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Targeted Antibacterial Strategies: Evaluating the Efficacy of Phthalocyanine-Based Conjugates in Combating Biofilm Formation

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Description

This study sheds light on how to rationally design efficient Photodynamic Antimicrobial Chemotherapy(PACT) agents by covalently linking Phthalo Cyanines(PCS) as photosensitizers with an antibiotic: Ciprofloxacin (CIP). Pcs used are zinc (II) 3-(4-((3,17,23-tris(4-(Benzo(d)thiazol-2-yl] thiol) phthalocyanine-9-yl) oxy) phenyl) propanoic acid (1) and zinc (II) 3-(4-(3,17,23-tris(3-(4-(triphenylphosphine) butyl) benzo[d]thiazol-3-ium bromide phthalocyanine-9-yl) oxy) phenyl) propanoic acid (2). High singlet oxygen quantum yields are observed in the presence of CIP. Square wave voltammetry was used to analyse the Pc-CIP uptake by bacteria biofilms of *Streptococcus pneumoniae (S. pneumonia)* and *Escherichia coli (E. coli)*. Electrochemical impedance spectroscopy and scanning electron spectroscopy were used to study the stability of the biofilms in the presence PC-CIP complexes and when exposed to light. Raman and time of flight-secondary ion mass spectrometry (TOF-SIMS) are used to identify the breakdown of cellular components of the biofilm and penetration of the PC-CIP into the biofilms, respectively. Oxygen and other Reactive Oxygen Species (ROS). In PACT, nonspecific oxidative damage by ROS, such as singlet oxygen, eliminates microbes. Hence microbial resistance group contains COOH for linking to the CIP. This is the first time that PCS have been covalently linked to CIP. 2 and 2-CIP contain cationic amphiphilic groups which enhance the electrostatic interactions to the biofilms. Hence producing singlet oxygen (which is cytotoxic to bacteria) in the proximity of the bacteria cell.

Electrochemical biofilm analysis

Charge transfer processes are important in biological and chemical systems. In this work, Pc-CIP uptake by *E. coli* and *S. pneumoniae* biofilms and degradation (removal) of the biofilms are studied using Square Wave Voltammetry (SWV) and Electrochemical Impedance Spectroscopy (EIS). EIS has been previously used to study the removal of biofilm cells hence, in this study, EIS are used to monitor the PACT activity of phthalocyanines against biofilm cells grown on Indium Tin Oxide (ITO) electrode. This is the first time that the uptake of PCS by bacteria and the degradation of the latter in the presence of the Pcs will be studied using EIS. The changes in the bacterium biofilms in the presence of the PC-CIP before and after illumination with laser PCS. Rapid and sensitive bacteria detection and identification are becoming increasingly relevant in a variety of fields, including food safety control, infectious disease prevention, and environmental monitoring. Analytical techniques such as Raman spectroscopy and TOF-SIMS are important for imaging of antibiotics and bacterium-derived metabolites in complex biological samples. This could help in understanding how sample matrices impact the survival of bacteria Raman Spectroscopy in particular gives full information for bacterial analysis in a short period of time and with excellent sensitivity and has potential applications in antibiotic susceptibility testing.

Phthalocyanine biofilm dynamics

Details of equipment items employed in this work can be found in the supporting information. The experimental conditions were as follows: The ground-state absorption spectra were recorded in solution using a 1 cm path length cuvette. The absorption spectra were normalized to 1 at the Q band and the data was baseline corrected using the solvent only. Infra-red spectra of complexes 1, 2 and their CIP derivatives were recorded as powders. A Bruker ALPHA FT-IR spectrometer equipped with this work reports on the synthesis of amphiphilic asymmetrical phthalocyanines for use in photodynamic antimicrobial chemothe -rapy. Drug responses to established S.pneumoniae and E.coli biofilms can be modulated synergistically by administering Ciprofloxacin in conjunction with phthalocyanine complexes. Drug uptake by S. pneumoniae and E. coli was monitored using square wave voltammetry. In the presence of light, the conjugates exhibit strong antibacterial action. In the dark, cell viability Conventional bacteria studies are usually performed in the free-floating, planktonic state. Multicellular aggregated communities of cells which occur on surfaces where they enclose themselves within extracellular polymeric substances comprising of proteins and nucleic acids, are termed biofilms. Unlike planktonic bacteria, biofilms exhibit altered growth and gene expression phenotypes, rendering them insensitive to antimicrobial techniques such as antibiotics and UV treatment and posing a threat to public health.

Alternative antimicrobial strategies

In recent years, micro-organisms have developed resistance to traditional antibiotics such as Methicillin-resistant *Staphylococcus aureus* (*S.aureus*), multidrug resistance *Escherichia coli* (*E. coli*) fluconazole-resistant *Candida albicans* (*C. albicans*). Furthermore, *E. coli*, *S. aureus*, and *C. albicans* biofilms are known to cause chronic infections associated with contamination of medical devices and in the urinary tract. As an alternative, Photodynamic Antimicrobial Chemotherapy (PACT) is proposed as a treatment method for removing infectious biofilm and planktonic micro-organisms. This approach is adopted from Photo Dynamic therapy (PDT-a cancer treatment). In PACT micro-organism inhibition is achieved by using a light source at a specific wavelength to activate a Photo Sensitizer (PS) to an excited state. The excited PS undergoes various photochemical processes of energy or electron transfer resulting in the production of Reactive Oxygen Species (ROS), including singlet oxygen, hydroxyl radical and superoxide anion. The generated species cause irreversible damage to cell membranes and cause protein inactivation, preventing resistance.