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ORIGINAL PAPER

New Proof-of-Concept in Viral Inactivation: Virucidal Efficacy of 405 nm Light Against Feline Calicivirus as a Model for Norovirus Decontamination

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Abstract The requirement for novel decontamination technologies for use in hospitals is ever present. One such system uses 405 nm visible light to inactivate microorganisms via ROS-generated oxidative damage. Although effective for bacterial and fungal inactivation, little is known about the virucidal effects of 405 nm light. Norovirus (NoV) gastroenteritis outbreaks often occur in the clinical setting, and this study was designed to investigate potential inactivation effects of 405 nm light on the NoV surrogate, feline calicivirus (FCV). FCV was exposed to 405 nm light whilst suspended in minimal and organically-rich media to establish the virucidal efficacy and the effect biologically-relevant material may play in viral susceptibility. Antiviral activity was successfully demonstrated with a 4 Log_{10} (99.99%) reduction in infectivity when suspended in minimal media evident after a dose of 2.8 kJ cm^{-2}. FCV exposed in artificial faeces, artificial saliva, blood plasma and other organically rich media exhibited an equivalent level of inactivation using between 50–85% less dose of the light, indicating enhanced inactivation when the virus is present in organically-rich biologically-relevant media. Further research in this area could aid in the development of 405 nm light technology for effective NoV decontamination within the hospital environment.

Keywords 405 nm Light · Viral inactivation · Feline calicivirus · Saliva · Faeces · Plasma

Views expressed in this article are an informal communication and represent the authors' own best judgment. These comments do not bind or obligate FDA.

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Introduction

Norovirus (NoV), one of the most common causes of epidemic acute gastroenteritis (Hall et al. 2013), can be transmitted via food and water, person-to-person contact or contact with environmental surfaces (Robilotti et al. 2015). Environmental stability and resistance to disinfection further aid the transmission of NoV, with viral particles detected on surfaces up to 42 days after contamination (Escudero et al. 2012). If environmental decontamination is deficient, this can lead to ward closures which has substantial operational and financial implications for health boards (Wu et al. 2005; Danial et al. 2011). NoV outbreaks in the healthcare setting and other densely populated areas such as nursing homes, schools and restaurants (Robilotti et al. 2015) have driven the need for new decontamination systems.

Advanced decontamination technologies used to overcome nosocomial outbreaks include ozone, hydrogen peroxide vapour and UV-light systems (Maclean et al. 2015).

These technologies are time consuming with hospital wards required to be vacated to prevent harmful effects to patients and staff (Otter et al. 2013), and are therefore suited to terminal cleaning. A technology using 405 nm violet-blue visible light has been developed to provide continuous decontamination of occupied hospital environments (Maclean et al. 2014). Application of 405 nm light for decontamination in hospitals has been demonstrated, with levels of bacterial contamination on environmental surfaces around occupied isolation rooms reduced by up to 86% over and above reductions achieved by traditional cleaning alone (Maclean et al. 2010, 2013a; Bache et al. 2012).

It has been demonstrated that 405 nm violet-blue light has germicidal activity against a range of bacteria and fungi (Guffey and Wilborn 2006; Enwemeka et al. 2008; Maclean et al. 2009, 2013b; Murdoch et al. 2013), effected through excitation of endogenous photosensitive porphyrin molecules within microbial cells, causing the production of singlet oxygen and other reactive oxygen species (ROS), resulting in oxidative damage and microbial cell death (Hamblin and Hasan 2004; Maclean et al. 2008; Murdoch et al. 2013). A study investigating the efficacy of 405 nm light on the bacteriophage ϕC31 indicated that the phage was susceptible to high doses of 405 nm light, with susceptibility significantly enhanced when exposed in nutrient-rich media (Tomb et al. 2014). However, as virions do not contain endogenous porphyrins (Gelderblom 1996), current knowledge on the antiviral efficacy of 405 nm light on medically important human and animal viruses is lacking and requires investigation.

This study was designed to provide the first proof-of-concept of the interaction of narrowband 405 nm light with feline calicivirus (FCV) as a model to study the antiviral effects of this light on NoV. Feline calicivirus was selected as a NoV surrogate, as there is currently no standardised cell culture system for NoV (Duizer et al. 2004a; Richards 2012; Cromeans et al. 2014). Our data demonstrate the influence of the suspending media, including biologically-relevant fluids, on viral susceptibility. As such, this study provides evidence of the antiviral efficacy and discusses the potential mechanism of 405 nm light viral inactivation.

Methodology

Cell and Virus Culture

Feline embryonic cells, strain FEA (Jarrett et al. 1973), were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 2 mM L-glutamine, 1 mM sodium pyruvate and 240 U mL^{-1} penicillin streptomycin (Gibco, Life Technologies, UK), to form 10% FBS-DMEM. Cells were maintained at 37 °C in 5% CO_2.

To prepare a virus pool of the FCV vaccine strain F9, virus inoculum (School of Veterinary Medicine, University of Glasgow) was added to FEA monolayers in 850 cm^2 cell culture roller flasks (Corning, USA). After 90 min incubation of the inoculated cells on a rotating roller stand at 37 °C in 5% CO_2, fresh culture medium was added and flasks incubated for 24 h. This resulted in virus-induced destruction of nearly 90% of the cell monolayer.

The tissue culture supernatant, and medium from a single wash step, was collected from each roller bottle and subjected to two freeze–thaw cycles before clarification by centrifugation at $3300 \times g$ for 10 min. The virus-containing supernatant was then stored at −80 °C until required. The infectious titre of FCV was approximately 2×10^7 plaque-forming units per millilitre (PFU mL^{-1}), determined by standard plaque assay techniques (Ormerod and Jarret 1978).

