Antimicrobial photodynamic inactivation of planktonic and biofilm cells by covalently immobilized porphyrin on polyethylene terephthalate surface

Fatima Shatila, Grace M. O. Tieman, Stefania F. Musolino, Jeremy E. Wulff, & Heather L. Buckley

Antimicrobial Photodynamic Inactivation of Planktonic and Biofilm Cells by Covalently Immobilized Porphyrin on Polyethylene Terephthalate Surface

Shatila, F.[bc], Tieman G.M.O. [acd], Musolino, S.F.[acd], Wulff, J.E.[acd], Buckley, H.L.[abcd]*

AUTHOR ADDRESS

a. Department of Chemistry, University of Victoria, 3800 Finnerty Road, Victoria, BC, Canada, V8P 5C2

b. Department of Civil Engineering, University of Victoria, 3800 Finnerty Road, Victoria, BC, Canada, V8P 5C2

c. Centre for Advanced Materials and Related Technologies (CAMTEC), University of Victoria, 3800 Finnerty Road, Victoria, BC, Canada, V8P 5C2

d. Institute for Integrated Energy Systems (IESVic), University of Victoria, 3800 Finnerty Road, Victoria, BC, Canada, V8P 5C2

fatimashatila@uvic.ca; orcid.org/0000-0001-9246-8847

gracetieman@uvic.ca

stefmusolino@uvic.ca; orcid.org/0000-0002-0990-1855

wulff@uvic.ca

hbuckley@uvic.ca; orcid.org/0000-0001- 7147-0980

Corresponding author Heather L. Buckley − Department of Chemistry, Department of Civil Engineering, Centre for Advanced Materials and Related Technologies (CAMTEC), and Institute for Integrated Energy Systems (IESVic), University of Victoria, Victoria, BC V8P 5C2, Canada; orcid.org/0000-0001- 7147-0980; Email: hbuckley@uvic.ca

Abstract

The appearance of resistant strains and the persistence of biofilms on different surfaces in a wide range of settings represent serious public health threats. Antimicrobial photodynamic inactivation (aPDI) is a promising alternative technology to overcome these challenges. The current study assessed the antimicrobial effect of polyethylene terephthalate (PET) discs covalently functionalized with a cationic porphyrin, against *E. coli* and *P. aeruginosa* growth. Irradiation with white LED light for 6 h resulted in 1.51 ± 0.03 and 3.26 ± 0.24 log reduction of planktonic *P. aeruginosa* and *E. coli*, respectively. The study also assessed the effect of the functionalized discs on biofilm formation by *E. coli*, *P. aeruginosa,* and *S. aureus.* The biovolumes of *S. aureus*, *P. aeruginosa,* and *E. coli* biofilms were decreased by 0.6 ± 0.1, 0.56 ± 0.13 and 0.74 ± 0.06 log reduction, respectively. These results emphasize the ability of porphyrin-functionalized photoactive surfaces to kill bacterial cells and consequently prevent biofilm formation.

Key words: Biofilms, photodynamic inactivation, porphyrin, photoactive surfaces, antimicrobial surfaces

1. Introduction

Chemical disinfection is the main technique used to clean different contaminated surfaces in a variety of environments. However, chemical disinfection can be problematic due to its hazards and is challenged by decreased efficiency due to the emergence of multidrug antimicrobial resistant strains (Spagnul et al., 2015; Silva et al., 2018; Zhang et al., 2020; Pinna, 2022; Van Dijk et al., 2022) . This has consequently resulted in cross resistance to antibiotics and contributed to the appearance of antibiotic resistant strains (Brovko et al., 2009; Colclough et al., 2019; Naseer et al., 2021). The problem is further aggravated by the ability of microorganisms to form biofilms, which are resistant sessile microbial communities embedded in the exopolysaccharide (EPS) matrix. The persistence of biofilms on different biotic and abiotic surfaces represents a serious public health threat (Chitlapilly Dass and Wang, 2022; Funari and Shen, 2022). Microbial biofilms are responsible for water borne diseases, more than 60% of hospital acquired infections and 60% of foodborne outbreaks (Han et al., 2017; Jamal et al., 2018; Hemdan et al., 2021; Devanga Ragupathi et al., 2022). The US National Institute of Health (NIH) reported that microbial biofilms are associated with 80% of chronic infections and 65% of microbial infections in humans (Han et al., 2017; Jamal et al., 2018; Vishwakarma et al., 2021). Moreover, it has been reported that biofilms are responsible for half a million deaths per year globally (Brinkman et al., 2016). The economic losses caused by biofilms also should not be underestimated. Biofilm-associated infections cost annually around $ 94 billion (Brinkman et al., 2016). Determining the financial burden imposed by biofilms in different industries can be challenging; however, a market analysis estimated that biofilms have an economic significance in excess of $ 5000 billion per year (Cámara et al., 2022). Therefore, developing novel green antimicrobial approaches and disinfection protocols is required to mitigate the consequences of microbial biofilms and emergence of antimicrobial resistant strains (Lekbach et al., 2018; Singh et al., 2018; Verderosa et al., 2019).

The development of antimicrobial surfaces has been among the approaches that are receiving increasing interest (Merchán et al., 2013; Elbourne et al., 2017). This approach depends on the

decreased capacity of microbial cells to attach to a surface, lethal contact, biocide leaching or antimicrobial light activated coatings (Akarsu and Uslu, 2018; Cerchier et al., 2018). Photoactive self-sterilizing surfaces represent a distinctive system of antimicrobial Photodynamic Inactivation (aPDI) that emerged from the development and assessment of new photosensitizers for PDI along with their potential incorporation or deposition on different supports (Q. Mesquita et al., 2018; Youf et al., 2021).

Photodynamic inactivation has been described as a rapid, efficient and environmentally friendly method of decontamination (Wang et al., 2021). Moreover, it has demonstrated efficiency against microorganisms that are resistant to conventional antimicrobials. The approach depends on the presence of a photosensitizer that can release reactive oxygen species (ROS) upon activation by visible light at appropriate wavelength in presence of molecular oxygen. The ROS can exert irreversible oxidative damage to multiple nonspecific cell targets (cell membrane, lipids proteins, DNA) leading to the inactivation of a wide spectrum of pathogens whether in their planktonic or biofilm state (Q. Mesquita et al., 2018).

Porphyrins are among the promising photosensitizers that are finding applications in aPDI because of their photochemical properties and excellent singlet oxygen generation quantum yield (Zhao et al., 2016; Sułek et al., 2020). Various methods for incorporation, addition, immobilization, and deposition of porphyrin on different supports have been reported previously. These supports include cellulose, chitosan, cotton, multi-walled carbon nanotubes, polystyrene and nylon films (Spagnul et al., 2015). Moreover, these porphyrin-enhanced surfaces demonstrated antimicrobial effect against different Gram negative and Gram positive bacteria including *E. coli* (Ringot et al., 2011; Castro et al., 2017; Nyga et al., 2021), and *S. aureus* (Ringot et al., 2011; Dastgheyb et al., 2015).

