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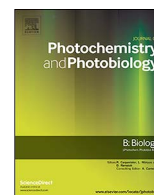

Visible and UVA light as a potential means of preventing *Escherichia coli* biofilm formation in urine and on materials used in urethral catheters

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A B S T R A C T

Catheter-associated urinary tract infections are the most common hospital-acquired infection, for which *Escherichia coli* is the leading cause. This study investigated the efficacy of 385 nm and 420 nm light for inactivation of *E. coli* attached to the silicone matrix of a urinary catheter. Using urine mucin media, inactivation of planktonic bacteria and biofilm formation was monitored using silicone coupons. Continuous irradiance with both 385 nm and 420 nm wavelengths with starting cell density population 10^3 CFU ml^{-1} reduced planktonic suspensions of *E. coli* to below the detection level after 2 h and 6 h, respectively. Bacterial attachment to silicone was successfully prevented during the same treatment. Inactivation by 385 nm and 420 nm was found to be dependent on media, cell density and oxygen, with less inhibition on planktonic suspensions when higher starting cell densities were used. In contrast to planktonic suspensions in PBS, continuous irradiance of pre-established biofilms showed a greater reduction in survival compared to urine mucin media after 24 h. Enhanced inhibition for 385 nm and 420 nm light in urine mucin media was associated with increased production of reactive oxygen species. These findings suggest 385 nm and 420 nm light as a promising antimicrobial technology for the prevention of biofilm formation on urethral catheters.

1. Introduction

Catheter-associated urinary tract infections (CAUTIs) are the most common type of hospital-acquired infection for which *Escherichia coli* is the most common etiological agent. Infection is a major problem in long term urinary catheterisation, and is associated with significantly higher morbidity and mortality [1]. Among catheterised patients the risk of urinary tract infection (UTI) is estimated to be up to 10% per day, with most patients with an indwelling urinary catheter for 30 days or longer developing a CAUTI [1]. Certain types of bacteria are able to attach to the catheter surface and replicate to form bacterial communities known as a biofilm [2,3]. It is estimated that > 60% of human infections involve biofilm formation [4,5]. Biofilm infections, such as in implant- and catheter-associated infections, affect millions of people in the developed world. When existing as part of a biofilm, bacteria show greater resistance to antibiotics and host immune defences compared to planktonic bacteria [6]. These factors make biofilm-mediated infections more difficult to treat, and many can develop into a chronic persistent infection despite antibiotic therapy.

Light-based technology is a promising therapeutic strategy for microbial disinfection of medical devices. Recent advances in fibre optics can allow for the introduction of light into medical devices. We recently described a side-emitting polymer optical fibre that could potentially be used to introduce up to 5.5 mW cm^{-2} 420 nm LED and laser light into a range of medical devices [7]. This side emitting optical fibre makes it possible to deliver uniform light into medical devices for antimicrobial applications. Ultraviolet (UV) and visible light have been demonstrated to inactivate clinically relevant microorganisms in planktonic suspensions [8–11] and those adhered to surfaces as part of a biofilm [11–14]. Several studies have investigated the potential for UVA and visible light to treat biofilm on artificial surfaces including medical-devices and for decontamination of food packaging material [13,15–18]. Photoinactivation by blue light is superior to light in the UV region in terms of patient safety and photodegradation of material, making it attractive for clinical applications [8].

The mechanism of action of light inactivation primarily occurs when photons interact with oxygen leading to the creation of reactive oxygen species (ROS), which in turn oxidise a range of cellular targets including lipids, proteins and DNA. The bactericidal effect of UVA and visible light is attributed to the formation of ROS generated by the

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absorption of photons by naturally occurring intracellular molecules, or endogenous photosensitisers, in the presence of oxygen [19]. Bacteria naturally possess endogenous photosensitisers, such as porphyrins, cytochromes, flavins and NADH [19]. When the photosensitiser absorbs a photon this excites the sensitizer to an excited state, which can then undergo intersystem crossing to the triplet state. In type I reactions, the photosensitiser can then react with an organic substrate to produce radicals, which in the presence of oxygen, further react to produce superoxide anion radicals ($O_2^{\cdot-}$), which in turn leads to the production of other ROS such as hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH). This reaction leads to the loss of the photosensitiser. In type II reactions, the excited photosensitiser transfers its energy to molecular oxygen ($^3O_2$) to produce an excited state singlet oxygen ($^1O_2$), regenerating the ground-state sensitiser. Hydrogen peroxide and singlet oxygen are the main ROS produced in response to light [20,21] and are the major species responsible for phototoxic effects. The production of ROS leads to non-specific damage to multiple cell targets including oxidative damage to proteins, DNA and lipids, ultimately leading to cell death [22]. Due to the multiple unspecific targets for generated ROS, microorganisms are unlikely to acquire resistance towards light-based therapies [13,23].

In view of the high level of antimicrobial resistance of biofilms, light-based antimicrobial therapy is gaining attention as a promising alternative approach to biofilm-mediated infections. This study investigates the potential for UVA and visible light to eradicate biofilms on materials used in medical devices under clinically relevant conditions. The ability for light to prevent biofilm was assessed by measuring bacterial adherence to silicone coupons. Increased formation of ROS in urine mucin media compared to PBS was shown using the nitro blue tetrazolium (NBT) reduction assay and bleaching of indigo carmine. Irradiance with visible and UVA light successfully decreased planktonic bacteria and prevented biofilm formation, suggesting light is a promising antimicrobial technology to prevent biofilm in urethral catheters. This study highlights the potential for light to excite naturally occurring photosensitisers in urine and lead to the formation of ROS.