405 nm Light Source

The light source used was a 405 nm light emitting diode (LED) array (ENFIS PhotonStar Innovate UNO 24; PhotonStar Technologies, UK) powered by a 40 V Phillips Xitanium LED Driver (Phillips, Netherlands). The array had a peak wavelength around 405 nm and a bandwidth of approximately 19 nm (Fig. 1) but will, for convenience, be referred throughout this text as 405 nm light. The array was attached to a heatsink and cooling fan, to minimise heat transfer to test samples, so that no significant heating of the sample occurred. The light source was held on a PVC stand at a distance of 4 cm from the microbial samples, giving an irradiance of 155.8 mW cm^{-2} at the sample surface [measured using a radiant power meter and photodiode detector (LOT Oriel, USA)].

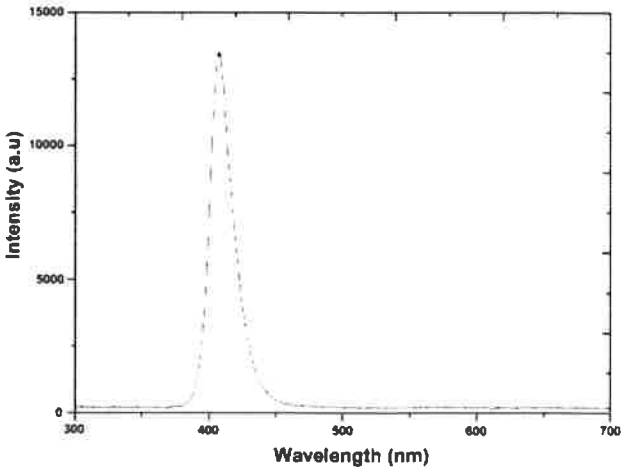

Fig. 1 Optical emission spectrum of the 405 nm LED array, measured using an HR4000 spectrometer (Ocean Optics, Germany) and Spectra Suite software version 2.0.151

405 nm Light Exposure of Viral Suspensions

Feline calicivirus stock virus was defrosted at room temperature and diluted to 2×10^5 PFU mL^{-1} in Dulbecco's phosphate-buffered saline, supplemented with calcium and magnesium (DPBS; Hyclone, Thermo Fischer Scientific, UK). This was used as a 'minimal medium' (MM). Viral suspension of 1.5 mL were transferred into the central four wells of a 24-well plate (Techno Plastic Products, Switzerland) and the plate positioned on a raised stand, with the sample wells at 4 cm directly below the light source and the plate lid kept on to prevent evaporation. Test samples were exposed to increasing doses of 405 nm light at room temperature, with the dose calculated as the product of irradiance (mW cm^{-2}) × exposure time (s). Control samples were set up under identical environmental conditions but without 405 nm light illumination. Post-exposure, FCV samples were serially diluted in MM for enumeration by plaque assay.

Exposures were repeated with FCV suspended in 'organically-rich media' (ORM): DMEM, 10% FBS-DMEM, artificial saliva, artificial faeces and blood plasma. The artificial saliva was a modified version of that used by Margomenou et al. (2000) [5.2 g $NaHCO_3$, 0.88 g NaCl, 1.36 g K_2HPO_4, 0.48 g KCl, 2000 units α-amylase and 2 g pig gastric mucin (Sigma-Aldrich, UK) in 1 L sterile water], and was adjusted to pH of 7–7.5 to emulate the variability of pH in human saliva, and also to ensure that no FCV inactivation occurred (Duizer et al. 2004b; Edgar et al. 2004). The artificial faeces was a modified version of that by Colón et al. (2015) [30 g inactivated yeast (Marigold, UK), 7 g physillum (Buy Whole Foods Online, UK), 11 g miso paste (Yutaka, UK), 8 g cellulose, 1.6 g NaCl, 0.8 g CaCl, 1.6 g KCl (Sigma-Aldrich) in 920 mL sterile water], and was also adjusted to pH 7. The modifications to the formulations of artificial saliva and faeces were to ensure compatibility with the FEA cells. Fresh frozen human blood plasma was obtained from the Scottish National Blood Transfusion Service (SNBTS, UK), and defrosted before use. FCV was also exposed when suspended in MM supplemented with riboflavin, with and without tyrosine, tryptophan, pyridoxine and folic acid (used at the same concentrations as found in DMEM: 0.4, 104, 16, 4 and 4 mg L^{-1} respectively).

Plaque Assay

Prior to experiments, 6-well cell culture plates (Thermo Fischer Scientific) were seeded with 7.5×10^5 FEA cells per well. 3 mL of the cell suspension in growth medium was pipetted into each well, and incubated at 37 °C in 5% CO_2 for 20 h, resulting in confluent monolayers.

Post-exposure of FCV, the growth medium was aspirated from the FEA cells and replaced with 1 mL FCV sample. Plates were co-incubated at 37 °C in a humidified 5% CO_2 incubator for 90 min, with the plates gently rocked every 15 min to ensure even distribution of the inoculum over each monolayer.

After the viral incubation period, the inoculum was aspirated and the well washed with medium (10% FBS-DMEM or DPBS) before adding 4 mL overlay mixture consisting of 2× supplemented DMEM 1:1 with 2× agarose. 2× supplemented DMEM was prepared using 20 mL from a filter-sterilised stock of 10× DMEM, adding the same supplements as detailed earlier, plus 9.86 mL sodium bicarbonate solution (Gibco), and was made up to 100 mL with sterile water. 2× agarose was prepared by dissolving 2 g agarose (Sigma-Aldrich) in 100 mL deionised distilled water and then sterilised by autoclaving. The overlay was left to set before the plates were incubated for 44–48 h at 37 °C in 5% CO_2.

Post-incubation, the monolayers were fixed and stained overnight with 0.5% crystal violet in 10% neutral buffered formalin. The agarose plugs and stain were then removed, the plates left to dry, plaques counted, and the virus infectivity titre expressed as PFU mL^{-1}.

Spectrophotometry

The transmission of 405 nm light through the suspending media was measured using a Biomate 5-UV–Visible spectrophotometer (Thermo Fischer Scientific). The presence of porphyrins, or other components with the ability to absorb 405 nm light and emit fluorescence, within the suspending media was determined by fluorescence spectrophotometry. Media were freshly prepared, and fluorescence measurements were carried out using a RF-5301 PC spectrofluorophotometer (Shimadzu, USA). Excitation was carried out at 405 nm and emission spectra recorded between 425 and 700 nm.