In previous work by Cuthbert et al. (2021), the synthesis and covalent attachment of a zinc porphyrin molecule, zinc(II)5,10,15,20-tetrakis((*N*-4-[3-(trifluoromethyl)-3*H*-diazirin-3yl] benzyl) -4-pyridyl)21*H*,23 *H*- porphine tetrabromide using thermal activation of the diazirine functionalities to induce reaction with carbon-based polymers were reported (Fig. 1), and the material was demonstrated to be effective for the inactivation of *Influenza A* virus under visible light irradiation. A recent study by Musolino et al. (2022) elaborated the crosslinking of the aforementioned porphyrin to PET (polyethylene terephthalate) discs and demonstrated both singlet oxygen production and the antibacterial activity of the functionalized discs against *S. aureus* under visible light irradiation. The current study further determines the antimicrobial effect of the same covalently tethered porphyrin-functionalized PET discs against two Gram negative bacterial strains (*E. coli* and *P. aeruginosa*), and its consequences on *S. aureus*, *P. aeruginosa* and *E. coli* biofilms.

2. Materials and methods

2.1 Microorganisms

The antibacterial activity of the functionalized PET discs was determined against one Gram positive (*Staphylococcus aureus* subsp. *aureus* (ATCC® 6538P™) as well as two Gram negative bacteria (*Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 10145™) and *E. coli* ATCC 25922). The bacterial cells were kindly provided by the Department of Biochemistry and Microbiology at the University of Victoria. The cells were maintained in 15% glycerol and preserved at -80 ºC.

2.2 Irradiation conditions

Photodynamic inactivation experiments were performed using a white LED light (75 W, 1800 lumens, Satco). The distance between the plates and the light source was ~ 35 cm with illuminance equivalent to 26400 ± 200 Lux (irradiance 276 ± 2 W/m^2). The plates were irradiated for either 6 h for photodynamic inactivation of planktonic cells, or 24 h to determine the effect of photodynamic inactivation on biofilm formation.

PET discs were prepared as mentioned previously by Musolino et al., (2022). Briefly, 20 µl of a 10 mg/mL methanol solution of zinc(II)5,10,15,20-tetrakis((*N*-4-[3-(trifluoromethyl)-3*H*-diazirin-3yl] benzyl)-4-pyridyl)21*H*,23 *H*- porphine tetrabromide ("porphyrin") was spin coated onto each disc (diameter 15.6 mm). The discs were heated overnight at 120 ºC to activate the diazirine for covalent attachment. The functionalized discs were used in one of two ways. For one set of experiments, they were added into assigned wells in 24 microtiter plates, soaked in 95% ethanol, left to dry under sterile conditions in a laminar flow hood and then used to determine the bactericidal effect against planktonic cells. For the other set, the functionalized discs were autoclaved before their transfer into culture plates and inoculation with bacterial cells to determine the effect of photoactivation against the tested bacterial biofilms.

2.3 Antimicrobial Photodynamic Inactivation (aPDI) of planktonic *P. aeruginosa* and *E. coli*

Antimicrobial photodynamic inactivation experiments against planktonic *P. aeruginosa* and *E. coli* were performed as described previously (Musolino et al., 2022). Briefly, overnight seed culture grown in LB (Luria-Bertani Broth, Fisher Bioreagents, Canada) with OD $_{600\ nm}$ ~ 1 was diluted 1:1000. 24 well plates with wells containing functionalized PET discs and wells with no added material were inoculated with 400 µL from standardized bacterial suspension. One plate was irradiated for 6 h while the other one was wrapped with aluminum foil and otherwise kept under the same conditions. After this 6 h period, serial dilutions followed by plating on Tryptone Soya Agar (TSA, Oxoid, United Kingdom) were performed. Cell counts were recorded after overnight incubation. Each experiment included duplicates and was repeated three times. The results were expressed as the logarithm reduction of cell counts in CFU/ mL.

2.4 Biofilm formation

The effect of the photoactive discs on *S. aureus*, *P. aeruginosa* and *E. coli* biofilms was also assessed. *E. coli* biofilms were cultivated in Tryptic Soy Broth (TSB, Sigma- Aldrich, USA), *P. aeruginosa* and *S. aureus* were grown in TSB supplemented with 1% glucose. Functionalized and unmodified PET discs, previously sterilized by autoclaving, were separately added to culture plates (35 mm) aseptically. Each plate was inoculated with 2 mL from the standardized bacterial suspension ($OD_{600\ nm}$ ~ 0.13) and irradiated for 24 h under the same conditions as described above. Similarly, another set of plates was prepared and covered with aluminum foil. Consequently, the control wells in each experiment included unmodified discs that were exposed to light, or kept in dark. Extra control wells, containing functionalized discs, were added to the "dark plate" to determine the effect of the porphyrin layer on biofilm formation. After incubation, the biofilms were observed with SEM (Scanning Electron Microscope) and CLSM (Confocal Laser Scanning Microscope) (Preuß et al., 2016; Castro et al., 2017; Vollmerhausen et al., 2017; Nyga et al., 2021).

2.4.1 Scanning Electron Microscopy of the bacterial biofilms

After incubation, the discs were rinsed with sterile PBS (3X) and fixed in glutaraldehyde (2.5%) at 4 ºC for 4 hours. After fixation, the discs were rinsed with PBS and then with distilled water. Each rinsing step was performed twice. The discs were transferred into ethanol gradient (30, 50, 70, 80, 90, 96 and 100%) for 5 minutes at each concentration. After this, the discs were soaked in 50% HMDS and then 100% HMDS respectively for 30 minutes. The discs were left overnight in a desiccator, sputter coated with gold (Anatech Hummer VI Sputter Coater), and examined under SEM (Hitachi S-4800-Japan).

2.4.2 Confocal Laser Scanning Microscopy of the bacterial biofilms

After incubation, the discs supporting the biofilms were rinsed with sterile distilled water (3X), transferred to miniature petri dish and stained with LIVE/DEAD™ BacLight™ Bacterial Viability Kit, for microscopy & quantitative assays (L7012, Thermofisher Scientific, Canada) at room temperature in the dark for 20 min.

Images were obtained with Zeiss LSM 880 Confocal Laser Scanning Microscope at excitation 488 nm with an argon laser and emission wavelengths (live 494 – 554 nm; dead 579- 701 nm). Images were obtained using (LD- Plan-Neofluar 20x/0.4KorrM27). Image capture and Z-stacks were performed using Zen black software (v3.6).

The 3D images were constructed using a microscopy image analysis software (Imaris) (version 9.8.0, Bitplane). The software allows the visualization and analysis of 3D images including the estimation of the biovolume, which is the amount of biofilm (μm^3) in the observation field. At least three images were captured randomly for each specimen and each experiment was repeated at least twice.

2.5 Statistical analysis

Data were analyzed using GRAPHPAD. Results presented are means ± standard deviation (SD). The “Percentage Inhibition” represents the average of different “Percentage Inhibition” values calculated with each control. One way ANOVA and T test were used to detect significant differences. Significance was determined at 5% ($P<0.05$) level.

3. Results

3.1 Bactericidal activity

The antibacterial effect of the functionalized PET discs against the planktonic cells of *P. aeruginosa* and *E. coli* was determined quantitatively by performing cell counts. After 6 h irradiation with white LED light, 1.51 ± 0.03 and 3.26 ± 0.24 log reduction (percentage inhibition equivalent to 97 ± 0.3 and 99.95 ± 0.02%) were recorded against *P. aeruginosa* and *E. coli*, respectively (Fig. 2; a and b).

