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In Vitro Cytotoxicity of Antimicrobial Conjugated Electrolytes: Interactions with Mammalian Cells

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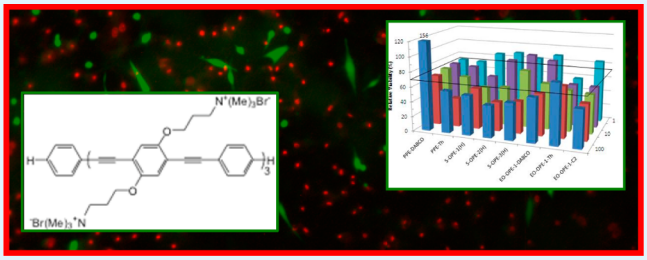
Supporting Information

ABSTRACT: An estimated 19 000 deaths and $3−4 billion in health care costs per year in the United States are attributed to methicillin-resistant Staphlococcus aureus (MRSA) infections. Certain conjugated phenylene ethynylene (CPE)-based polymers (PPE) and oligomers (OPE) have been demonstrated to exhibit dark and light-activated antimicrobial activity. Until recently, the relative cytotoxicity of these PPEs and OPEs toward mammalian cells haas been unknown, limiting the applications for which they may be used (e.g., reducing and/or preventing the spread of untreatable bacterial strains). In this work, we examine the toxicity of CPEs to mammalian cells using cytotoxicity assays of cellular monolayers. Eight CPEs, two PPEs and six OPEs, were selected for these studies based on their biocidal activity, and diversity of repeat unit number and functional groups. Briefly, two cell types were exposed to CPEs at concentrations ranging from 1−100 ug/mL for 24 h. We find that concentration largely determines the resulting viability of cells, although at intermediate concentrations (5−10 ug/mL), the effect of light on light-activated compounds is very important. Furthermore, we find that the longer-chained compounds are cytotoxic at much higher concentrations, and therefore have the widest range of concentrations available for potential applications.



KEYWORDS: *antimicrobial, amphiphilic, biocide, cytotoxicity, live/dead, phenylene ethynylene*

INTRODUCTION

An estimated 19,000 deaths and $3−4 billion in health care costs per year in the USA are attributed to methicillin-resistant *Staphlococcus aureus* (MRSA) infections.[1] At the same time, the development of new classes of antibiotics slowed significantly after the introduction of quinolones, gylcopeptides, and streptogramins in the mid 20th century, with no new classes of drugs entering the clinic until the introduction of oxazolidinines in 2000.[1] However, in 1987, a promising new class of compounds known as antimicrobial peptides (i.e., host-defense peptides) were discovered.[2] These compounds, which are generally short peptides (10−50 amino acids), have a net positive charge (+2 to +9), and have a significant (∼30%) fraction of residues that are hydrophobic.[3]

Subsequently, a number of peptidomimetics were developed in an effort to increase the stability of antimicrobial peptides, while still retaining their biocidal activity. These antimicrobial peptidomimetics include α-peptides,[4,5] β-peptides,[6−8] peptoids (oligo-*N*-substituted glycines),[9,10] and cyclic peptides.[11] In addition, facially-amphiphilic polymers, which have only the facially-amphiphilic feature of the antimicrobial peptides, and facially-symmetric polymers have been developed by the Tew group,[12−14] as well as the Whitten and Schanze groups,[15] respectively. These synthetic analogues show a great deal of promise as antibacterial and antiviral agents, as they are theoretically easily substituted, and will not run the risk of inducing adapted resistance by bacteria.

Recently, the Schanze and Whitten groups have worked to synthesize, characterize, and develop applications for conjugated phenylene ethynylene (CPE)-based polymers and oligomers (reviewed in Ji et al.).[15] All of these polymers and oligomers (PPEs and OPEs) are conjugated electrolytes, i.e., they have double or triple bonds separated by a single bond and form ions in solution. Furthermore, all of the compounds are fluorescent, and are soluble in water to some extent. Previous research has demonstrated that the PPEs have biocidal activity against both Gram-positive and Gram-negative bacteria, including *Bacillus anthracis* spores, *Bacillus atrophaeus*, *Cobetia marina*, and *Pseudomonas aeruginosa* strain PAO1.[16−19] Furthermore, the compounds were found to have differential cytotoxicity toward bacteria upon exposure to light ("light activation") versus under absence of light ("dark conditions"); in some cases, this amounted to 400× difference in the threshold for biocidal activity between light and dark conditions. In addition to their ability to kill Gram-positive

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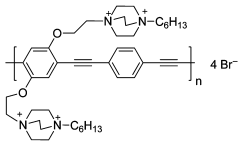
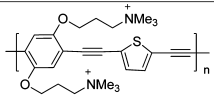
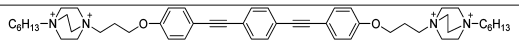
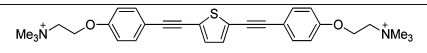
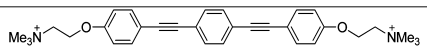
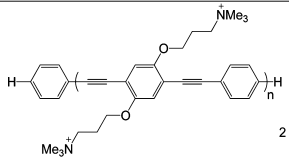
and Gram-negative bacteria as well as viruses, these CPEs may be readily attached to surfaces or incorporated into fibers. These traits make them ideally suited for biomedical devices or other applications. However, prior to use in such applications, the polymers and oligomers must be tested for toxicity toward mammals.