Data Analysis

Data points represent mean results ± standard deviation (SD), taken from triplicate independent experiments ($n = \geq 3$). The antiviral activity of 405 nm light was determined by calculating the reduction in the level of infectivity from the difference between Log_{10} values for exposed and control samples. Significant differences were calculated at a 95% significance level, using paired *t*-tests or one-way ANOVA (Minitab 16 Statistical Software), with results found to be significant when $P < 0.05$.

Results

Feline calicivirus was suspended in MM and ORM and exposed to increasing doses of 405 nm light at an irradiance of 155.8 mW cm^{-2}. Results (Fig. 2) show that when

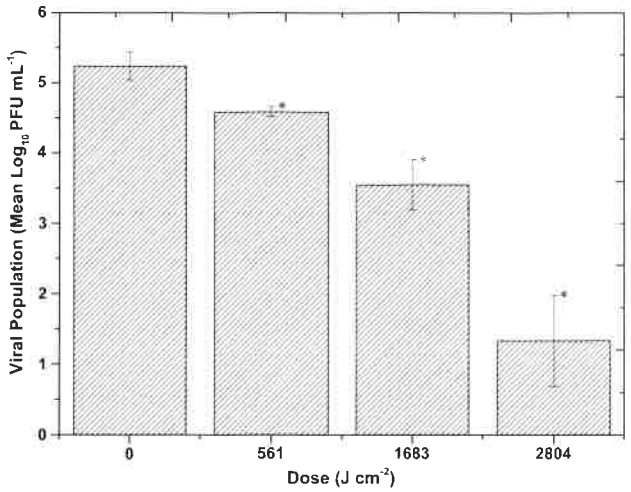

Fig. 2 Inactivation of feline calicivirus when suspended in minimal medium (Dulbecco's phosphate buffered saline), upon exposure to 405 nm light at an irradiance of 155.8 mW cm^{-2}. Data points show the mean counts ($n = 6$) ± SD. *Asterisks* indicate light-exposed samples that were significantly different to the non-exposed final control samples ($P \leq 0.05$), using one-way ANOVA. No significant decrease was observed in the final control populations ($P \geq 0.05$)

suspended in MM, significant FCV inactivation was achieved after exposure to 561 J cm^{-2} ($P = 0.043$), and relatively linear inactivation kinetics were observed, with a dose of 2.8 kJ cm^{-2} required for a 3.9 Log_{10} inactivation. The non-exposed control samples showed no significant change over the course of the experiment ($P > 0.05$).

Antiviral efficacy was found to differ significantly when suspended in ORM. When exposed in 10% FBS-DMEM, a significantly lower dose was required for viral inactivation (Fig. 3), with a 4.8 Log_{10} reduction achieved after a dose of 421 J cm^{-2}. As the presence of FBS in DMEM is thought to reduce the level of oxidation upon exposure to normal laboratory lighting (Grzelak et al. 2001), the exposure was repeated with FCV suspended in DMEM without FBS to observe any differences in inactivation kinetics. Although slightly greater inactivation was observed with each applied dose, results (Fig. 3) demonstrate no significant differences in the inactivation kinetics of FCV when the virus is exposed in DMEM in the presence or the absence of 10% FBS ($P > 0.05$). Control samples showed no significant decrease ($P > 0.05$).

Furthermore, components of DMEM have been shown to be photosensitive to light (Grzelak et al. 2001), and therefore, exposures were repeated with riboflavin added to MM with and without tyrosine, tryptophan, pyridoxine and folic acid in the same concentrations as found in DMEM (Table 1). Results demonstrated that exposure of FCV suspended in MM with riboflavin only resulted in a 1.3 Log_{10} reduction after 421 J cm^{-1}; however, when all components were present, enhanced inactivation occurred and a 5.1 Log_{10} inactivation was achieved.

Artificial saliva, artificial faeces and blood plasma were selected as ORM which are biologically relevant in terms of media in which viral particles may be found in the environment, with NoV being regularly identified in faeces. Exposure of FCV when suspended in artificial saliva yielded results similar to those in DMEM, with a 5.1 Log_{10} reduction of infectivity achieved after a dose of

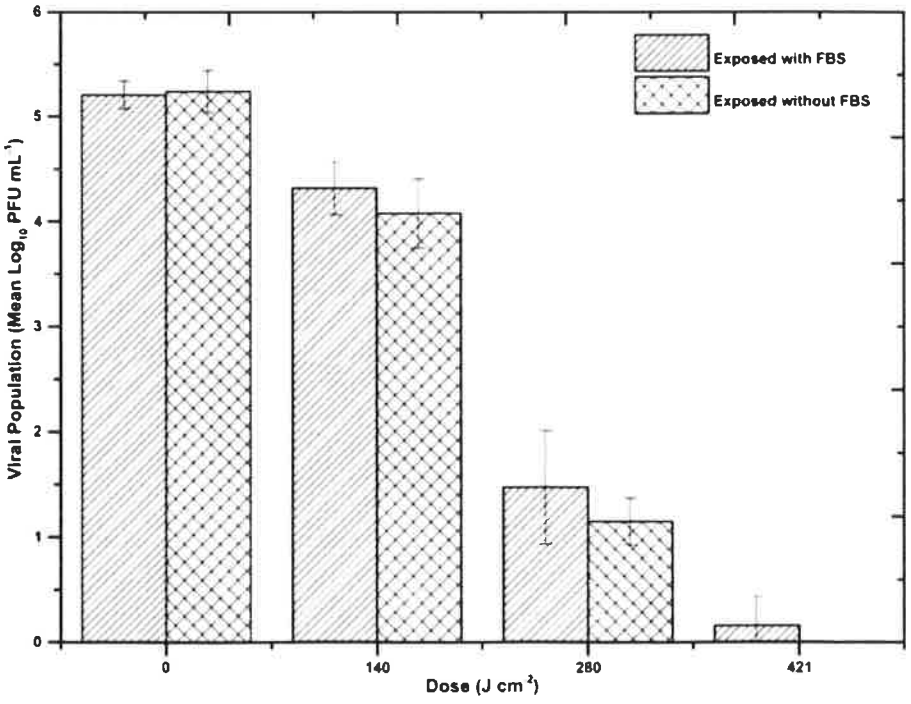

Fig. 3 Comparison of the inactivations of feline calicivirus when suspended in organically-rich media [supplemented Dulbecco's modified eagle's medium, without and without 10% fetal bovine serum (FBS)], upon exposure to 405 nm light at an irradiance of 155.8 mW cm^{-2}. Data points show the mean counts ($n = 3$) ± SD. Statistical analysis, using a paired *t* test, showed no significant difference between inactivations in the two media ($P > 0.05$). No significant decrease was observed in the final control populations ($P \geq 0.05$)