3.2. Antibiofouling activity

The effect of photodynamic inactivation on biofilm formation by the tested bacterial strains was determined using SEM (Scanning Electron Microscopy) and CLSM (Confocal Laser Scanning Microscopy). Scanning electron micrographs demonstrated morphological changes in the

S. aureus cells grown on porphyrin-functionalized surfaces upon exposure to light as compared to the three control conditions (Fig. 3a); however, the presence of morphological changes on *P. aeruginosa* (Fig. 4a) and *E. coli* (Fig. 5a) was less apparent. The electron micrographs also revealed that the biofilms formed on the PET discs exposed to light were reduced to cellular clusters characterized with less complex 3D structure and lower surface coverage when compared to the three controls of the tested bacterial strains. These results were also supported by the confocal images and subsequent biovolume measurements after construction of the images using Imaris. The confocal images as well as the biovolume measurements indicated that the biofilms formed on the irradiated functionalized PET surfaces were reduced by 0.6 ± 0.1, 0.56 ± 0.13 and 0.74 ± 0.06 log reduction (percentage inhibition 68 ± 5 %, 73 ± 8 % and 78 ± 3 %) for *S. aureus*, *P. aeruginosa*, and *E. coli*, respectively (Fig 3 b,c; 4 b,c and 5 b,c).

4. Discussion

Prevention of biofilms by killing planktonic bacterial cells is challenged by the inevitable development of strains that are resistant to the drug targeting the vital processes of the bacterial cells (Di Somma et al., 2020). On the other hand, the scarcity of reports indicating resistance to aPDI suggests the possibility of using this method to manage adapted strains (Youf et al., 2021). This emphasizes aPDI, and consequently photoactive surfaces, as an attractive approach to control pathogens (Reynoso et al., 2021). These photoactive surfaces can find applications in different fields including healthcare environments, the food industry and different community settings (Spagnul et al., 2015; Q. Mesquita et al., 2018).

The current study evaluated the bactericidal activity of covalently bound porphyrin to PET discs, which were prepared as detailed in previous reports, against different bacterial strains (Cuthbert et al., 2021; Musolino et al., 2022). Musolino et al., (2022) reported the ability of the porphyrin-functionalized PET discs to cause ~ 1.76 - log reduction (percentage inhibition ~ 97.5%) against *S. aureus*. The current study extends this work and reports the antibacterial effect of the same porphyrin-functionalized PET discs against two gram negative bacterial strains (*E. coli* and *P. aeruginosa*) and potential effect on biofilm formation by the three tested bacterial strains.

The cell counts recorded in the irradiated wells that contained the functionalized discs were significantly lower than those in the control wells. On the other hand, the difference in cell counts between the controls was insignificant which indicates that the bactericidal effect does not occur in the presence of the functionalized discs or light separately, yet both parameters have to be combined to trigger the killing effect.

The recorded bactericidal effect against the test microorganisms can be attributed to singlet oxygen generation from the photoactive porphyrin bound surface upon irradiation as demonstrated in our previous study (Musolino et al., 2022). Taking into consideration the immobilized state of the porphyrin on PET discs, the inhibitory effect of the photoactive surface is likely due to cell envelope damage as a result of its interaction with singlet oxygen that is photo-generated at the top of the coating affecting the cells within close proximity (Dahl et al., 1987; Ringot et al., 2009; Akarsu and Uslu, 2018). Del Valle et al., (2020) also emphasized that the morphology and different features of the surface should be addressed as the electrostatic forces affect the ability of bacterial cells to bind to the surface leading to potential disruption of the lipopolysaccharide wall of gram negative bacteria. Therefore, interpretation of results should take into consideration the generated

singlet oxygen on the material surface and the presence of different functional cationic groups (Bonnett et al., 2006).

The inhibitory effect of different photoactive surfaces, reported in previous studies, demonstrates variations which can be attributed to different experimental conditions. Therefore, direct comparison remains challenging in lack of standardized procedure (Akarsu and Uslu, 2018). Sabbahi et al., (2013) tested the effect of different experimental conditions on the photodynamic inactivation of *S. aureus* and *P. aeruginosa*. The study concluded that the degree of microbial PDI depends on different parameters including the type, concentration and physicochemical properties of the photosensitizer as well as the type of bacteria and irradiation time.

The inhibition of *P. aeruginosa* and *E. coli* by different photoactive antimicrobial porphyrin-bound surfaces has been reported previously. Porphyrin-dyed paper resulted in 1.66 log reduction after irradiation with LED lamp for one hour (George et al., 2018). The grafting of protoporphyrin to nylon fibers killed 30% of *E. coli* after irradiation with incandescent light for 30 min (Bozja et al., 2003). Further, total killing of *E. coli* cells was reported after irradiation of tricationic porphyrin -cellulosic paper for 24 h (Mbakidi et al., 2013). Porphyrin-bearing Kraft pulp fibers resulted in a strong bactericidal effect against *P. aeruginosa* after irradiation with white light for 24 h (Nzambe Ta keki et al., 2016). A study performed by Fayyaz et al. (2021) reported the photoinactivation of *E. coli* and *P. aeruginosa* by three tetra-cationic porphyrins immobilized on cellulosic fabrics. The irradiation of the porphyrin- cellulosic fabrics resulted in percentage inhibition ranging between 10 and 100% depending on the type of porphyrin, concentration and irradiation time.

The bactericidal effect resulting from incorporating a photosensitizer into a polymer surface against planktonic bacterial cells suggests the presence of antibiofilm activity (Merchán et al., 2013; Sehmi et al., 2015). Similarly, the bactericidal effect of the porphyrin-functionalized discs recorded previously against *S. aureus*, and currently against the tested microorganisms (*P. aeruginosa* and *E. coli*), suggests the ability of these coatings to inhibit or reduce biofilm formation. The biofilms of the tested microorganisms were grown on unmodified PET discs and on porphyrin-functionalized discs in presence and absence of light. After overnight irradiation of bacterial cells, the modified and non-modified PET discs underwent microscopic analysis. Scanning Electron Microscope allowed the determination of surface coverage while Confocal Laser Scanning Microscope allowed the estimation of the biovolume. The reduced surface coverage and lower biovolume of the biofilms growing on the surfaces of the functionalized coupons upon irradiation can be attributed to the bactericidal effect of the PDI. On the other hand, among the controls, it was noted that the biovolumes of the biofilms formed on the functionalized discs (incubated in dark) were lower than those formed on the non-functionalized controls, which might indicate the presence of antibiofilm activity triggered by the porphyrin-functionalized surface. Nyga et al. (2021) reported that the presence of an organic moiety can affect the adhesion process between the bacteria and the surfaceThe application of different antimicrobial approaches, including aPDI, to prevent biofilm formation has been reported previously. A study by Chen et al. (2018) attributed biofilm prevention to the killing of planktonic cells by peptides. Similarly, Miñán et al., (2015) applied photodynamic inactivation against planktonic cells, which reduced the number of bacterial cells that reached the surface and attached to it. Vollmerhausen et al. (2017) also reported that the photo inactivation of an *E. coli* suspension prevented biofilm formation. These findings demonstrated the efficiency of PDI in reducing the number of adhering cells and as an approach to prevent biofilm formation.