In this work, two cell types were exposed to eight compounds at concentrations from 1-100 μg/mL for 24 h. During the last 50 min of the 24 h exposure period, half of the cells were irradiated with either visible or ultraviolet light, depending on the compound. PPEs absorb light in the visible region of the electromagnetic spectrum, whereas OPEs absorb light in the ultraviolet region of the spectrum. Following irradiation, the cells were assayed for viability relative to untreated cells. Relative viability was then compared among compounds with respect to light or dark conditions and concentration. Three runs of each set of conditions, all with triplicate samples, were performed on separate days.

2. MATERIALS AND METHODS

2.1. Synthesis of PPEs and OPEs. Eight phenylene ethynylene compounds were selected for this study, including two PPEs [PPE-DABCO, or 1,4-diazabicyclo[2.2.2]octane, and PPE-thiophene], three end-only oligo(phenylene ethynylene)s (EO-OPE-1-DABCO, EO-OPE-1-Th, and EO-OPE-1-C2), and three symmetric oligo(phenylene ethynylene)s (S-OPE-*n*(H), where *n* = 1, 2, or 3). The synthesis of these compounds has been described in detail previously.[20−25] The structure of each compound is given in Table 1.

Table 1. Chemical Structures of Phenylene Ethynylene Polymers and Oligomers, Categorized by Family[a]

Family	Structure	Name
PPEs		PPE-DABCO
PPEs		PPE-Th
EO-OPEs		EO-OPE-1-DABCO
EO-OPEs		EO-OPE-1-Th
EO-OPEs		EO-OPE-1-C2
S-OPEs		S-OPE-n(H) n = 1, 2, or 3

[a]PPEs = poly(phenylene ethynylene)s, EO-OPEs = end-only oligo(phenylene ethynylene)s, S-OPEs = symmetric oligo(phenylene ethynylene)s, DABCO = 1,4-diazabicyclo[2.2.2]octane; Me = methyl group, CH_3; Th = thiophene.

2.2. Cell Treatment with Polymer/Oligomer Solutions. Routine cell culture and cell culture for the MTS assays are described in the Supporting Information. After 24 h, the DMEM-based medium was exchanged for 1:1 DMEM/F-12-based medium. For each compound tested, three wells each at concentrations of 0 (negative control), 1, 5, 10, 50, and 100 μg/mL polymer or oligomer were prepared and three wells were left as-is (containing DMEM-based medium without phenol red). To facilitate testing in light and dark conditions, a second plate was prepared in an identical manner. Following medium exchange, the plates were returned to the incubator.

Solutions were prepared from polymers and oligomers synthesized as previously described. Polymer test solutions were prepared by diluting aqueous stock solutions to the desired test concentration by adding sterile 1:1 DMEM/F-12 medium (HyClone, Logan, UT). Stock solutions of the oligomers were prepared by weighing the oligomers, dissolving them in 100 μL DMSO (assisted by vortex mixing), adding 900 μL ultrapure water, and vortex mixing. The oligomer test solutions were prepared by diluting stock solutions to the desired test concentration by adding sterile 1:1 DMEM/F-12 medium. Polymer and oligomer test solutions were prepared within a day of the medium exchange.

2.3. Light Exposure. After 23 h in the incubator, the plates were removed from the incubator and allowed to cool for 10 min uncovered in a biosafety cabinet in the dark (to prevent later overheating above 39.5° C, when cells are adversely affected by temperature.)[26] Following this brief cooling period, the "dark" plate was covered in foil and the "light" plate was exposed to light for 50 min. Light plates containing polymer solutions were placed on a light box (Mini Light Box, Bel-Art Products, Pequannock, NJ) emitting visible light. Light plates containing oligomer solutions were placed beneath a 365 nm UV lamp (Model EA-140, 4 Watt, Spectroline, Westbury, NY) supported by two empty tissue culture flasks.

2.4. MTS Assay. Immediately following light exposure (for a total of 24 h of exposure to polymer or oligomer solutions), an MTS assay was conducted. 20 μL of MTS solution (Promega, Madison, WI), previously thawed for ∼1 h, was added to each sample well on the two plates. Following the addition of the MTS solution, the plates were returned to the incubator for 1 h. After exactly 1 h, the wells were quickly checked for bubbles, any bubbles were eliminated, and absorbance readings at 570 nm were measured on a microplate reader (Molecular Devices, Sunnyvale, CA) after a 10 s pre-mix. The plate reader was set to 37° C and allowed to warm up prior to use. Absorbance readings were measured one plate at a time in the order in which MTS solution was added to the plates.