Table 1 Comparison of the inactivations of feline calicivirus when suspended in minimal media supplemented with riboflavin alone or alongside tyrosine, tryptophan, pyridoxine and folic acid, upon exposure to 405 nm light at an irradiance of 155.8 mW cm^{-2}

Photosensitive components	Starting population, Log_{10} PFU mL^{-1} (± SD)	Exposed viral population, Log_{10} PFU mL^{-1} (± SD)	Non-exposed control population, Log_{10} PFU mL^{-1} (± SD)	Log_{10} reduction, PFU mL^{-1} (P value)
Riboflavin	5.01 ± 0.02	3.77 ± 0.61	5.05 ± 0.06	1.28* ($P = 0.00$)
Riboflavin Tyrosine Tryptophan Pyridoxine Folic acid	5.15 ± 0.03	0.00 ± 0.00	5.12 ± 0.07	5.12* ($P = 0.00$)

Data points represent the mean count ($n = 3$) ± SD

* Light-exposed samples that were significantly different to the non-exposed final control samples ($P \leq 0.05$)

421 J cm^{-2} (Fig. 4a). (In this case, inactivation was measured to a sensitivity of ten PFU mL^{-1}, as the artificial saliva in the undiluted samples adversely reacted with the FEA cells causing them to dislodge from the plate). The dose required for inactivation when suspended in blood plasma was slightly greater than that required when in artificial saliva, with 561 J cm^{-2} being required for 4.8 log_{10} inactivation of FCV (Fig. 4a). FCV inactivation in artificial faeces required greater doses, with 4.5 log_{10} inactivation achieved after 1.4 kJ cm^{-2} (Fig. 4b). Control samples in artificial saliva, plasma and artificial faeces showed no significant changes ($P = 0.618$, 0.101, 0.747, respectively).

Optical analysis of the suspending media demonstrated the transmission of 405 nm light to be 90% in DPBS, 40.6% in DMEM, 30.6% in 10% FBS-DMEM, 35.9% in artificial saliva, 0.05% in artificial faeces, and 2.1% in blood plasma ($n = 4$). The fluorescence emission spectra (Fig. 5) of MM (DPBS) and ORM (DMEM, 10% FBS-DMEM, artificial saliva, artificial faeces and blood plasma) when excited at 405 nm, show emission peaks for DMEM, 10% FBS-DMEM, artificial faeces and blood plasma observed between 510 and 520 nm and for artificial saliva at 460 nm.

Discussion

Although there has been a recent move towards using Murine Norovirus and Tulane Virus, alongside FCV, as NoV surrogates (Cromeans et al. 2014; Kniel 2014; Chui et al. 2015; Esseili et al. 2015; Zonta et al. 2016), FCV was chosen as it has physiochemical and genomic similarities to NoV, and is a well-established surrogate with a standardised cell culture protocol (Doultree et al. 1999; Bidawid et al. 2003; Duizer et al. 2004a, 2004b; Chander et al. 2012). Similarly, studies investigating the virucidal effects of UV-light, ozone, hydrogen peroxide vapour and cold atmospheric gas plasma technologies have also used FCV as a NoV surrogate (Nuswalen et al. 2002; Hudson et al. 2007; Bentley et al. 2012; Aboubaktar et al. 2015; Holmdahl et al. 2016).

The virucidal efficacy of 405 nm light was determined using FCV suspended in both MM and ORM. Exposure in MM would provide a better indication of the interaction of 405 nm light and the virus alone, when under suspension in ORM, which is likely to contain photosensitive components, and would assess how viral susceptibility can potentially be influenced by the surrounding media.

Successful FCV inactivation was achieved when suspended in MM, although the dose required was substantially great, with 2.8 kJ cm^{-2} achieving a 3.9 Log_{10} reduction (Fig. 2). In the case of bacteria and fungi in MM, doses in the range of 18–576 J cm^{-2} are typically required for 5 Log_{10} inactivations (Maclean et al. 2009; Murdoch et al. 2012, 2013). The increased susceptibility of bacteria and fungi compared with viruses is accredited to the presence of endogenous photosensitive porphyrins within these cells (Hamblin and Hasan 2004; Maclean et al. 2008; Murdoch et al. 2013). Low sensitivity of FCV in MM was anticipated due to the absence of porphyrins in the viral structure, coupled with the fact that MM does not contain any photosensitive substances which absorb light at 405 nm (Fig. 5), suggesting that viral inactivation, in this case, is due to a differing mechanism.

