499−513.
(14) Lesage, G.; Bussey, H. *Microbiol. Mol. Biol. Rev.* **2006**, *70*, 317−343.
(15) Klis, F. M.; Mol, P.; Hellingwerf, K.; Brul, S. *FEMS Microbiol. Rev.* **2002**, *26*, 239−256.
(16) Osumi, M. *Micron* **1998**, *29*, 207−233.
(17) Vanderrest, M. E.; Kamminga, A. H.; Nakano, A.; Anraku, Y.; Poolman, B.; Konings, W. N. *Microbiol. Rev.* **1995**, *59*, 304−322.
(18) Coluccio, A.; Neiman, A. M. *Microbiology* **2004**, *150*, 3189−3196.
(19) Feofilova, E. P.; Ivashechkin, A. A.; Alekhin, A. I.; Sergeeva, Y. E. *Appl. Biochem. Micro.+* **2012**, *48*, 1−11.
(20) Briza, P.; Ellinger, A.; Winkler, G.; Breitenbach, M. *J. Biol. Chem.* **1988**, *263*, 11569−11574.
(21) Neiman, A. M. *Genetics* **2011**, *189*, 737−765.
(22) Herman, P. K.; Rine, J. *EMBO J.* **1997**, *16*, 6171−6181.
(23) Banerjee, I.; Mehta, K. K.; Dordick, J. S.; Kane, R. S. *J. Appl. Microbiol.* **2012**, *113*, 1461−7.
(24) Tang, Y. L.; Hill, E. H.; Zhou, Z. J.; Evans, D. G.; Schanze, K. S.; Whitten, D. G. *Langmuir* **2011**, *27*, 4945−4955.
(25) Zhao, X. Y.; Pinto, M. R.; Hardison, L. M.; Mwaura, J.; Muller, J.; Jiang, H.; Witker, D.; Kleiman, V. D.; Reynolds, J. R.; Schanze, K. S. *Macromolecules* **2006**, *39*, 6355−6366.
(26) Cowan, M. K.; Talaro, K. P. *Microbiology: A Systems Approach*, 2nd ed.; McGraw-Hill Science: New York, 2008.
(27) Wang, Y.; Zhou, Z. J.; Zhu, J. S.; Tang, Y.; Canady, T. D.; Chi, E. Y.; Schanze, K. S.; Whitten, D. G. *Polymers* **2011**, *3*, 1199−1214.
(28) Wernerwashburne, M.; Braun, E.; Johnston, G. C.; Singer, R. A. *Microbiol. Rev.* **1993**, *57*, 383−401.
(29) Zhou, Z. J.; Corbitt, T. S.; Parthasarathy, A.; Tang, Y. L.; Ista, L. F.; Schanze, K. S.; Whitten, D. G. *J. Phys. Chem. Lett.* **2010**, *1*, 3207−3212.
(30) Bozzola, J. J.; Russell, L. D., *Electron Microscopy: Principles and Techniques for Biologists*, 1st ed.; Jones & Bartlett: London, 1992.
(31) Corbitt, T. S.; Ding, L. P.; Ji, E. Y.; Ista, L. K.; Ogawa, K.; Lopez, G. P.; Schanze, K. S.; Whitten, D. G. *Photochem. Photobiol. Sci.* **2009**, *8*, 998−1005.