Supporting Information for:

Contact Active Antibacterial Phosphonium Coatings Cured with UV Light

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Coating preparation

Formulations containing 5 wt% of the photoinitiator 2-hydroxy-2-methyl-1-phenylpropanone (**3**) (Cytec Industries, Georgia, USA), and varying ratios of cross-linker tricyclodecanedimethanol diacrylate (**2)** (Cytec Industries, Georgia, USA) and ((3-acryloyloxypropyl)tributyl)phosphonium chloride (**1**)[1] were mixed by stirring for five minutes. The formulations were then deposited on either Melinex 725 Teijin PET films purchased from Tekra (Wisconsin, USA), glass slides, or silicon wafers and cast using a 25-μm Meyer Rod purchased from Gardco (Florida, USA). The glass slides and silicon wafers were first cleaned by immersion in 1:2 H_2SO_4:H_2O_2 vol/vol (Piranha solution; attention – strong acid, strong oxidizer!) then they were rinsed with water, ethanol, and then dried under vacuum. If under an inert atmosphere, the film was inserted into an inert atmosphere cell and purged with N_2 for 30 seconds before irradiation. Free standing films were produced by drop casting the mixture on a clean glass slide and then adding spacers and another glass slide on top to create films in an air-free atmosphere, which were then removed from the glass using a razor blade. Photopolymerization was performed using a modified UV-curing system purchased form UV Process and Supply Inc. (Illinois, USA) equipped with a mercury bulb. Samples were irradiated with an energy density of 1878 mJ/cm^2 and an irradiance of 3325 mW/cm^2, as determined by a PP2-H-U Power Puck II purchased from EIT Instrument Markets (Virginia, USA). Coatings cast on PET or glass were ~25 μm thick, while the free standing films were ~250 μm thick, as measured using a micrometer. Properties of the films on PET and glass were summarized in Table 2 of the manuscript, while those of the free standing films are shown in Table S2. The coatings and free standing films had very similar properties.

Atomic force microscopy (AFM) and hardness testing

AFM was performed using a Park Systems XE-100 instrument (Suwon, Korea). The cantilever had a nominal spring constant of 40 N/m, resonant frequency of 300 kHz, and a tip apex radius of 10 nm. Hardness was evaluated using a KLA Tencor P-10 Surface Profiler equipped with a diamond tip and

applied with a 0.5 mN force to scratch the surface. The resulting surface was imaged by AFM. The measured surfaces were prepared on a glass substrate as it is flat, allowing accurate assessment of film roughness.

Measurement of surface adhesion

Testing was performed in accordance with ASTM D3359 – 09e2. Briefly, the surfaces coated with the phosphonium thin films were cut through to the substrate with a scalpel, in a pattern of 6 parallel lines. Another set of 6 parallel lines perpendicular to the initial lines were then cut. The lines were approximately 0.25 cm apart. A line of tape was put across the lines and pressed down and left for 30 seconds. The tape (3M Scotch #2020; 35 oz/in adhesion strength to steel) was then removed from one end, and the number of removed squares compared to the number of starting squares was counted to provide the percentage of removed squares. The results are provided in Table S1.

Measurement of accessible surface charges using the fluorescein dye assay

This procedure was based on the previously reported protocol.[2] The surface (~ 1 cm^2) was immersed in a 1 w/v% solution of fluorescein sodium salt in deionized water for 10 minutes. The resulting surface was rinsed with deionized water, then immersed in 10 mL of a 0.1 v/v% solution of cetyltrimethylammonium chloride in deionized water and sonicated for 30 minutes. The surface was then removed and 10% v/v phosphate buffer added and the absorbance of the solution at 501 nm was measured using a Varian Cary 300 UV-visible spectrometer. The concentration of accessible charges on the surface was then determined based on the extinction coefficient of fluorescein (ε = 77,000 $M^{-1}cm^{-1}$), assuming that each molecule of fluorescein binds to one accessible cation. The measured coatings were prepared on the PET substrate.

Measurement of cure percentage

Infrared spectra (IR) were recorded using a Bruker Tensor 27 spectrometer in attenuated total reflectance mode (ATR) using a ZnSe crystal. The peak corresponding to C=O (1720 cm^{-1}) was used as

an internal standard as its intensity does not change upon curing. The intensity of the C=C peak (810 cm^{-1}) was compared to that of the internal standard before curing, after curing, and after incubation in water for a total of 12 h, with three water changes. The cure percentage was calculated as the percent decrease in the relative intensity of the C=C peak. Measurements were performed in triplicate. Samples for this experiment (Table 2) were cast on a PET substrate due to its flexibility, which allows ATR-IR to be easily performed. Free standing films were measured using the same procedure (Table S2).