3. RESULTS AND DISCUSSION

3.1. Effect of Concentration on Viability. In this work, mammalian cells were exposed to eight related synthetic compounds, including two PPEs [PPE-DABCO and PPE-Thiophene], three end-only oligo(phenylene ethynylene)s [EO-OPE-1-DABCO, EO-OPE-1-Th, and EO-OPE-1-C2], and three symmetric oligo(phenylene ethynylene)s [S-OPE-n(H), where *n* = 1, 2, or 3]. (See Table 1 for structures) Each compound was tested at concentrations from 1−100 μg/mL for 24 h, and exposed to both endothelial and epithelial mammalian cells. Previously reported antimicrobial activity has been observed at concentrations at or below 10 μg/mL; however, live/dead assays with mammalian cells showed that a five-fold increase in concentration (50 vs. 10 μg/mL) was necessary to observe any significant cytotoxicity in mammalian cells. See the Supporting Information, Figure 5, for examples of live/dead assay results.

In general, we found that viability decreases with increasing concentration. The decrease in viability with increasing concentration is consistent in dark and light conditions. This decrease in viability is also consistent in both cell types. Table 2

Table 2. Relative Viabilities (%) for Endothelial Cells Exposed to Each of Eight Test Compounds for 24 h, with the Final 50 min of Exposure in Dark or Light Conditions[a]

		Concentration (μg/mL)				
		1	5	10	50	100
PPE-DABCO	dark	72 ± 14	71 ± 8	**68 ± 13**	**62 ± 23**	192 ± 24
	light	73 ± 6	72 ± 6	71 ± 10	**68 ± 15**	156 ± 19
PPE-Th	dark	75 ± 11	71 ± 17	72 ± 11	**34 ± 22**	**69 ± 46**
	light	72 ± 10	70 ± 13	**62 ± 15**	**39 ± 10**	**57 ± 43**
S-OPE-1(H)	dark	85 ± 10	87 ± 10	81 ± 8	**60 ± 9**	**56 ± 8**
	light	85 ± 11	**58 ± 16**	**50 ± 9**	**55 ± 7**	**54 ± 6**
S-OPE-2(H)	dark	91 ± 9	87 ± 16	78 ± 14	**62 ± 15**	**55 ± 16**
	light	89 ± 11	84 ± 8	**52 ± 10**	**40 ± 4**	**43 ± 6**
S-OPE-3(H)	dark	87 ± 7	86 ± 10	87 ± 13	75 ± 10	**59 ± 14**
	light	83 ± 6	93 ± 10	78 ± 17	**43 ± 4**	**50 ± 8**
EO-OPE-1-DABCO	dark	87 ± 17	86 ± 12	71 ± 12	**31 ± 3**	**33 ± 6**
	light	89 ± 4	87 ± 8	**62 ± 9**	**57 ± 7**	**59 ± 8**
EO-OPE-1-Th	dark	85 ± 18	89 ± 14	91 ± 12	**40 ± 28**	**29 ± 3**
	light	**58 ± 9**	**56 ± 9**	**56 ± 10**	**69 ± 7**	81 ± 10
EO-OPE-1-C2	dark	89 ± 18	102 ± 15	113 ± 18	**22 ± 6**	**28 ± 3**
	light	84 ± 10	**56 ± 10**	**53 ± 6**	**49 ± 8**	**51 ± 8**

[a]Viabilities ≤70% are shown in bold type. The underlined entries highlight specific data points discussed in Sections 3.1 and 3.2.

Table 3. Relative Viabilities (%) for Epithelial Cells Exposed to Each of Eight Test Compounds for 24 h, with the Final 50 min of Exposure in Dark or Light Conditions[a]