2. Materials and Methods

2.1. Bacterial Culture

E. coli UTI 11380, which was isolated from an adult with cystitis, was selected to investigate inactivation by light for this study (obtained from Innovatech, US). The strain was stored at − 80 °C in LB broth (Sigma) containing 7% DMSO and recovered on Luria agar grown overnight at 37 °C. Due to the higher incidence of biofilm on the outer surface of urethral catheters, we aimed to mimic the environmental conditions pathogens may encounter on this surface. To achieve this we adapted an artificial sputum medium recipe [24] mixed with human urine to mimic the mucous layer of the urinary tract. This urine mucin media consisted of 50% filter sterilised pooled human urine from three healthy individuals (Innovative Research, Patricell Limited, Nottingham, UK) with autoclaved 43.9% deionised water, 2.5% (w/v) porcine stomach mucin (Sigma), 2.5% (w/v) NaCl (Sigma) and 1.1% (w/v) KCl (Sigma). Pooled human urine had a specific gravity 1.015. All experiments were performed in this urine mucin media unless otherwise stated. Colonies were then cultivated overnight in urine mucin media prior to light exposure. For suspension of bacterial cells in PBS, an overnight culture in urine mucin media was washed twice in phosphate-buffered saline (PBS) by centrifugation at 5000 × *g* for 5 min with the resultant pellet resuspended in PBS. The bacterial starting density was determined using a spectrophotometer at 600 nm and diluted to the required starting population. Experiments investigating the involvement of ROS included the addition of 125 U ml^{-1} catalase (Sigma) or 20 mM dimethylthiourea (Sigma) to the media.

2.2. Light Apparatus

Light exposure was achieved using light emitting diodes (LEDs) (M385 L2 and M420 L2; Thorlabs Ltd) with a nominal wavelength of 385 nm (FWHM 10 nm) and 420 nm (FWHM 12 nm). An aspheric condenser lens (Ø50 mm, focal length 40 mm, ACL5040-A, Thorlabs Ltd) was used to create uniform distribution of light over the desired area of the wellplate. To ensure that the transmittance of the media did not significantly impact the intensity of the light impinging on the sample surface, an upward configuration was utilised whereby the light passed through the bottom of a 96-well plate and then the silicone (polydimethylsiloxane; PDMS) coupon (supplied by Teleflex, Cambridge, US) before passing through the media (Fig. S1). The 96-well plate (Thermo Scientific, Microwell Plate Nunclon flat bottom 400 μl, Fisher Scientific, Ireland) was fixed at a distance of 1 cm above the LED light source. The light setup was encased in a light-tight enclosure that utilised a built in fan to enable the temperature to be equalised with the incubation room environment in which the experiment was conducted. The irradiance was measured with a wellplate and silicone sample *in situ* using a power meter and sensor (PM100D and S120VC model Thorlabs). Dose (J cm^{-2}) was calculated as irradiance (W cm^{-2}) × exposure time (s). The addition of silicone disks decreased the transmission of light through the wellplate to the media by 20%. Trials indicate that no deterioration of the PDMS is caused by exposure to either 385 nm or 420 nm light after 24 h at the power densities used in this study.

2.3. Microtiter Plate Method to Measure Bacterial Adhesion

Biofilm formation was assessed by the ability of *E. coli* to adhere and form biofilm on silicone coupons with a thickness of 0.5 mm and diameter of 6 mm placed into the bottom of a 96-well microtitre plate (Thermo Scientific) [25]. Prior to the experiment bacteria were grown overnight at 37 °C in 2 ml of urine mucin media as described above. Bacterial concentration was determined by optical density (OD) at 600 nm and was diluted to the appropriate starting density. Wells containing silicone disks were preconditioned with urine mucin media for 1 h at 37 °C prior to inoculation. Media was then removed and aliquots of 200 μl of bacterial suspension were added per well containing silicone coupons and plates were incubated at 37 °C without shaking for 24 h.

2.4. Bacterial Enumeration

At each sampling interval, the culture media was removed and was serially diluted in PBS and 10 μl was spotted in triplicate on Luria agar (Sigma) to determine the survival of *E. coli* in the planktonic suspension. The plates were then incubated at 37 °C for 24–48 h. Plates were incubated up to 48 h to allow sublethally injured cells to grow to size large enough to be counted. Colonies were then counted and the number of viable cells (CFU ml^{-1}) was determined. The detection limit for individual experiments was 10^2 CFU ml^{-1} unless otherwise stated. In some instances, pooled independent experiments reduce the average counts below 10^2 CFU ml^{-1}.

For adherent bacteria, the silicone disks were carefully removed from the wells and were gently washed three times in 500 μl of PBS in a 1.5 ml tube. The silicone disks were then vortexed for 30 s, followed by 4 min of sonication in an Elmasonic P30 ultrasonic bath (ultrasonic frequency 37 kHz, 100% intensity, pulse mode, Elma, Singen, Germany) at room temperature, followed by an additional 30 s of vortexing. Samples were then serially diluted and plated as described above and CFU per disk was determined. Complete removal of cells was confirmed after sonication by microscopic evaluation of surfaces. This treatment was previously shown not to decrease bacterial viability [26]. To confirm sonication did not reduce viability, disks were sonicated at 30 s intervals for 10 min. No significant reduction in CFU ml^{-1} was

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