Measurement of gel content

Surfaces with dimensions of 3 cm^2 cast on glass were used, due to the ease of creating a uniform coating over a large area on this highly flat substrate (Table 2). The large dimension was required for accurate measurement of coating mass for these films. Free standing films were also measured (Table S2). Cured but unwashed coatings were weighed then incubated in 10 mL of deionized water for 12 hours (water changed three times). The surfaces were then dried to constant mass in a vacuum oven overnight at 50 °C. The gel content was calculated as the (initial dry weight/extracted dry weight) × 100%.

Measurement of water contact angle

Water contact angle measurements were performed using a Kruss DSA100 Drop Shape Analyzer and analyzed using drop shape analysis. Values were obtained two minutes after droplet application and the measurements were performed in triplicate. Coatings on PET were used to obtain the values in Table 2, though the water contact angle has been observed to not depend on the underlying substrate.

Agar plate antibacterial procedure

This procedure was based on the Kirby-Bauer protocol.[3] A loop of precultured *E. coli* (ATCC 29425) or *S. aureus* (ATCC 6538) was freshly cultured in LB broth (VWR International, Mississauga, Canada) for 18 - 24 hours at 37 °C in a shaker at 175 rpm. The resulting suspension was centrifuged for 10 minutes, decanted, and resuspended in phosphate buffered saline (PBS), vortexed, and centrifuged for 10 minutes. This was repeated twice. The resulting solid was resuspended in PBS and diluted to a

concentration of 10^8 colony forming units (CFU)/mL (calculated based on *E. coli* optical density of 0.2 at 600 nm and a *S. aureus* optical density of 0.3 at 600 nm). The suspension was further diluted to a concentration of 10^6 CFU/mL and 0.1 mL was plated onto an agar plate (Difco™ plate agar, BD, Sparks, MD, USA) and spread thoroughly and evenly on the plate. The plate was allowed to dry for 5 minutes. Free standing phosphonium films prepared with 47.5 wt% **1**, cured under nitrogen, pre-washed by 12 hour incubation in deionized water (with 3 water changes), and dried were placed on the agar and pressed into the agar to ensure contact. The agar plate was then incubated at 37 °C for 24 hours. The films were carefully removed with tweezers and the agar was imaged. The experiment was performed on at least 4 films for each strain of bacteria.

Evaluation of potential antibacterial properties of surface leachable molecules

A 1 cm^2 phosphonium surface (47.5 wt% **1**, cured under N_2) was prepared on a PET substrate and pre-washed for 12 hours with three water changes in the same manner as for the agar protocol above. It was then placed in an Erlenmeyer flask containing 5 mL of 0.3 mM pH 7.4 phosphate buffer. A control sample contained no surface. The samples were agitated using a wrist action shaker at 75 rpm for 1 hour then the surface was removed. A suspension of *S. aureus* in PBS at a concentration of 10^6 CFU/mL was prepared as described above. 100 μL of this suspension was added to the 5 mL of leachate described above, providing a final concentration of 10^5 CFU/5 mL. This solution was then agitated using a wrist action shaker at 75 rpm for 1 hour, diluted to 10^2 CFU/mL, pour plated into growth agar in triplicate, and incubated at 37 °C for 24 hours. CFUs were then counted. No significant differences between the sample and control were observed, confirming the absence of leachable biocides in the coatings. The experiment was performed in triplicate.

LIVE/DEAD® BacLight bacterial viability assay

A suspension of *S. aureus* in PBS at a concentration of 10^8 CFU/mL was prepared as described above. 1 mL of this suspension was placed on each 4 cm^2 phosphonium coating (prepared on an activated

silicon wafer and washed for 12 hours with three water changes). The control surface was an activated silicon wafer (washed with water and dried under vacuum). The surfaces were placed in a petri dish, the dish was sealed with parrafilm, and the samples were incubated for either 4 or 24 hours at 37 °C at 40-50% humidity. The surfaces were then washed with 10 mL of PBS, then incubated with a mixture of the LIVE/DEAD® BacLight bacterial viability assay dyes (SYTO 9 and propidium iodide, Life Technologies, Burlington, Canada) for 30 minutes in the dark according to the manufacturer's directions. The surfaces were then gently washed with deionized water and dried in the dark overnight. The dry surfaces were then mounted on glass slides and imaged by laser confocal microscopy using an LSM 510 multichannel point scanning confocal microscope (Zeiss, Oberkochen, Germany) (Laser 488 nm for the SYTO 9 with a pass filter of 505-530 nm and a laser at 543 nm for the propidium iodide with a pass filter of 615 nm, magnification 40×). All the images were obtained and refined with the ZEN software. Each time point and surface was tested in triplicate.