An alternative mechanism of inactivation when FCV is suspended in MM may be associated with the LED emission spectrum extending slightly into the UVA region (Fig. 1), meaning the virus is exposed to very low-level UVA photons (~390 nm). Over an extended period, this could cause oxidative damage to proteins (Girard et al. 2011), for example, to the viral capsid, and therefore contribute to the observed inactivation. Another possibility is that the small amount of 420–430 nm light emitted from the source may contribute to viral inactivation. Antiviral effects of 420–430 nm have been demonstrated against murine leukaemia virus, with long exposures thought to cause photo-damage to the virion-associated reverse

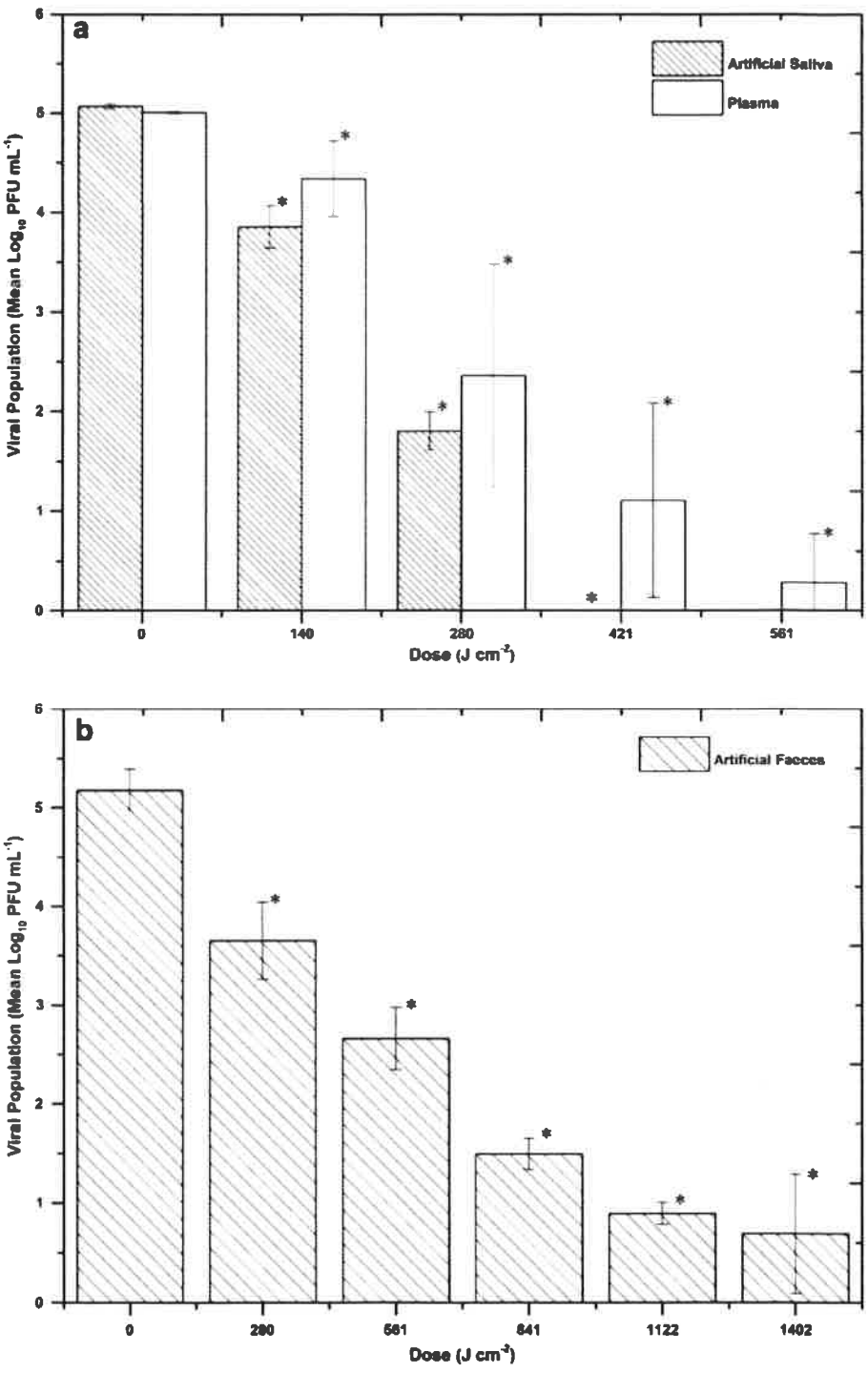

Fig. 4 Inactivation of feline calicivirus suspended in **a** artificial saliva or plasma and **b** artificial faeces, upon exposure to increasing doses of 405 nm light at an irradiance of 155.8 mW cm^{-2}. Data points show the mean counts ($n = 3$) ± SD. *Asterisks* indicate light-exposed samples that were significantly different to non-exposed final control samples ($P \leq 0.05$), using one-way ANOVA. No significant decrease was observed in the final control populations ($P \geq 0.05$)

transcription complex (Richardson and Porter 2005). Although the virus differs in structure to FCV, these findings suggest that the prolonged exposure to wavelengths at the tail ends of the 405 nm LED emission spectrum such as 390 and 420 nm, as well as 405 nm, may affect the viruses' ability to infect and replicate in host cells, and have a role in the inactivation of FCV by the LEDs used in this study.

To investigate whether exposure in ORM had any effect on viral susceptibility, FCV was first suspended in DMEM with and without 10% FBS, thought to aid protection against ROS (Grzelak et al. 2001). Results (Fig. 3) demonstrated near complete reduction in infectivity of a 10^5 PFU mL^{-1} population after a dose of 421 J cm^{-2}. As can be seen in Fig. 3, slightly greater inactivation occurred