The covalent immobilization of porphyrin on PET discs using the diazirine functional group confers longer term stability of the tethered molecule and prevents its leaching, avoiding potential environmental and health concerns. Future work will focus on assessing the performance of the newly designed photoactive surface under different irradiation conditions. Such data should be useful in determining the durability of the surface and the conditions resulting in optimal antimicrobial activity. Moreover, determining the antimicrobial effect of porphyrin tethered to different surfaces with diazirine crosslinkers should demonstrate the capacity of this approach for mitigating pathogen growth in a wide range of settings.

5. Conclusion

The present work represents a significant step in development of porphyrin-functionalized photoactive surfaces, by demonstrating their capacity for aPDI on multiple species of planktonic bacterial cells and their biofilms. The porphyrin-functionalized discs demonstrated antibacterial effect against *P. aeruginosa* and *E. coli*. Moreover, the photo activated surfaces reduced the biovolumes of *S. aureus*, *P. aeruginosa* and *E. coli* biofilms. These results emphasize the ability of the porphyrin bound photoactive surface to kill bacterial cells and consequently prevent biofilm formation.

Declaration of Competing Interest

Authors H.B., J.W. and S.M. are listed inventors on patent WO/2021/179064 claiming the use of the diazirine–photosensitizer conjugate described in the current work. There are no additional conflicts to declare.

Funding

We gratefully acknowledge Mitacs Canada (grant IT17318) and our industry partners, Epic Ventures, as well as the IESVic Clean Energy Systems Accelerator Program (IESVic-CESAP) IRAP, for funding.

Acknowledgement

The authors would like to thank the Department of Microbiology and Biochemistry for providing the bacterial strains, and the CAMTEC Advanced Microscopic Facility at the University of Victoria for providing training on the Scanning Electron Microscope and Confocal Laser Scanning Microscope. We also thank Rebecca Hof for support and access to CAMTEC BioCore facilities, Luiz Henrique da Silva Correa for his support with Imaris software, and Epic Ventures as partners in this research.

References

Akarsu, E., Uslu, R., 2018. Light-activated hybrid organic/inorganic antimicrobial coatings. J. Sol-Gel Sci. Technol. 87, 183–194. https://doi.org/10.1007/s10971-018-4714-y

Bonnett, R., Krysteva, M.A., Lalov, I.G., Artarsky, S.V., 2006. Water disinfection using photosensitizers immobilized on chitosan. Water Res. 40, 1269–1275. https://doi.org/10.1016/j.watres.2006.01.014

Bozja, J., Sherrill, J., Michielsen, S., Stojiljkovic, I., 2003. Porphyrin-based, light-activated antimicrobial materials. J. Polym. Sci. Part Polym. Chem. 41, 2297–2303. https://doi.org/10.1002/pola.10773

Brinkman, C.L., Schmidt-Malan, S.M., Karau, M.J., Greenwood-Quaintance, K., Hassett, D.J., Mandrekar, J.N., Patel, R., 2016. Exposure of bacterial biofilms to electrical current leads to cell death mediated in part by reactive oxygen species. PLOS ONE 11 (12), e0168595. https://doi.org/10.1371/journal.pone.0168595

Brovko, L.Y., Meyer, A., Tiwana, A.S., Chen, W., Liu, H., Filipe, C.D.M., Griffiths, M.W., 2009. Photodynamic treatment: A novel method for sanitation of food handling and food processing surfaces. J. Food Prot. 72, 1020–1024. https://doi.org/10.4315/0362-028X-72.5.1020

Cámara, M., Green, W., MacPhee, C.E., Rakowska, P.D., Raval, R., Richardson, M.C., Slater-Jefferies, J., Steventon, K., Webb, J.S., 2022. Economic significance of biofilms: A multidisciplinary and cross-sectoral challenge. Npj Biofilms Microbiomes 8, 42. https://doi.org/10.1038/s41522-022-00306-y

Castro, K.A.D.F., Moura, N.M.M., Fernandes, A., Faustino, M.A.F., Simões, M.M.Q., Cavaleiro, J.A.S., Nakagaki, S., Almeida, A., Cunha, Â., Silvestre, A.J.D., Freire, C.S.R., Pinto, R.J.B., Neves, M. da G.P.M.S., 2017. Control of *Listeria innocua* biofilms by biocompatible photodynamic antifouling chitosan based materials. Dyes Pigments 137, 265–276. https://doi.org/10.1016/j.dyepig.2016.10.020

Cerchier, P., Pezzato, L., Moschin, E., Coelho, L.B., Olivier, M.G.M., Moro, I., Magrini, M., 2018. Antifouling properties of different Plasma Electrolytic Oxidation coatings on 7075 aluminium alloy. Int. Biodeterior. Biodegrad. 133, 70–78. https://doi.org/10.1016/j.ibiod.2018.06.005

Chen, H., Wubbolts, R.W., Haagsman, H.P., Veldhuizen, E.J.A., 2018. Inhibition and eradication of *Pseudomonas aeruginosa* biofilms by host defence peptides. Sci. Rep. 8, 10446. https://doi.org/10.1038/s41598-018-28842-8

Chitlapilly Dass, S., Wang, R., 2022. Biofilm through the looking glass: A microbial food safety perspective. Pathogens 11, 346. https://doi.org/10.3390/pathogens11030346

Colclough, A., Corander, J., Sheppard, S.K., Bayliss, S.C., Vos, M., 2019. Patterns of cross-resistance and collateral sensitivity between clinical antibiotics and natural antimicrobials. Evol. Appl. 12, 878–887. https://doi.org/10.1111/eva.12762

Cuthbert, T.J., Ennis, S., Musolino, S.F., Buckley, H.L., Niikura, M., Wulff, J.E., Menon, C., 2021. Covalent functionalization of polypropylene filters with diazirine–photosensitizer conjugates producing visible light driven virus inactivating materials. Sci. Rep. 11, 19029. https://doi.org/10.1038/s41598-021-98280-6

Dahl, T.A., Midden W.R., Hartman, P.E., 1987. Pure singlet oxygen cytotoxicity for bacteria. Photochem. Photobiol. 46, 345–352. https://doi.org/10.1111/j.1751-1097.1987.tb04779.x

Dastgheyb, S.S., Toorkey, C.B., Shapiro, I.M., Hickok, N.J., 2015. Porphyrin-adsorbed Allograft Bone: A Photoactive, Antibiofilm Surface. Clin. Orthop. 473, 2865–2873. https://doi.org/10.1007/s11999-015-4299-5

Del Valle, C.A., Pérez-Laguna, V., Resta, I.M., Gavara, R., Felip-León, C., Miravet, J.F., Rezusta, A., Galindo, F., 2020. A cost-effective combination of rose bengal and off-the-shelf cationic polystyrene for the photodynamic inactivation of *Pseudomonas aeruginosa.* Mater. Sci. Eng. C 117, 111302. https://doi.org/10.1016/j.msec.2020.111302

Devanga Ragupathi, N.K., Veeraraghavan, B., Karunakaran, E., Monk, P.N., 2022. Editorial: Biofilm-mediated nosocomial infections and its association with antimicrobial resistance: Detection, prevention, and management. Front. Med. 9, 987011. https://doi.org/10.3389/fmed.2022.987011.