		Concentration (μg/mL)				
		1	5	10	50	100
PPE-DABCO	dark	84 ± 7	84 ± 6	84 ± 6	74 ± 20	**62 ± 12**
	light	90 ± 4	95 ± 10	89 ± 6	77 ± 24	**57 ± 10**
PPE-Th	dark	**67 ± 36**	79 ± 4	83 ± 7	**70 ± 9**	**35 ± 17**
	light	**60 ± 39**	89 ± 8	**66 ± 16**	**22 ± 6**	**20 ± 11**
S-OPE-1(H)	dark	91 ± 10	82 ± 9	89 ± 4	**63 ± 9**	**47 ± 6**
	light	78 ± 14	**44 ± 16**	**29 ± 4**	**28 ± 4**	**27 ± 2**
S-OPE-2(H)	dark	102 ± 14	105 ± 7	92 ± 18	**58 ± 9**	**36 ± 10**
	light	81 ± 9	**64 ± 18**	**29 ± 6**	**24 ± 3**	**24 ± 1**
S-OPE-3(H)	dark	96 ± 11	99 ± 7	97 ± 19	**60 ± 13**	**26 ± 5**
	light	95 ± 11	**59 ± 13**	**49 ± 11**	**33 ± 13**	**25 ± 3**
EO-OPE-1-DABCO	dark	81 ± 5	102 ± 17	98 ± 9	**23 ± 14**	**14 ± 1**
	light	87 ± 22	**65 ± 23**	**42 ± 15**	**27 ± 7**	**29 ± 3**
EO-OPE-1-Th	dark	85 ± 10	92 ± 7	101 ± 8	**33 ± 19**	**12 ± 1**
	light	65 ± 13	**29 ± 6**	**29 ± 7**	**32 ± 4**	**39 ± 3**
EO-OPE-1-C2	dark	95 ± 7	96 ± 5	101 ± 11	**44 ± 24**	**11 ± 2**
	light	81 ± 14	**65 ± 12**	**32 ± 8**	**23 ± 6**	**24 ± 5**

[a]Viabilities ≤70% are shown in bold type. The underlined entries highlight specific data points discussed in Sections 3.1 and 3.2.

lists relative viabilities of endothelial cells after 24 h exposure to eight test compounds in both light and dark conditions at five concentrations: 1, 5, 10, 50, and 100 μg/mL. Table 3 lists relative viabilities of epithelial cells tested similarly. The decrease in viability with concentration occurs with all compounds tested. However, there is one exception to this trend. Endothelial cells exposed to PPE-DABCO appear to have a higher viability at 100 μg/mL than 50 μg/mL (refer to red box in Table 2). Because this trend does not occur with epithelial cells exposed to the same concentrations of the same polymer, the apparent increase is most likely due to aggregation of the polymer in solution, which would affect the absorbance readings on which the viabilities are based. Aggregation of the PPE-DABCO in solution could be related to cellular products of endothelial cells or a slight difference in growth medium composition between cells (medium used for culture of endothelial cells contained 1% MEM NEAA).

In cell-based assays, the onset of cytotoxicity for a given compound for a given exposure time is defined as the concentration at which relative viability is ≤70%. In most cases, the onset of cytotoxicity occurs between 10 and 50 μg/mL. At the lowest concentration tested, 1 μg/mL, one of the oligomers, EO-OPE-1-Th, is cytotoxic to both cell types in certain conditions. At a concentration of 5 μg/mL, two additional oligomers, S-OPE-1(H) and EO-OPE-1-C2, are cytotoxic to both cell types in certain conditions.

At a concentration of 100 μg/mL, only three of the 32 cell type/compound/condition combinations have viabilities greater than 70%: endothelial PPE-DABCO dark and PPE-DABCO light, and endothelial EO-OPE-1-Th light. However, these three combinations have viabilities less than 70% at concentrations less than 100 μg/mL, so the higher-than-expected viabilities at 100 μg/mL are probably not a true

Table 4. Relative Viabilities (%) for Bovine Aortic Endothelial Cells (BAECs) and Vero (epithelial) Cells Exposed to Each of Eight Test Compounds for 24 h, with the Final 50 min of Exposure in Dark

		Concentration (μg/mL)				
		1	5	10	50	100
PPE-DABCO	BAEC	72 ± 14	71 ± 8	68 ± 13	62 ± 23	192 ± 24
	Vero	84 ± 7	84 ± 6	84 ± 6	74 ± 20	62 ± 12
PPE-Th	BAEC	75 ± 11	71 ± 17	72 ± 11	34 ± 22	69 ± 46
	Vero	67 ± 36	79 ± 4	83 ± 7	70 ± 9	35 ± 17
S-OPE-1(H)	BAEC	85 ± 10	87 ± 10	81 ± 8	60 ± 9	56 ± 8
	Vero	91 ± 10	82 ± 9	89 ± 4	63 ± 9	47 ± 6
S-OPE-2(H)	BAEC	91 ± 9	87 ± 16	78 ± 14	62 ± 15	55 ± 16
	Vero	102 ± 14	105 ± 7	92 ± 18	58 ± 9	36 ± 10
S-OPE-3(H)	BAEC	87 ± 7	86 ± 10	87 ± 13	75 ± 10	59 ± 14
	Vero	96 ± 11	99 ± 7	97 ± 19	60 ± 13	26 ± 5
EO-OPE-1-DABCO	BAEC	87 ± 17	86 ± 12	71 ± 12	31 ± 3	33 ± 6
	Vero	81 ± 5	102 ± 17	98 ± 9	23 ± 14	14 ± 1
EO-OPE-1-Th	BAEC	85 ± 18	89 ± 14	91 ± 12	40 ± 28	29 ± 3
	Vero	85 ± 10	92 ± 7	101 ± 8	33 ± 19	12 ± 1
EO-OPE-1-C2	BAEC	89 ± 18	102 ± 15	113 ± 18	22 ± 6	28 ± 3
	Vero	95 ± 7	96 ± 5	101 ± 11	44 ± 24	11 ± 2
Vero < BAEC	2/8	2/8	1/8	3/8	7/8	