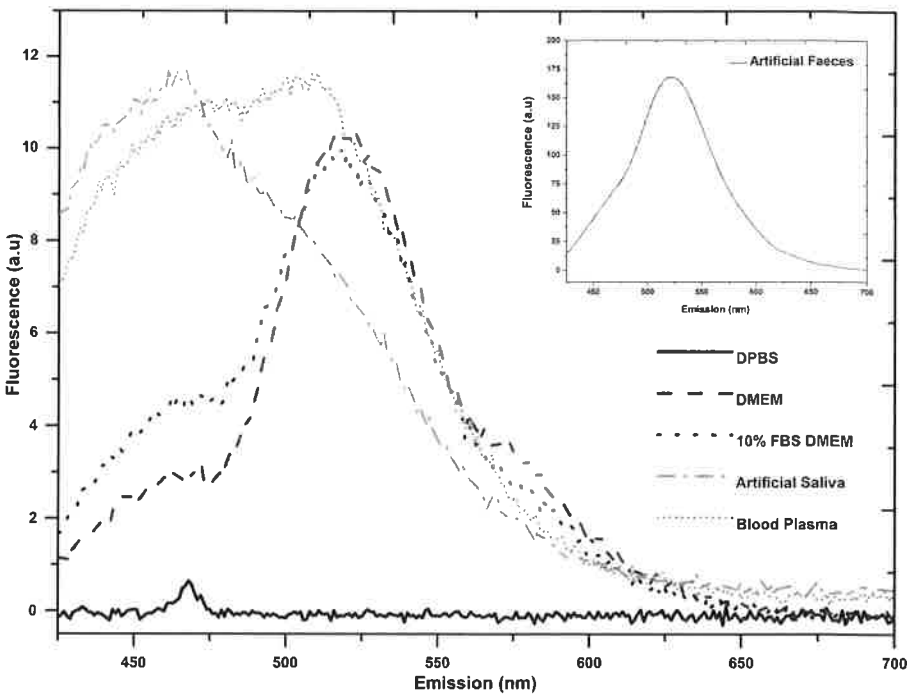

Fig. 5 Fluorescence spectra of minimal medium [Dulbecco's phosphate buffered saline (DPBS)] and organically-rich media [Dulbecco's modified eagle's medium (DMEM), 10% foetal bovine serum-supplemented DMEM (10% FBS-DMEM), artificial saliva, artificial faeces and blood plasma] using an excitation wavelength of 405 nm

when FCV was suspended in DMEM without the FBS serum additive; however, no significant difference was seen between the inactivation kinetics. As the inactivation dose of 421 J cm^{-2} is 85% less than that required for a similar level of inactivation in MM, it is likely that components of the ORM are influencing FCV inactivation. A study investigating the susceptibility of bacteriophage ϕC31 (Tomb et al. 2014) demonstrated similar results to those of the current study: little inactivation was observed when exposed in a simple salt solution; however, susceptibility was significantly enhanced when suspended in a nutrient-rich medium, with a 5.4 Log_{10} reduction of ϕC31 achieved after exposure to 510 J cm^{-2}. This was hypothesised to be due to the complex protein and amino acid-rich composition of the nutrient-rich medium, suggesting that some components could be photosensitive and when exposed to 405 nm light in the presence of oxygen, would produce ROS, damaging the bacteriophage (Tomb et al. 2014). This same phenomenon is likely to account for the enhanced inactivation of FCV when suspended in DMEM and 10% FBS-DMEM, as these contain a complex mixture of amino acids, vitamins and sugar, which have the potential to absorb 405 nm light (Fig. 5) and act as photosensitisers.

The photosensitisation of components of DMEM has also been demonstrated upon exposure to light, with riboflavin being shown to produce ROS which is further enhanced by tryptophan, tyrosine, pyridoxine and folic acid (Grzelak et al. 2001). Furthermore, blue-light wavelengths are thought to be the most efficient for the photo-decomposition of riboflavin and generation of ROS (Cheng et al. 2015). To investigate this, riboflavin was added to MM with and without tyrosine, tryptophan, pyridoxine and folic acid in the same concentrations found in DMEM (Table 1). Results support this, with only 1.3 Log_{10} reduction when only riboflavin was present; however, when all vitamins and amino acids (riboflavin, tyrosine, tryptophan, pyridoxine and folic acid) were present, enhanced inactivation of FCV was achieved with complete inactivation of a 10^5 PFU mL^{-1} population.

It is important to consider how light-induced inactivation would be influenced when viral particles were suspended in more biologically-relevant, naturally occurring matrices such as body fluids or secretions. As artificial saliva and artificial faeces can be prepared, these were used alongside human blood plasma, as model human secretions in which many viruses can be transmitted (Aitken and Jeffries 2001).

Results (Fig. 4) demonstrated that, similar to inactivation in ORM (DMEM and 10% FBS-DMEM), viral susceptibility was significantly increased when suspended in these biologically-relevant fluids. Of the three model fluids used, sensitivity was the highest when suspended in artificial saliva, with a 5.1 Log_{10} reduction of FCV infectivity being achieved after a dose of 421 J cm^{-2}—the same as that observed when suspended in ORM. Susceptibility was slightly reduced when suspended in blood plasma (4.8 Log_{10} inactivation with 561 J cm^{-2}), and further reduced when suspended in artificial faeces, with more than three times the dose required to achieve a 4.5 Log_{10} reduction. The reduced levels of 405 nm light transmission through the blood plasma and artificial faeces will contribute

to these slower inactivation rates, with average values of 2.12 and 0.05% transmission levels of 405 nm recorded for blood plasma and artificial faeces, respectively, compared to 30–40% transmission levels in all other ORM used. Overall, the susceptibility values of FCV to 405 nm light when suspended in artificial faeces, artificial saliva, blood plasma and other organically rich media were significantly increased compared to the susceptibility in minimal media, with 50–85% less dose being required for similar levels of viral inactivation. Inactivation when suspended in these ORM is likely due to the proteins contained within the media, for example, the mucin in the artificial saliva, proteins within the plasma, and inactivated yeast within the artificial faeces, which may all be predisposed to photosensitisation (demonstrated by the fluorescence peaks around 460 and 510–520 nm in Fig. 5). These results indicate the potential for NoV susceptibility to 405 nm light to be enhanced when suspended in ORM, or host secretions in which they are released, such as faeces, blood and vomit. Although the consistency and transparency/opacity may differ to those used in this study, these fluids are likely to be rich in molecules which could potentially be sensitive to 405 nm light, thereby aiding in the NoV inactivation.