Di Somma, A., Moretta, A., Canè, C., Cirillo, A., Duilio, A., 2020. Inhibition of bacterial biofilm formation, in: Dincer, S., Sümengen Özdenefe, M., Arkut, A. (Eds.), Bacterial biofilms. IntechOpen. https://doi.org/10.5772/intechopen.90614

Elbourne, A., Crawford, R.J., Ivanova, E.P., 2017. Nano-structured antimicrobial surfaces: From nature to synthetic analogues. J. Colloid Interface Sci. 508, 603–616. https://doi.org/10.1016/j.jcis.2017.07.021

Fayyaz, F., Rassa, M., Rahimi, R., 2021. Antibacterial photoactivity and thermal stability of tetra-cationic porphyrins immobilized on cellulosic fabrics. Photochem. Photobiol. 97, 385–397. https://doi.org/10.1111/php.13353

Funari, R., Shen, A.Q., 2022. Detection and characterization of bacterial biofilms and biofilm-based sensors. ACS Sens. 7, 347–357. https://doi.org/10.1021/acssensors.1c02722

George, L., Hiltunen, A., Santala, V., Efimov, A., 2018. Photo-antimicrobial efficacy of zinc complexes of porphyrin and phthalocyanine activated by inexpensive consumer LED lamp. J. Inorg. Biochem. 183, 94–100. https://doi.org/10.1016/j.jinorgbio.2018.03.015

Han, Q., Song, X., Zhang, Z., Fu, J., Wang, X., Malakar, P.K., Liu, H., Pan, Y., Zhao, Y., 2017. Removal of foodborne pathogen biofilms by acidic electrolyzed water. Front. Microbiol. 8, 988. https://doi.org/10.3389/fmicb.2017.00988

Hemdan, B.A., El-Taweel, G.E., Goswami, P., Pant, D., Sevda, S., 2021. The role of biofilm in the development and dissemination of ubiquitous pathogens in drinking water distribution systems: An overview of surveillance, outbreaks, and prevention. World J. Microbiol. Biotechnol. 37, 36. https://doi.org/10.1007/s11274-021-03008-3

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M.A., Hussain, T., Ali, M., Rafiq, M., Kamil, M.A., 2018. Bacterial biofilm and associated infections. J. Chin. Med. Assoc. 81, 7–11. https://doi.org/10.1016/j.jcma.2017.07.012

Lekbach, Y., Xu, D., El Abed, S., Dong, Y., Liu, D., Khan, M.S., Ibnsouda Koraichi, S., Yang, K., 2018. Mitigation of microbiologically influenced corrosion of 304L stainless steel in the presence of *Pseudomonas aeruginosa* by *Cistus ladanifer* leaves extract. Int. Biodeterior. Biodegrad. 133, 159–169. https://doi.org/10.1016/j.ibiod.2018.07.003

Mbakidi, J.-P., Herke, K., Alvès, S., Chaleix, V., Granet, R., Krausz, P., Leroy-Lhez, S., Ouk, T.-S., Sol, V., 2013. Synthesis and photobiocidal properties of cationic porphyrin-grafted paper. Carbohydr. Polym. 91, 333–338. https://doi.org/10.1016/j.carbpol.2012.08.013

Merchán, M., Ouk, T.S., Kubát, P., Lang, K., Coelho, C., Verney, V., Commereuc, S., Leroux, F., Sol, V., Taviot-Guého, C., 2013. Photostability and photobactericidal properties of porphyrin-layered double hydroxide–polyurethane composite films. J. Mater. Chem. B 1, 2139- 2146. https://doi.org/10.1039/c3tb20070a

Miñán, A., Lorente, C., Ipiña, A., Thomas, A.H., Fernández Lorenzo de Mele, M., Schilardi, P.L., 2015. Photodynamic inactivation induced by carboxypterin: a novel non-toxic bactericidal strategy against planktonic cells and biofilms of *Staphylococcus aureus*. Biofouling 31, 459–468. https://doi.org/10.1080/08927014.2015.1055731

Musolino, S.F., Shatila, F., Tieman, G.M.O., Masarsky, A.C., Thibodeau, M.C., Wulff, J.E., Buckley, H.L., 2022. Light-induced anti-bacterial effect against *Staphylococcus aureus* of

porphyrin covalently bonded to a polyethylene terephthalate surface. ACS Omega 7, 29517–29525. https://doi.org/10.1021/acsomega.2c04294
Naseer, M., Ramadan, R., Xing, J., Samak, N.A., 2021. Facile green synthesis of copper oxide nanoparticles for the eradication of multidrug resistant *Klebsiella pneumonia* and *Helicobacter pylori* biofilms. Int. Biodeterior. Biodegrad. 159, 105201. https://doi.org/10.1016/j.ibiod.2021.105201
Nyga, A., Czerwińska-Główka, D., Krzywiecki, M., Przystaś, W., Zabłocka-Godlewska, E., Student, S., Kwoka, M., Data, P., Blacha-Grzechnik, A., 2021. Covalent Immobilization of Organic Photosensitizers on the Glass Surface: Toward the Formation of the Light-Activated Antimicrobial Nanocoating. Materials 14, 3093. https://doi.org/10.3390/ma14113093
Nzambe Ta keki, J.K., Ouk, T.-S., Zerrouki, R., Faugeras, P.-A., Sol, V., Brouillette, F., 2016. Synthesis and photobactericidal properties of a neutral porphyrin grafted onto lignocellulosic fibers. Mater. Sci. Eng. C 62, 61–67. https://doi.org/10.1016/j.msec.2016.01.028
Pinna, D., 2022. Can we do without biocides to cope with biofilms and lichens on stone heritage? Int. Biodeterior. Biodegrad. 172, 105437. https://doi.org/10.1016/j.ibiod.2022.105437
Preuß, A., Bornhütter, T., Färber, A., Schaller, C., Röder, B., 2016. Photodynamic inactivation of biofilm building microorganisms by photoactive facade paints. J. Photochem. Photobiol. B 160, 79–85. https://doi.org/10.1016/j.jphotobiol.2016.04.008
Q. Mesquita, M., J. Dias, C., P. M. S. Neves, M., Almeida, A., F. Faustino, M., 2018. Revisiting Current Photoactive Materials for Antimicrobial Photodynamic Therapy. Molecules 23, 2424. https://doi.org/10.3390/molecules23102424
Reynoso, E., Durantini, A.M., Solis, C.A., Macor, L.P., Otero, L.A., Gervaldo, M.A., Durantini, E.N., Heredia, D.A., 2021. Photoactive antimicrobial coating based on a PEDOT-fullerene C_{60} polymeric dyad. RSC Adv. 11, 23519–23532. https://doi.org/10.1039/D1RA03417K
Ringot, C., Sol, V., Barrière, M., Saad, N., Bressollier, P., Granet, R., Couleaud, P., Frochot, C., Krausz, P., 2011. Triazinyl porphyrin-based photoactive cotton fabrics: Preparation, characterization, and antibacterial activity. Biomacromolecules 12, 1716–1723. https://doi.org/10.1021/bm200082d
Ringot, C., Sol, V., Granet, R., Krausz, P., 2009. Porphyrin-grafted cellulose fabric: New photobactericidal material obtained by “Click-Chemistry” reaction. Mater. Lett. 63, 1889–1891. https://doi.org/10.1016/j.matlet.2009.06.009
Sabbahi, S., Ben Ayed, L., Boudabbous, A., 2013. Cationic, anionic and neutral dyes: Effects of photosensitizing properties and experimental conditions on the photodynamic inactivation of pathogenic bacteria. J. Water Health 11, 590–599. https://doi.org/10.2166/wh.2013.219
Sehmi, S.K., Noimark, S., Bear, J.C., Peveler, W.J., Bovis, M., Allan, E., MacRobert, A.J., Parkin, I.P., 2015. Lethal photosensitisation of *Staphylococcus aureus* and *Escherichia coli* using crystal violet and zinc oxide-encapsulated polyurethane. J. Mater. Chem. B 3, 6490–6500. https://doi.org/10.1039/C5TB00971E
Silva, A., Borges, A., Freitas, C., Hioka, N., Mikcha, J., Simões, M., 2018. Antimicrobial photodynamic inactivation mediated by rose bengal and erythrosine is effective in the control of food-related bacteria in planktonic and biofilm states. Molecules 23, 2288. https://doi.org/10.3390/molecules23092288