Cell viability assay

C2C12 mouse myoblast cells were cultured in growth medium composed of Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and supplemented with 1% Glutamax (100×) solution and antibiotics (Penicillin and Streptomycin, 100 units/mL each) (Life Technologies, Burlington, Canada). Cells were seeded onto a 96-well plate (flat bottom, Corning, USA) at a density of 10,000 cells per well with a final volume of 100 μL of culture medium. Cells were allowed to adhere for 24 hours at 37 ^{o}C in a humidified incubator with 5% CO_2. After 24 hours the growth medium was aspirated and replaced with either the positive control sodium dodecyl sulfate (SDS) in the growth medium at concentrations of 0.2, 0.15, 0.10, 0.05 mg/ml, compound **1** at serial two-fold dilutions from 0.8 mg/mL to 0.007 mg/mL, or benzyldimethylhexadecylammonium chloride at serial two-fold dilutions starting from 1 mg/mL to 0.008 mg/mL. Control cells were grown in medium alone. The cells were then incubated for 24 hours under the conditions described above. All

medium was aspirated, and then 100 μL of fresh medium and 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, Mississauga, Canada) solution (5 mg/mL in growth medium) were added to each well. After incubation for 4 hours, the medium was aspirated and the formazan product was solubilized by addition of 50 μL of DMSO to each well. The absorbance of each well was measured at 540 nm using a plate reader (M1000-Pro, Tecan, Mannedorf, Switzerland) and after subtraction of the blank, the absorbance was compared to that of the control cells that were not exposed to micelles in order to calculate the relative cell viability. Less than 2% cell viability was detected for the cells exposed to the highest concentrations of the positive control SDS, confirming the sensitivity of the assay.

Table S1. Results of surface adhesion testing by ASTM D3359 – 09e2. *The test was repeated 5 times on the same surface without any loss of adhesion.

Formulation; curing atmosphere; substrate	% squares removed	Cross hatch adhesion classification
2-95wt%, **3**-5wt%; Air; PET	0%*	5B - 0% removed
1-47.5wt%, **2**-47.5wt%, **3**-5%; N_2; PET	0%*	5B - 0% removed
1-47.5wt%, **2**-47.5wt%, **3**-5%; Air; PET	0%*	5B - 0% removed
2-95wt%, **3**-5wt%; N_2; glass	87%	0B - >65% removed
2-95wt%, **3**-5wt%; Air; glass	100%	0B - >65% removed
1-47.5wt%, **2**-47.5wt%, **3**-5%; N_2; glass	88%	0B - >65% removed
1-47.5wt%, **2**-47.5wt%, **3**-5%; Air; glass	100%	0B - >65% removed

Table S2. Properties of free standing films prepared from 47.5 wt% of **1**, 47.5 wt% **2**, and 5 wt% **3**.

Cure % (ATR-FTIR) before washing	Cure % (ATR-FTIR) after washing	Gel content
91 ± 5%	90 ± 4 %	83 ± 1%