Table 5. Relative Viabilities (%) for Bovine Aortic Endothelial Cells (BAECs) and Vero (epithelial) Cells Exposed to Each of Eight Test Compounds for 24 h, with the Final 50 min of Exposure in Light

		Concentration (μg/mL)				
		1	5	10	50	100
PPE-DABCO	BAEC	73 ± 6	72 ± 6	71 ± 10	68 ± 15	156 ± 19
	Vero	90 ± 4	95 ± 10	89 ± 6	77 ± 24	57 ± 10
PPE-Th	BAEC	72 ± 10	70 ± 13	62 ± 15	39 ± 10	57 ± 43
	Vero	60 ± 39	89 ± 8	66 ± 16	22 ± 6	20 ± 11
S-OPE-1(H)	BAEC	85 ± 11	58 ± 16	50 ± 9	55 ± 7	54 ± 6
	Vero	78 ± 14	44 ± 16	29 ± 4	28 ± 4	27 ± 2
S-OPE-2(H)	BAEC	89 ± 11	84 ± 8	52 ± 10	40 ± 4	43 ± 6
	Vero	81 ± 9	64 ± 18	29 ± 6	24 ± 3	24 ± 1
S-OPE-3(H)	BAEC	83 ± 6	93 ± 10	78 ± 17	43 ± 4	50 ± 8
	Vero	95 ± 11	59 ± 13	49 ± 11	33 ± 13	25 ± 3
EO-OPE-1-DABCO	BAEC	89 ± 4	87 ± 8	62 ± 9	57 ± 7	59 ± 8
	Vero	87 ± 22	65 ± 23	42 ± 15	27 ± 7	29 ± 3
EO-OPE-1-Th	BAEC	58 ± 9	56 ± 9	56 ± 10	69 ± 7	81 ± 10
	Vero	65 ± 13	29 ± 6	29 ± 7	32 ± 4	39 ± 3
EO-OPE-1-C2	BAEC	84 ± 10	56 ± 10	53 ± 6	49 ± 8	51 ± 8
	Vero	81 ± 14	65 ± 12	32 ± 8	23 ± 6	24 ± 5
Vero < BAEC	5/8	5/8	6/8	7/8	7/8	

indicator that these compounds are nontoxic at that concentration.

3.2. EFFECT OF LIGHT ON VIABILITY

In general, we find that adding light during cell exposure to the test compound decreases viability, particularly at concentrations from 5 to 10 μg/mL. At the lowest concentration tested, 1 μg/mL, the effect of light on viability is negligible for all compounds except one of the oligomers, EO-OPE-1-Th. For 1 μg/mL EO-OPE-1-Th, viability decreases from 85 to 58% for endothelial cells and 85 to 65% for epithelial cells with the addition of light. At 5 μg/mL, light decreases viability of both cell types exposed to EO-OPE-1-Th and two additional oligomers, S-OPE-1(H) and EO-OPE-1-C2. At 10 μg/mL, light decreases viability of both cell types exposed to all compounds tested except PPE-DABCO. At 50 μg/mL, light decreases viability of both cell types exposed to the three symmetric oligomers. At 100 μg/mL, light increases viability of both cell types exposed to the three "end-only" oligomers. The viability of cells exposed to PPE-DABCO is unaffected or slightly increased by light, except at 100 μg/mL, where a decrease in viability is observed in both cell types. The observed increases in viability of cells with light at high polymer or oligomer concentrations may be due to the inner filter effect, where excess CPE in solution acts to filter incident light. Enhanced bacterial survival with at moderate or high concentrations of CPEs has been previously reported.[16] The effect of light on the viability of cells exposed to PPE-Th is unclear.

3.3. Comparison between Cell Types. In general, we find that relative viability is somewhat cell-type dependent, particularly at higher polymer or oligomer concentrations. Table 4 compares viabilities of endothelial cells and viabilities of epithelial cells in the dark. Table 5 compares viabilities between

the two cell types in the light. The instances where epithelial cell viability is less than endothelial cell viability are tabulated at the bottom of each table. Supporting Information Figures 1−4 provide graphical comparisons of relative viabilities for the two cell types.

For the lower concentrations, 1−10 μg/mL, epithelial cells do not have lower viabilities than endothelial cells overall (seven of the 16 compound/condition combinations for 1, 5, and 10 μ/mL). However, where viability is lower for epithelial cells than endothelial cells at lower concentrations, the majority of occurrences are in the light (five of seven, five of seven, and six of seven for 1, 5, and 10 μ/mL, respectively). At 50 μg/mL, epithelial cells have lower relative viabilities then endothelial cells for ten of the 16 compound/condition combinations. Of these ten occurrences, seven are in the light. At 100 μg/mL, epithelial cells have lower relative viabilities for 14 of the 16 compound/concentration combinations. Of these 14 occurrences, seven are in the light and seven are in the dark.