Singh, J., Dutta, T., Kim, K.-H., Rawat, M., Samddar, P., Kumar, P., 2018. 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation. J. Nanobiotechnology 16, 84. https://doi.org/10.1186/s12951-018-0408-4

Spagnul, C., Turner, L.C., Boyle, R.W., 2015. Immobilized photosensitizers for antimicrobial applications. J. Photochem. Photobiol. B 150, 11–30. https://doi.org/10.1016/j.jphotobiol.2015.04.021

Sułek, A., Pucelik, B., Kobielusz, M., Barzowska, A., Dąbrowski, J.M., 2020. Photodynamic inactivation of bacteria with porphyrin derivatives: Effect of charge, lipophilicity, ROS generation, and cellular uptake on their biological activity in vitro. Int. J. Mol. Sci. 21, 8716. https://doi.org/10.3390/ijms21228716

Van Dijk, H.F.G., Verbrugh, H.A., Ad hoc advisory committee on disinfectants of the Health Council of the Netherlands, 2022. Resisting disinfectants. Commun. Med. 2, 6. https://doi.org/10.1038/s43856-021-00070-8

Verderosa, A.D., Totsika, M., Fairfull-Smith, K.E., 2019. Bacterial biofilm eradication agents: A current review. Front. Chem. 7, 824. https://doi.org/10.3389/fchem.2019.00824

Vishwakarma, A., Dang, F., Ferrell, A., Barton, H.A., Joy, A., 2021. Peptidomimetic polyurethanes inhibit bacterial biofilm formation and disrupt surface established biofilms. J. Am. Chem. Soc. 143, 9440–9449. https://doi.org/10.1021/jacs.1c02324

Vollmerhausen, T.L., Conneely, A., Bennett, C., Wagner, V.E., Victor, J.C., O'Byrne, C.P., 2017. Visible and UVA light as a potential means of preventing *Escherichia coli* biofilm formation in urine and on materials used in urethral catheters. J. Photochem. Photobiol. B 170, 295–303. https://doi.org/10.1016/j.jphotobiol.2017.04.018

Wang, D., Kyere, E., Ahmed Sadiq, F., 2021. New trends in photodynamic inactivation (PDI) combating biofilms in the food industry—A review. Foods 10, 2587. https://doi.org/10.3390/foods10112587

Youf, R., Müller, Max, Balasini, A., Thétiot, F., Müller, Mareike, Hascoët, A., Jonas, U., Schönherr, H., Lemercier, G., Montier, T., Le Gall, T., 2021. Antimicrobial photodynamic therapy: Latest developments with a focus on combinatory strategies. Pharmaceutics 13, 1995. https://doi.org/10.3390/pharmaceutics13121995

Zhang, G., Li, W., Chen, S., Zhou, W., Chen, J., 2020. Problems of conventional disinfection and new sterilization methods for antibiotic resistance control. Chemosphere 254, 126831. https://doi.org/10.1016/j.chemosphere.2020.126831

Zhao, Q., Wang, Y., Xu, Y., Yan, Y., Huang, J., 2016. Out-of-plane coordinated porphyrin nanotubes with enhanced singlet oxygen generation efficiency. Sci. Rep. 6, 31339. https://doi.org/10.1038/srep31339

Figures

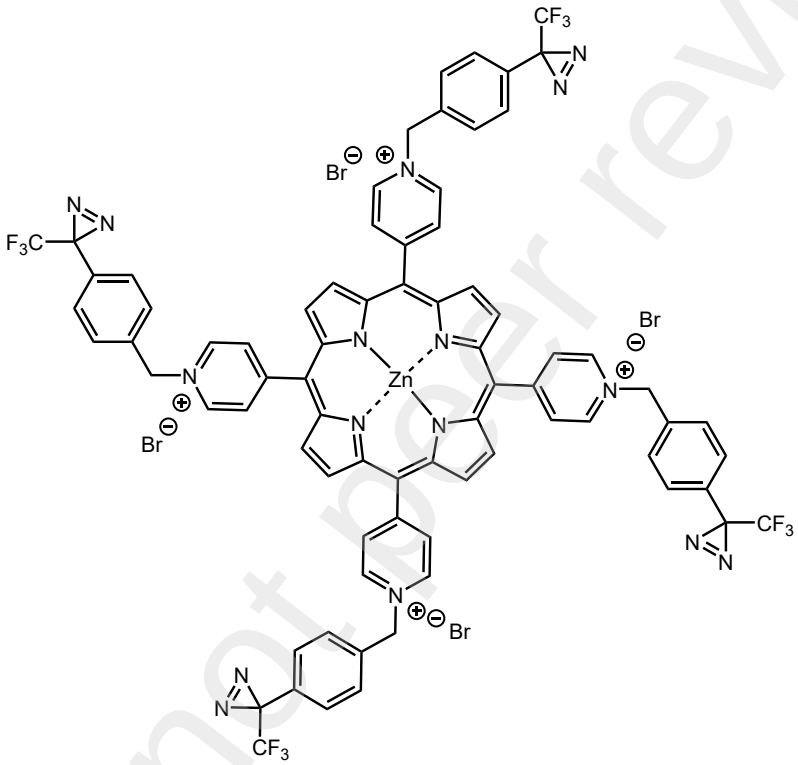
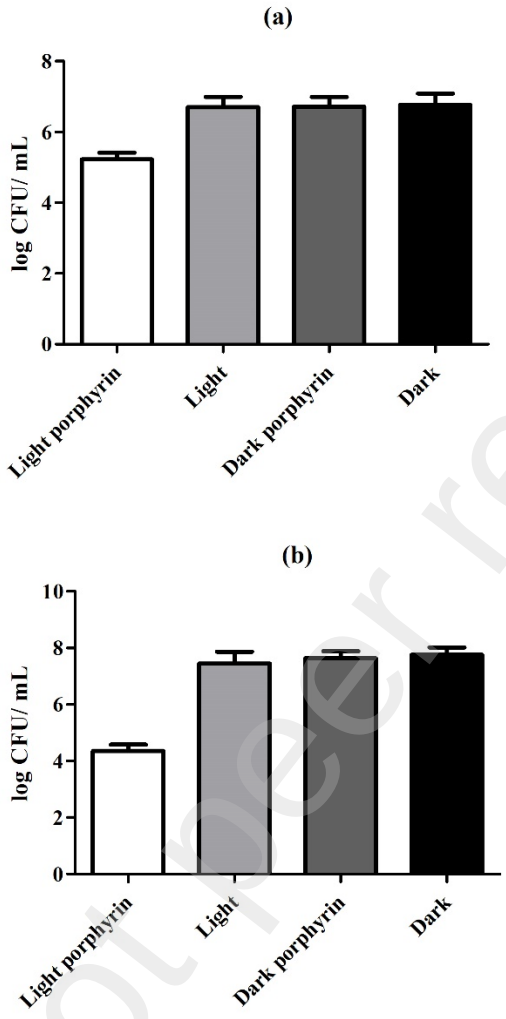