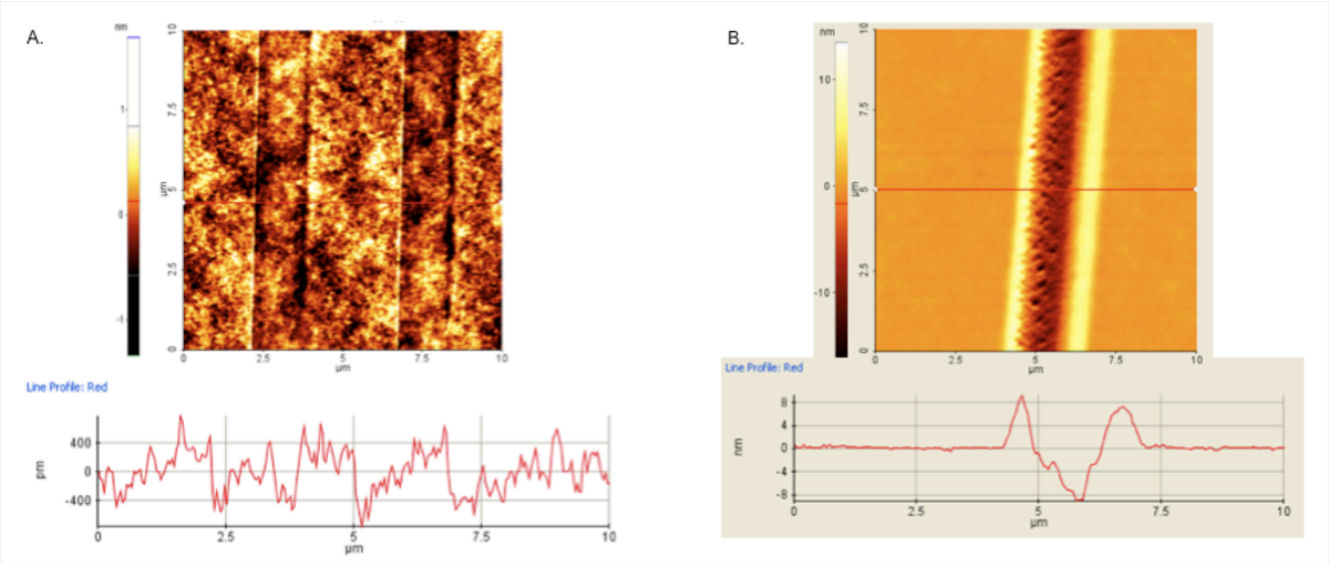

Figure S1. AFM images and corresponding height profiles of a) a 47.5 wt% **1** coating cured under N_2 and b) a 47.5 wt% **1** coating cured under air. These images show that upon application of a diamond tip surface profiler with a force of 0.5 mN, the etching could not be distinguished from the background surface roughness in the case of the surface cured under N_2, whereas a 10 nm groove was etched in the surface cured under air. These images also show the low roughness of the initial films.

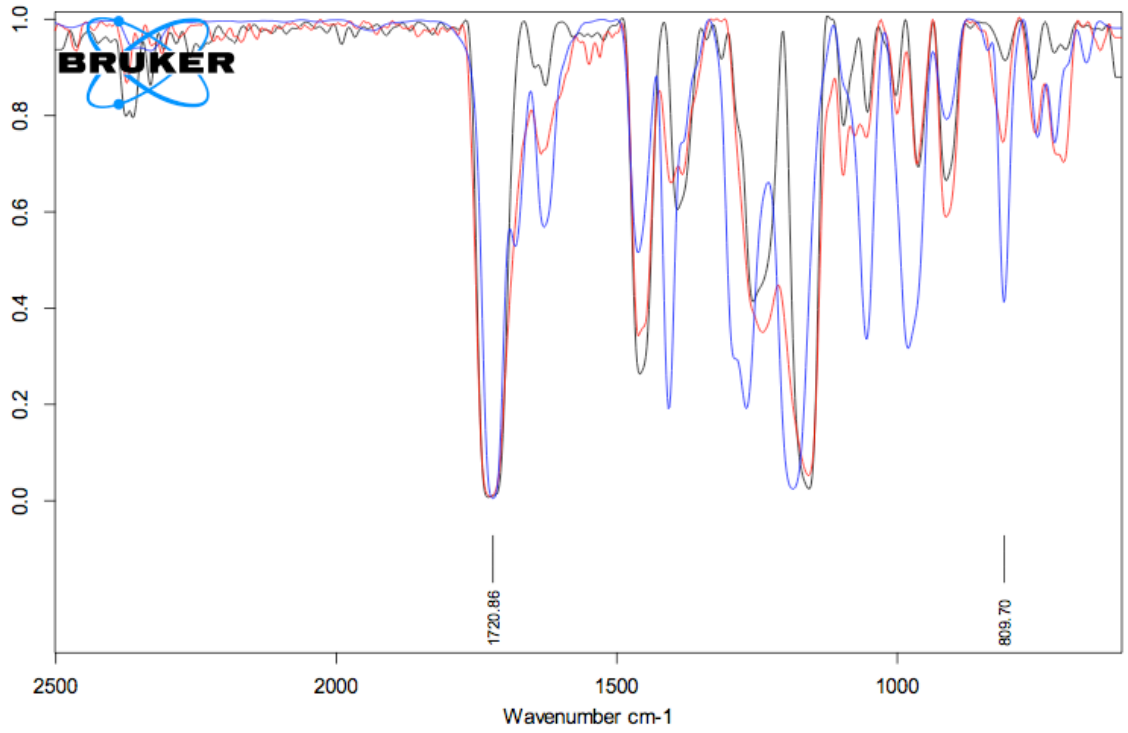

Figure S2. Example ATR-FTIR spectra of a 47.5 wt% **1** formulation (blue), cured film (red), and cured and washed film (black), showing the decrease in the intensity of the peak at 810 cm^{-1} corresponding to C=C, in comparison to the internal standard C=O peak at 1720 cm^{-1}.