3.4. Comparison among Individual Compounds. The DABCO polymer is unique in that it is cytotoxic at a lower concentration in the dark. All other compounds are cytotoxic at lower concentrations in the light. In the dark, the DABCO-containing polymers and oligomers are cytotoxic at the lowest concentrations, the thiophene-substituted polymers and oligomers are cytotoxic at intermediate concentrations, and oligomers with neither DABCO nor thiophene functional groups are cytotoxic at the highest concentrations. In light conditions, the thiophene-substituted polymers and oligomers are cytotoxic at the lowest concentrations, the oligomers with neither DABCO nor thiophene functional groups are cytotoxic at intermediate concentrations, and DABCO-containing polymers and oligomers are cytotoxic at the highest concentrations. Previous work comparing the antimicrobial activity of the DABCO polymer and the thiophene-substituted polymer found that the thiophene polymer killed significantly more bacteria in dark conditions than light conditions.[18] The enhanced bacterial killing in the dark was attributed to the lipophilic character of PPE-Th. The opposite trend observed with mammalian cells may be correlated to differences in phospholipid composition between mammalian and bacterial membranes.

In both dark and light conditions, with both cell types, the S-OPE-3(H) oligomer is cytotoxic at higher concentrations than its shorter analogs, S-OPE-1(H) and S-OPE-2(H). The lower relative cytotoxicity of S-OPE-3(H) is a trend also observed with bacteria. The antimicrobial activity of S-OPE-3(H) is less than its shorter analogs, S-OPE-1(H) and S-OPE-2(H).[15] The relative triplet yield and the sensitized $^1O_2$ yield decrease with oligomer length for this series of S-OPEs.

3.5. Comparison among Families Based on Chemical Structure. The eight compounds tested fall into three families of phenylene ethynylenes: PPEs, S-OPEs, and EO-OPEs. Viability data can be compared across these families to look for trends attributable to characteristic chemical structures. Figures 1 and 2 show relative viabilities after exposure to PPEs as a function of concentration. Figure 1 shows data for endothelial cells and Figure 2 shows data for epithelial cells. Neglecting the endothelial cell data for 100 μg/mL, viability after exposure to PPE-DABCO is not significantly affected by concentration or light conditions. For PPE-Th, more cells are killed in the light with increasing concentration.

Figures 3−6 present similar data for the two families of oligomers. Figures 3 and 4 show relative viabilities after exposure to S-OPEs as a function of concentration. Symmetric oligomers (S-OPEs) follow a trend similar to PPE-Th. More cells are killed in the light with increasing concentration. Further, for S-OPEs, the number of repeat units factors into differences in viability with increasing concentration. Viability for S-OPE-1(H) in light is significantly lower than viability in the dark at 5 μg/mL. For S-OPE-2(H) and S-OPE-3(H) viabilities are significantly lower at 10 and 50 μg/mL, respectively. Figures 5 and 6 show relative viabilities after exposure to EO-OPEs as a function of concentration. The end-only oligomers (EO-OPEs) follow a trend opposite PPE-Th and the S-OPEs in that more cells are killed in the dark with increasing concentration. The EO-OPEs are different from the other families in that they do not have any side chains. Model membrane studies with EO-OPEs similar to EO-OPE-1-Th and EO-OPE-1-C2 show that these EO-OPEs are particularly effective at interacting with negatively-charged membrane lipids

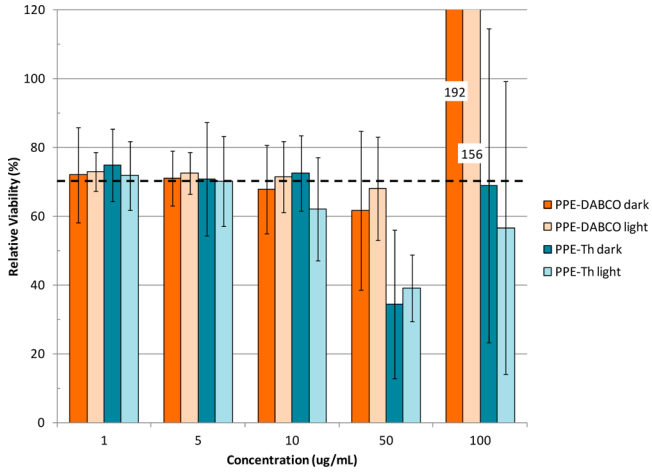


Figure 1. Relative viabilities (in %) of bovine aortic endothelial cells exposed to varying concentrations (1, 5, 10, 50, and 100 μg/mL, horizontal axis) of two PPEs for 24 h (in the dark or with the final 50 min in light). The dashed black line indicates 70% viability, below which the compounds are considered cytotoxic.