The results of this study provide first proof-of-concept demonstrating that the antimicrobial efficacy of 405 nm light can be extended to medically important viruses, with the susceptibility being significantly enhanced when the viral particles are contained within biologically-relevant media. Further work should be carried out to establish the effects of 405 nm light on other NoV surrogates, such as Murine Norovirus and Tulane Virus, which may be more resistant to decontamination. This will ensure that the antiviral efficacy of 405 nm light is not over/under-estimated and allows for a more accurate quantification of the dose required for NoV inactivation. In addition, as this work used a small-scale LED source with a high irradiance output to establish the inactivation kinetics, and further investigations are therefore required to investigate the effectiveness of 405 nm light against airborne and surface-deposited viruses, using low irradiance light applied continuously over long periods, similar to that employed in clinical decontamination evaluations (Maclean et al. 2010, 2013a; Bache et al. 2012). Further studies could lead to the beneficial application of 405 nm light for the decontamination of air, surfaces and equipment in healthcare settings, as well as in other indoor locations, where transmission of viral pathogens is a significant occurrence.

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Antimicrobial effect of blue light using Porphyromonas gingivalis pigment.

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Abstract

The development of antibiotics cannot keep up with the speed of resistance acquired by microorganisms. Recently, the development of **antimicrobial** photodynamic therapy (aPDT) has been a necessary **antimicrobial** strategy against antibiotic resistance. Among the wide variety of bacteria found in the oral **flora**, Porphyromonas gingivalis (P. gingivalis) is one of the etiological **agents** of periodontal disease. aPDT has been studied for periodontal disease, but has risks of cytotoxicity to **normal** stained tissue. In this study, we performed aPDT using protoporphyrin IX (PpIX), an intracellular pigment of P. gingivalis, without an external photosensitizer. We confirmed singlet oxygen generation by PpIX in a **blue-light** irradiation intensity-dependent manner. We discovered that **blue-light** irradiation on P. gingivalis is potentially bactericidal. The sterilization mechanism seems to be oxidative DNA damage in bacterial cells. Although it is said that no resistant bacteria will emerge using aPDT, the conventional method relies on an added photosensitizer dye. PpIX in P. gingivalis is used in energy production, so aPDT applied to PpIX of P. gingivalis should limit the appearance of resistant bacteria. This approach not only has potential as an effective treatment for new periodontal diseases, but also offers potential antibacterial treatment for multiple drug resistant bacteria.

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The effects of 405 nm light on bacterial membrane integrity determined by salt and bile tolerance assays, leakage of UV-absorbing material and SYTOX green labelling.

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Abstract

Bacterial inactivation by 405 nm light is accredited to the photoexcitation of intracellular porphyrin molecules resulting in energy transfer and the generation of reactive oxygen species that impart cellular oxidative damage. The specific mechanism of cellular damage, however, is not fully understood. Previous work has suggested that destruction of nucleic acids may be responsible for inactivation; however, microscopic imaging has suggested membrane damage as a major constituent of cellular inactivation. This study investigates the membrane integrity of Escherichia coli and Staphylococcus aureus exposed to 405 nm light. Results indicated membrane damage to both species, with loss of salt and bile tolerance by S. aureus and E. coli, respectively, consistent with reduced membrane integrity. Increased nucleic acid release was also demonstrated in 405 nm light-exposed cells, with up to 50 % increase in DNA concentration into the extracellular media in the case of both organisms. SYTOX green fluorometric analysis, however, demonstrated contradictory results between the two test species. With E. coli, increasing permeation of SYTOX green was observed following increased exposure, with >500 % increase in fluorescence, whereas no increase was observed with S. aureus. Overall, this study has provided good evidence that 405 nm light exposure causes loss of bacterial membrane integrity in E. coli, but the results with S.

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Assessment of the potential for resistance to antimicrobial violet-blue light in Staphylococcus aureus.

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Abstract

BACKGROUND: **Antimicrobial** violet-**blue light** in the region of 405 nm is emerging as an alternative technology for hospital decontamination and clinical treatment. The mechanism of action is the excitation of endogenous porphyrins within exposed microorganisms, resulting in ROS generation, oxidative damage and cell death. Although resistance to 405 nm **light** is not thought likely, little evidence has been published to support this. This study was designed to establish if there is potential for tolerance development, using the nosocomial pathogen *Staphylococcus aureus* as the model organism.

METHODS: The first stage of this study investigated the potential for *S. aureus* to develop tolerance to high-intensity 405 nm **light** if pre-cultured in low-level stress violet-**blue light** ($\leq$1 mW/cm^2) conditions. Secondly, the potential for tolerance development in bacteria subjected to repeated sub-lethal exposure was compared by carrying out 15 cycles of exposure to high-intensity 405 nm **light**, using a sub-lethal dose of 108 J/cm^2. Inactivation kinetics and antibiotic susceptibility were also compared.

RESULTS: When cultured in low-level violet-**blue light** conditions, *S. aureus* required a greater dose of high-intensity 405 nm **light** for complete inactivation, however this did not increase with multiple (3) low-stress cultivations. Repeated sub-lethal exposures indicated no evidence of bacterial tolerance to 405 nm **light**. After 15 sub-lethal exposures 1.2 and 1.4 $\log_{10}$ reductions were achieved for MSSA and MRSA respectively, which were not significantly different to the initial 1.3 $\log_{10}$ reductions achieved (P = 0.242 & 0.116, respectively). Antibiotic susceptibility was unaffected,

with the maximum change in zone of inhibition being ± 2 mm.

CONCLUSIONS: Repeated sub-lethal exposure of non-proliferating *S. aureus* populations did not affect the susceptibility of the organism to 405 nm **light**, nor to antibiotics. Culture in low-level violet-**blue light** prior to 405 nm **light** exposure may increase oxidative stress responses in *S. aureus*, however, inactivation still occurs and results demonstrate that this is unlikely to be a selective process. These results demonstrate that tolerance from repeated exposure is unlikely to occur, and further supports the potential development of 405 nm **light** for clinical decontamination and treatment applications.

KEYWORDS: 405 nm **light**; Bacterial resistance; Bacterial tolerance; EMRSA-15; Staphylococcus aureus

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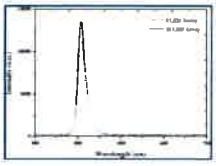

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Recent Pat Antiinfect Drug Discov. 2017 Nov 7. doi: 10.2174/1872213X11666171108104104. [Epub ahe

Recent Patents on Light-Based Anti-Infective Approaches.