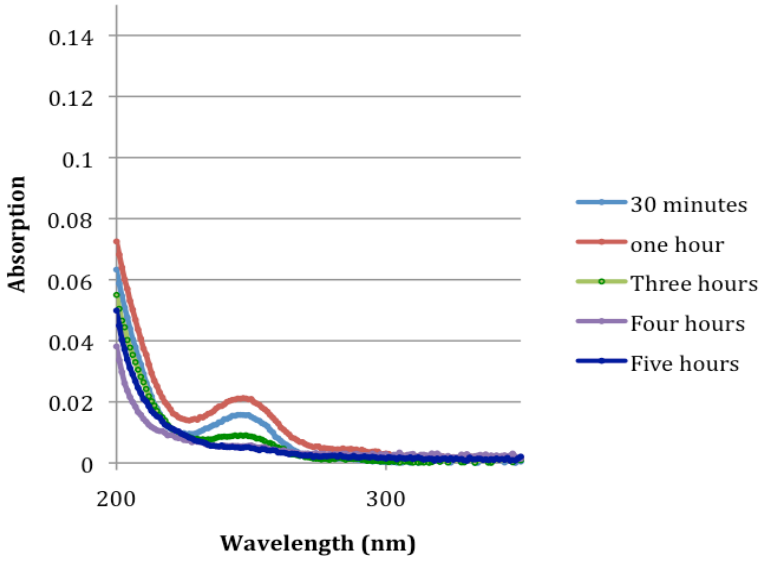

Figure S3. UV-Vis spectra of surface washings (47.5 wt% **1**, cured under N_2, PET substrate) after different time periods, confirming that a 12 hours incubation in water is more than sufficient to remove all leachable molecules. (Note: washings were discarded after each time point, so the lack of detectable molecules after 5 hours suggests that all leachable molecules had already been removed from the surface).

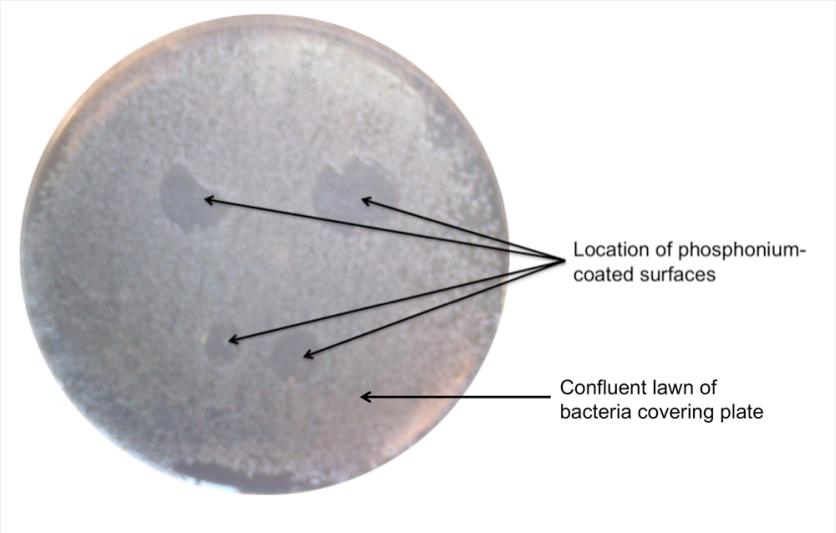

Figure S4. Antibacterial testing results for a coating containing 47.5 wt% of **1** and cured under N_2. The image shows an agar plate with absence of bacterial growth where the phosphonium surfaces were placed, following 24 h incubation with *E. coli.*