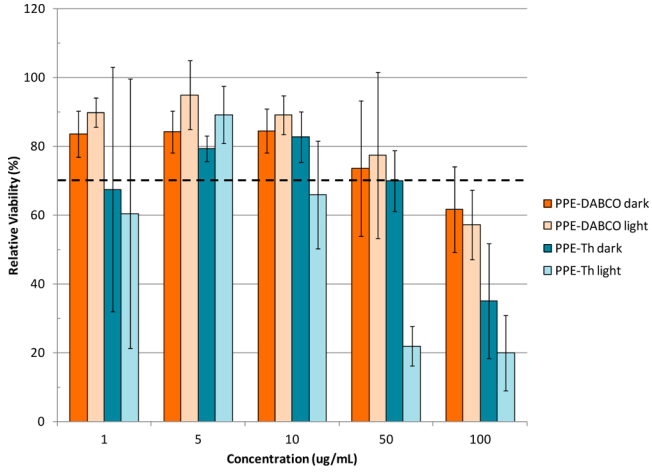


Figure 2. Relative viabilities (in %) of Vero (epithelial) cells exposed to varying concentrations (1, 5, 10, 50, and 100 μg/mL, horizontal axis) of two PPEs for 24 h (in the dark or with the final 50 min in light). The dashed black line indicates 70% viability, below which the compounds are considered cytotoxic.

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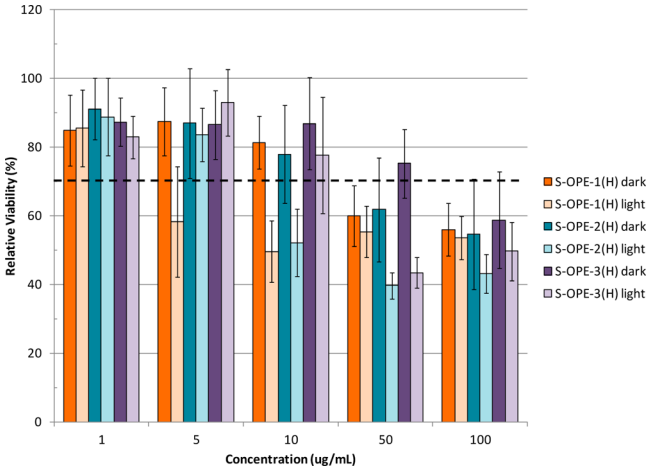

Figure 3. Relative viabilities (in %) of bovine aortic endothelial cells exposed to varying concentrations (1, 5, 10, 50, and 100 μg/mL, horizontal axis) of three S-OPEs for 24 h (in the dark or with the final 50 min in light). The dashed black line indicates 70% viability, below which the compounds are considered cytotoxic.

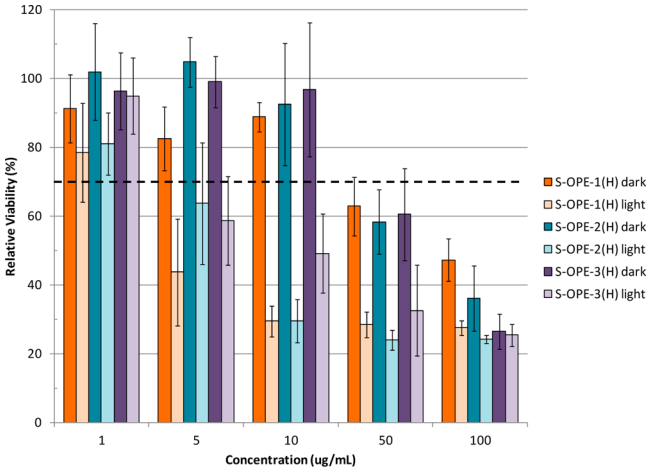

Figure 4. Relative viabilities (in %) of Vero (epithelial) cells exposed to varying concentrations (1, 5, 10, 50, and 100 μg/mL, horizontal axis) of three S-OPEs for 24 h (in the dark or with the final 50 min in light). The dashed black line indicates 70% viability, below which the compounds are considered cytotoxic.