Fig. 1. zinc(II) 5,10,15,20-tetrakis((N-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzyl)-4-pyridyl)-21H,23H-porphine tetrabromide, has four possible sites of attachment and can be used to produce singlet oxygen in the presence of light.



527

528 **Fig. 2. The bactericidal effect of crosslinked porphyrin surface, expressed in log reduction,**
529 **after irradiation with LED light (26,400 ± 200 lx for 6h) against: (a) *P. aeruginosa***
530 **(b) *E. coli*. Error bars represent the standard deviation**.

531

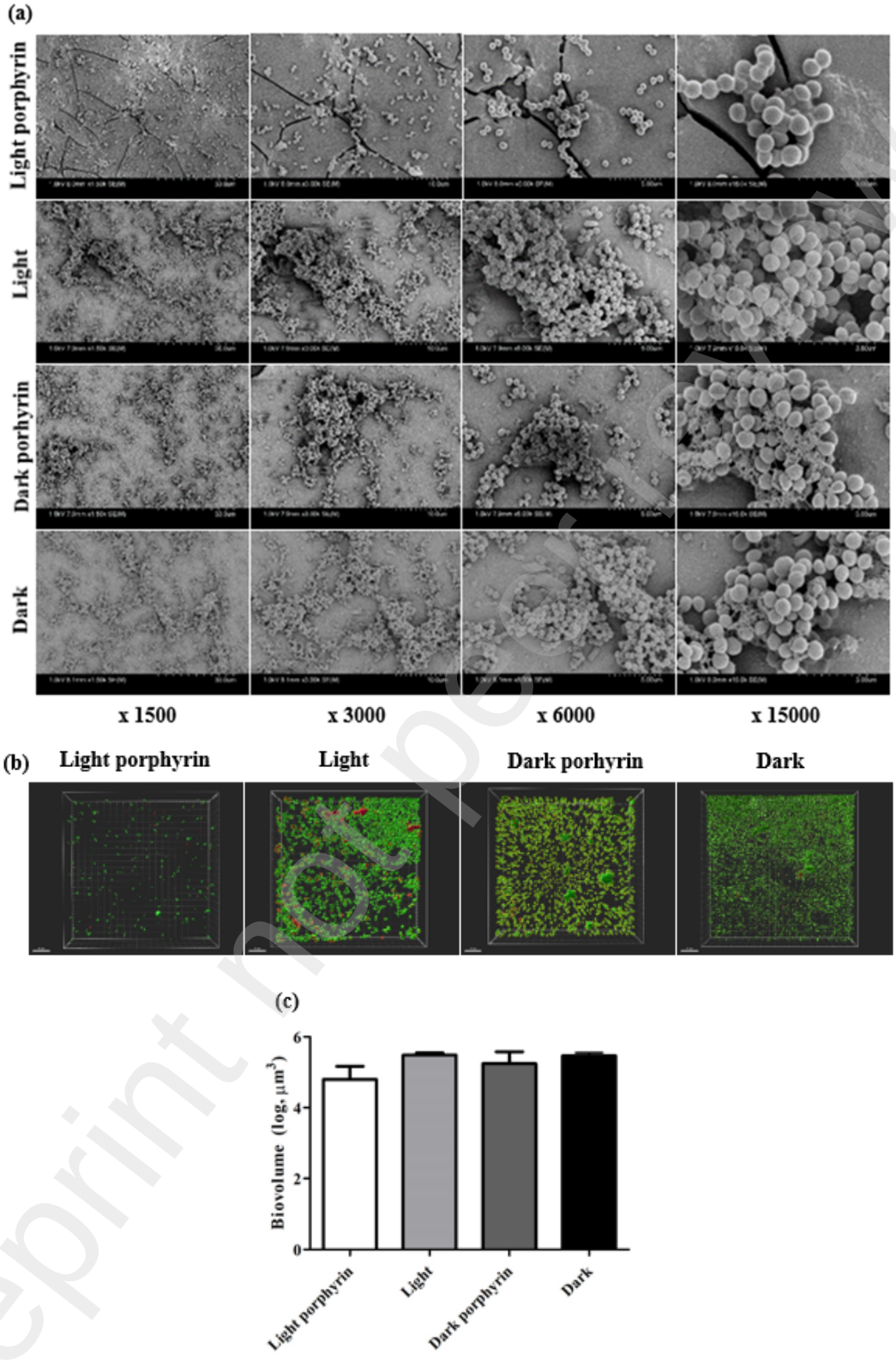


Fig. 3. The effect of the photoactive porphyrin discs on *S. aureus* biofilms a) Scanning Electron Micrographs (1500, 3000, 6000 and 15000X) b) Confocal Laser Scanning Microscope images reconstructed with Imaris c) Biovolume.