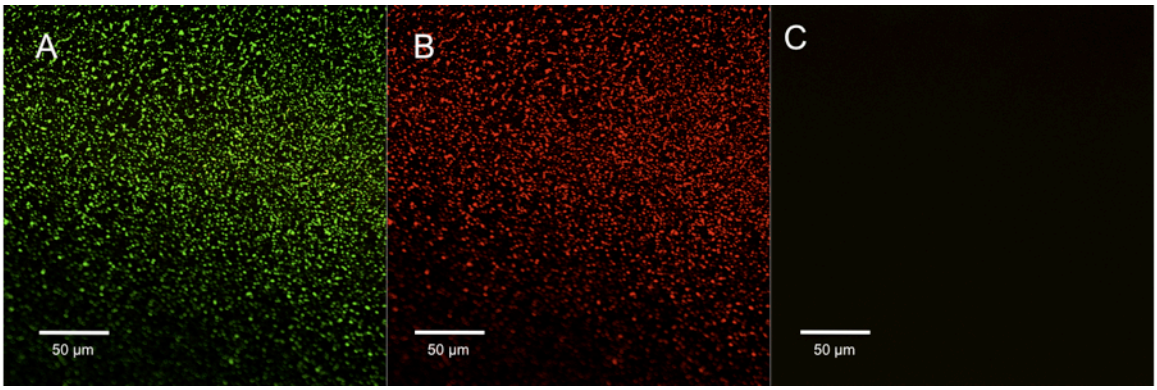

Figure S5. a) and b): clean silicon wafer control and c) phosphonium surface following LIVE/DEAD® analysis. The surfaces were incubated with *S. aureus* for 24 h. a) shows live bacteria stained in green; b) shows dead bacteria stained in red; c) is an overlay of red and green fluorescence images showing an absence of live or dead bacteria.

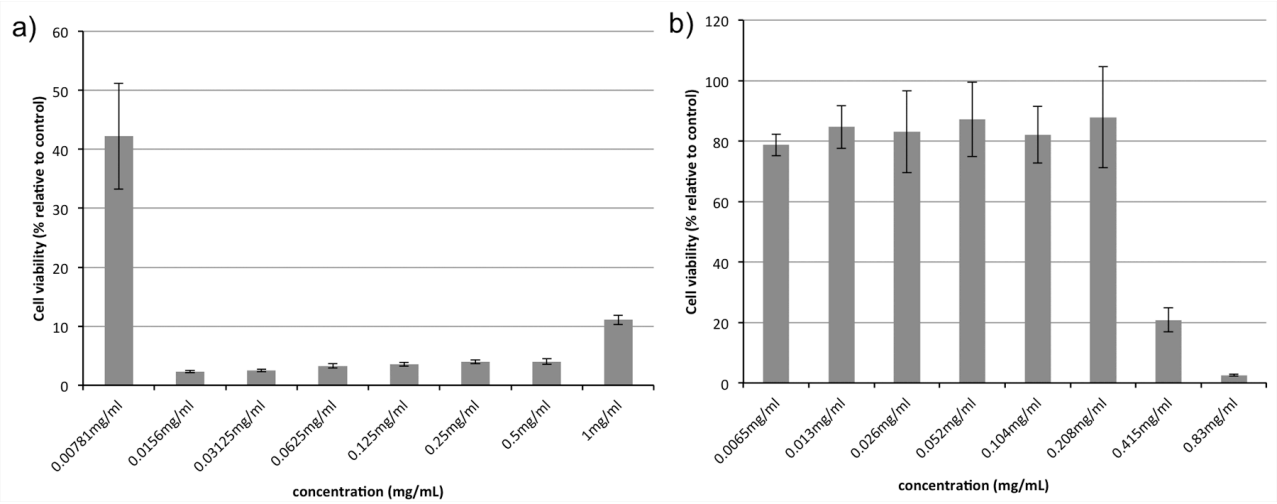

Figure S6. *In vitro* cytotoxicity of a) benzyldimethyl hexadecyl ammonium chloride and b) phosphonium monomer **1** as measured by the MTT assay following a 24 h incubation with C2C12 mouse myoblast cells. Data represent the mean and standard deviation of six replicates per concentration (N = 6).

References

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(2) Murata, H.; Koepsel, R. R.; Matyjaszewski, K.; Russell, I. J. Permanent, non-leaching antibacterial surfaces—2: How high density cationic surfaces kill bacterial cells. *Biomaterials*. **2007**, *28*, 4870–4879.

(3) Gaydos, J. M.; Harrington, B. J. Agar Disk Diffusion for the Quality Control Testing of Autobac Elution Disks. *Antimicrob. Agents Chemother*. **1982**, 21, 516–518.