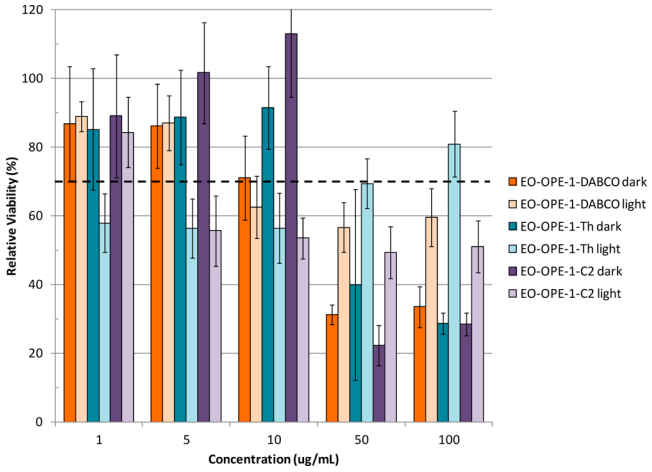

Figure 5. Relative viabilities (in %) of bovine aortic endothelial cells exposed to varying concentrations (1, 5, 10, 50, and 100 μg/mL, horizontal axis) of three EO-OPEs for 24 h (in the dark or with the final 50 min in light). The dashed black line indicates 70% viability, below which the compounds are considered cytotoxic.

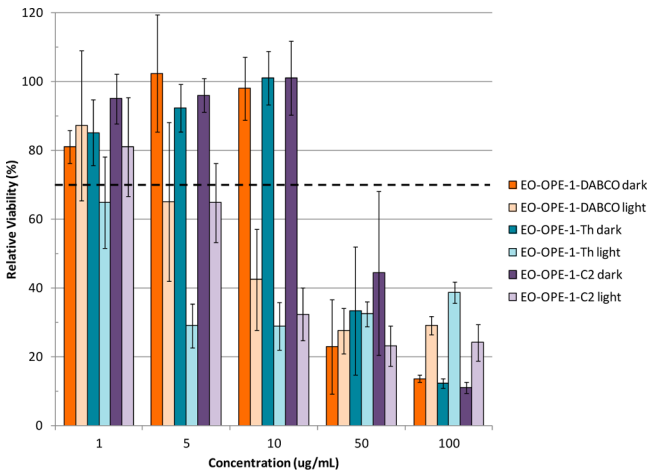

Figure 6. Relative viabilities (in %) of Vero (epithelial) cells exposed to varying concentrations (1, 5, 10, 50, and 100 μg/mL, horizontal axis) of three EO-OPEs for 24 h (in the dark or with the final 50 min in light). The dashed black line indicates 70% viability, below which the compounds are considered cytotoxic.

(i.e. bacterial lipids), possibly because of their linear structures.[27]

4. CONCLUSION

In this work, two types of mammalian cells were assayed for viability following exposure to five concentrations of eight light-activated phenylene ethynylene compounds for a period of 24 h. Half of the cells were irradiated with visible or UV light during the last 50 min of the 24 h exposure period, while the other half remained in the dark. The cytotoxicity testing described here represents a very conservative approach. Having potentially cytotoxic compounds present in growth medium is analogous to systemic (internal) exposure at a constant concentration. As all currently-envisioned applications are external to the body, receiving such constant, high-concentration exposure is unlikely. Also, any cytotoxic effects observed during these experiments are probably exaggerated because all compounds were dosed in serum-free medium. Serum, which is always present in vivo, is known to mask cytotoxic effects.[28]

As expected, concentration plays the largest role in determining viability. At low concentrations, light has a negligible effect on cell viability. Above a threshold concentration (which varies from compound to compound), light continues to affect viability, but concentration effects are predominant. At intermediate concentrations (5−10 μg/mL for most compounds), the interplay between light and the light-activated compounds is very important.

Viability trends were consistent across cell types, therefore the mode of action of mammalian cell killing appears to be independent of mammalian cell type, thus related to basal cell function. Ongoing work is expected to evaluate cytotoxicity with additional assays and to refine the mode of action against mammalian cells. For applications below cytotoxic concentrations, these compounds are safe for mammalian cells. The concentrations at which the longer S-OPEs and the DABCO-

containing compounds are cytotoxic are much higher than for the shortest S-OPE, PPE-Th, and the remaining two EO-OPEs; thus these compounds have the widest range of concentrations available for potential applications.

■ ASSOCIATED CONTENT

Supporting Information

Merged fluorescence microscopy images of endothelial cells after 24 h exposure to PPE-DABCO in serum-free medium, and after 24 h exposure to PPE-Th in serum-free medium. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

KNW designed the experiments, collected and analyzed the data, and drafted the manuscript. DGW supervised the experiments, and reviewed the manuscript. HEC conceived the study, supervised the experiments, and revised the manuscript. All authors have read and approved the final manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

MRSA, methicillin-resistant *Staphlococcus aureus*
CPE, conjugated phenlylene ethynylene
PPE, poly(phenylene ethynylene)
OPE, oligo(phenylene ethynylene)
S-OPE, symmetric oligo(phenylene ethynylene)
BAEC, bovine aortic endothelial cells
TCPS, tissue culture polystyrene
EDTA, ethylenediaminetetraacetic acid
DMEM, Dulbecco's modified Eagle medium
NEAA, non-essential amino acids
MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
DABCO, 1,4-diazabicyclo[2.2.2]octane
Th, thiophene
EO-OPE, end-only oligo(phenylene ethynylene)

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