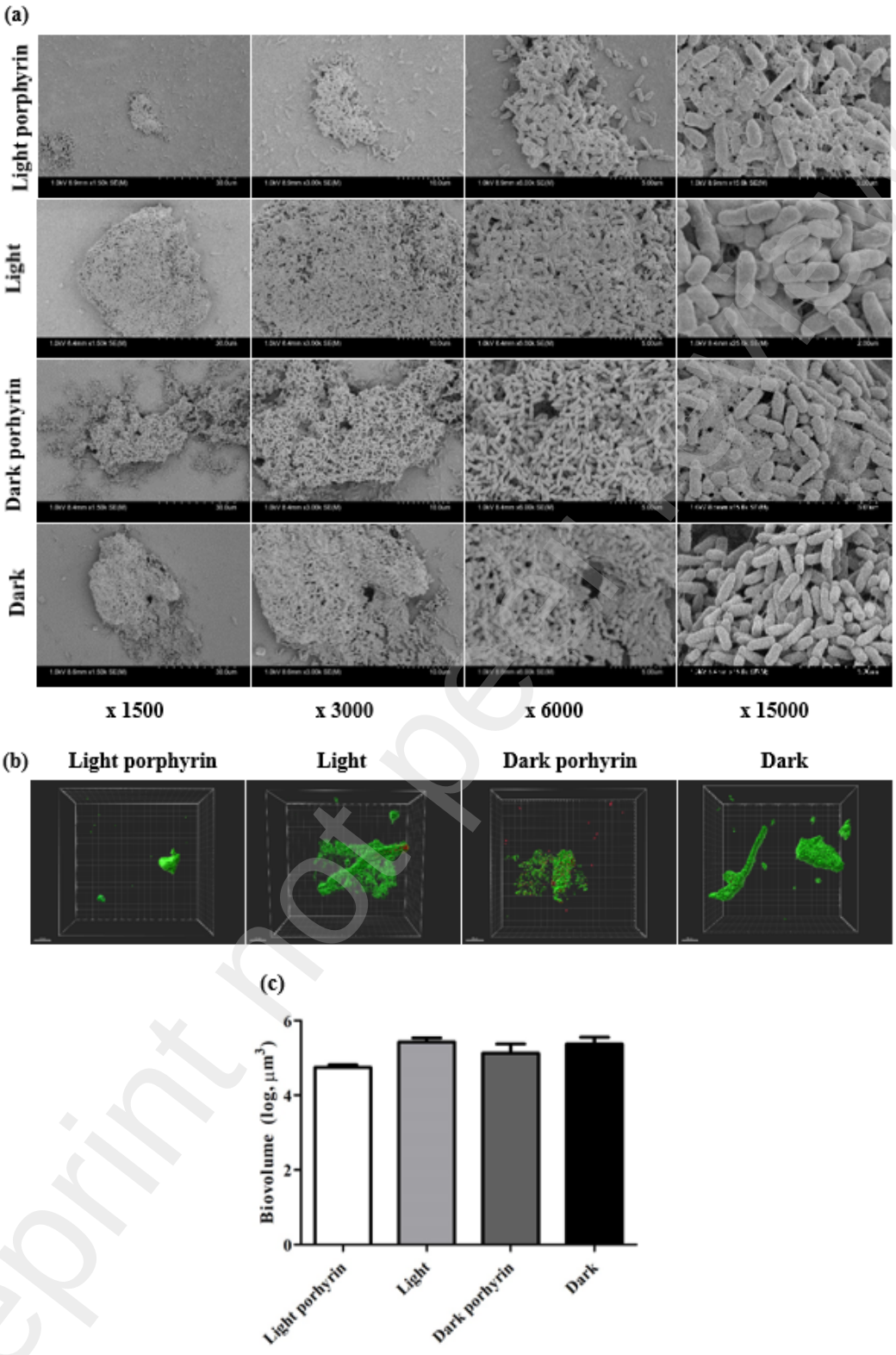


Fig. 4. The effect of the photoactive porphyrin discs on *P aeruginosa* biofilms a) Scanning Electron Micrographs (1500, 3000, 6000 and 15000X) b) Confocal Laser Scanning Microscope images reconstructed with Imaris c) Biovolume.

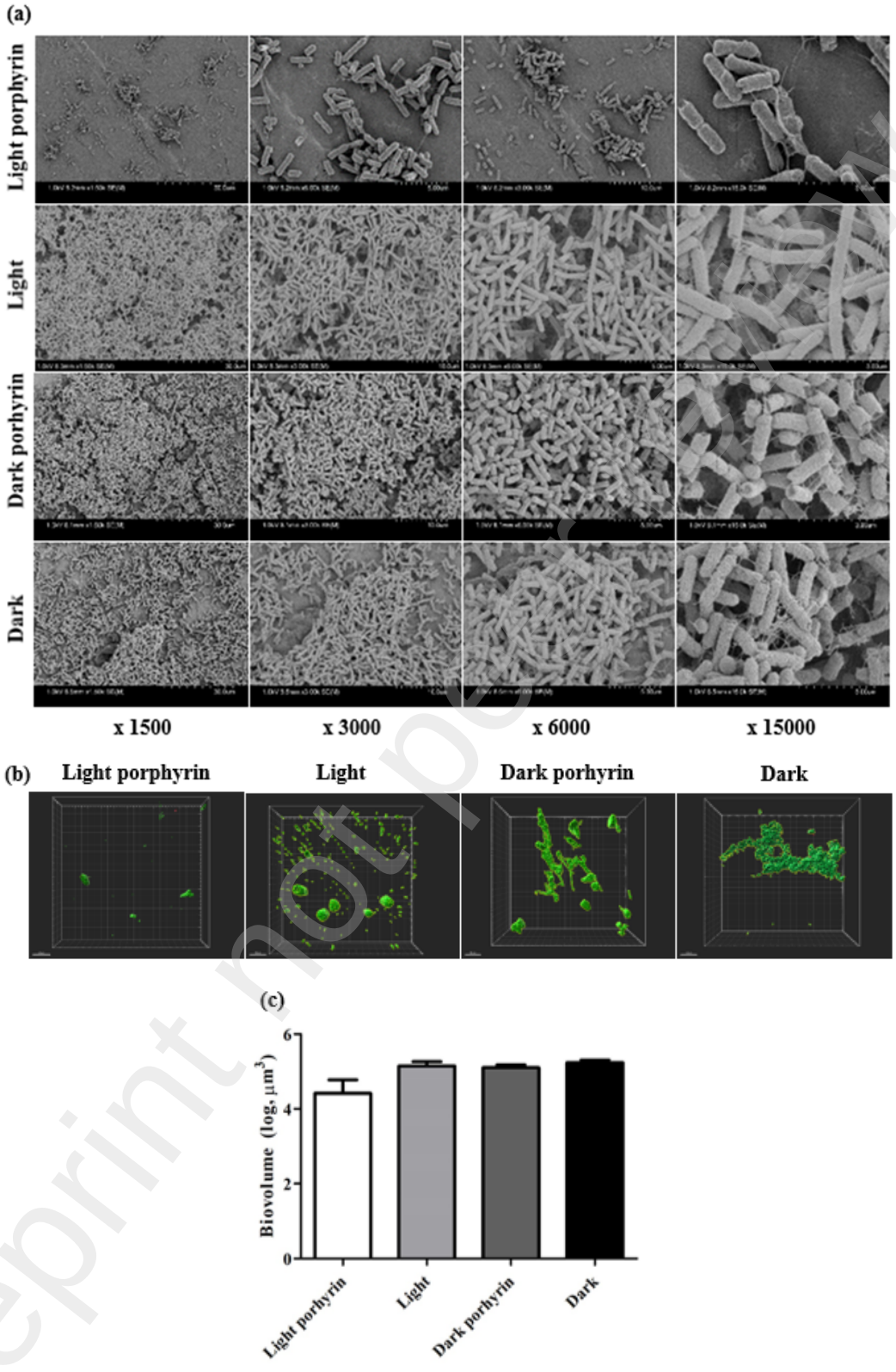


Fig. 5. The effect of the photoactive porphyrin discs one *E. coli* biofilms a) Scanning Electron Micrographs (1500, 3000, 6000 and 15000X) b) Confocal Laser Scanning Microscope images reconstructed with Imaris c) Biovolume.