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Abstract

BACKGROUND: Antibiotic resistance is one of the most serious health threats to modern medicine. The lack of potent antibiotics puts us at a disadvantage in the fight against infectious diseases, especially those caused by antibiotic-resistant microbial strains. To this end, an urgent need to search for alternative **antimicrobial** approaches has arisen. In the last decade, **light**-based therapy has made significant strides in this fight to combat antibiotic resistance among various microbial strains. This method includes utilizing **antimicrobial blue light**, **antimicrobial** photodynamic therapy, and germicidal ultraviolet irradiation, among others. **Light**-based therapy is advantageous over traditional antibiotic-based therapy in that it selectively eradicates microbial cells without harming human cells and tissues.

METHODS: This review highlights the patents on **light**-based therapy that were filed approximately within the last decade and are dedicated to eradicating pathogenic microbes.

RESULTS: The treatments and devices discussed in this review are interestingly enough to be used in various different functions and settings, such as dental applications, certain diseases in the eye, skin and hard surface cleansing, decontamination of internal organs (e.g., the stomach), decontamination of apparel and equipment, eradication of pathogenic microbes from buildings and rooms, etc. Most of the devices and inventions introduce methods of destroying pathogenic bacteria and fungi without harming human cells.

CONCLUSIONS: **Light**-based **antimicrobial** approaches hold great promise for the future in regards to treating antibiotic-resistant infections and diseases.

Antimicrobial Blue Light Therapy for Infectious Keratitis: Ex Vivo and In Vivo Studies

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Purpose. To investigate the effectiveness of antimicrobial blue light (aBL) as an alternative or adjunctive therapeutic for infectious keratitis.

Methods. We developed an ex vivo rabbit model and an in vivo mouse model of infectious keratitis. A bioluminescent strain of *Pseudomonas aeruginosa* was used as the causative pathogen, allowing noninvasive monitoring of the extent of infection in real time via bioluminescence imaging. Quantitation of bacterial luminescence was correlated to colony-forming units (CFU). Using the ex vivo and in vivo models, the effectiveness of aBL (415 nm) for the treatment of keratitis was evaluated as a function of radiant exposure when aBL was delivered at 6 or 24 hours after bacterial inoculation. The aBL exposures calculated to reach the retina were compared to the American National Standards Institute standards to estimate aBL retinal safety.

Results. *Pseudomonas aeruginosa* keratitis fully developed in both the ex vivo and in vivo models at 24 hours post inoculation. Bacterial luminescence in the infected corneas correlated linearly to CFU ($R^2 = 0.921$). Bacterial burden in the infected corneas was rapidly and significantly reduced (>2-$\log_{10}$) both ex vivo and in vivo after a single exposure of aBL. Recurrence of infection was observed in the aBL-treated mice at 24 hours after aBL exposure. The aBL toxicity to the retina is largely dependent on the aBL transmission of the cornea.

Conclusions. Antimicrobial blue light is a potential alternative or adjunctive therapeutic for infectious keratitis. Further studies of corneal and retinal safety using large animal models, in which the ocular anatomies are similar to that of humans, are warranted.

Keywords: antimicrobial blue light, keratitis, *Pseudomonas aeruginosa*, mouse model, rabbit model, bioluminescence imaging

Infectious keratitis is a potentially blinding ocular condition of the cornea. According to a recent report released by the U.S. Centers for Disease Control and Prevention (CDC), each year in the United States there are approximately 1 million clinical visits for keratitis, translating into an estimated total cost of $175 million per year.[1] The risk factors for infectious keratitis include ocular trauma, contact lens wear, recent ocular surgery, preexisting ocular surface disease, dry eyes, lid deformity, corneal sensational impairment, chronic use of topical steroids, and systemic immunosuppression.[2] The common causative pathogens of infectious keratitis are *Pseudomonas aeruginosa*,[2–5] *Staphylococcus aureus*,[2,5–7] *Streptococcus pneumonia*,[8,9] and *Fusarium solani*.[10,11] Treatment of infectious keratitis must be rapidly instituted to minimize the destruction of corneal tissue, limit the extent of corneal scarring, and prevent vision loss. The current standard of care for the treatment of infectious keratitis is the use of topical or systemic antibiotics.[12,13] However, the clinical management of keratitis has been significantly complicated by the increasing emergence of multidrug-resistant pathogens.[2–8,11,14] Pathogens replicate rapidly, and a mutation that helps a pathogen survive in the presence of antibiotic(s) will quickly become predominant throughout the microbial population, rendering infections that cannot be treated with available antibiotics. There is, consequently, a pressing need for the development of alternative treatment regimens to tackle drug resistance in infectious keratitis.

A novel light-based antimicrobial approach, antimicrobial blue light (aBL), has attracted increasing attention due to its intrinsic antimicrobial effect without the involvement of exogenous photosensitizers.[15–17] It also appears that pathogens are less able to develop resistance to aBL than to traditional antibiotics due to the multitarget characteristic of aBL.[15,18] The mechanism of action of aBL is still not fully understood. A common hypothesis is that aBL excites the naturally occurring endogenous porphyrins or/and flavins in microbial cells and subsequently leads to the production of cytotoxic reactive oxygen species (ROS).[15] The transparency of the cornea and

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Low-dose blue light irradiation enhances the antimicrobial activities of curcumin against Propionibacterium acnes.

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Abstract

Propionibacterium acnes (P. acnes) is an opportunistic infection in human skin that causes acne vulgaris. Antibiotic **agents** provide the effective eradication of microbes until the development of drug-resistant microbes. Photodynamic inactivation (PDI) is a non-antibiotic therapy for microbial eradication. In this study, the visible **blue light** (BL, λ_{max}=462nm) was used to enhance the **antimicrobial** activities of curcumin, a natural phenolic compound. Individual exposure to curcumin or BL irradiation does not generate cytotoxicity on P. acnes. The viability of P. acnes was decreased significantly in 0.09J/cm^2 BL with 1.52μM of curcumin. Furthermore, the low-dose **blue light** irradiation triggers a series of cytotoxic actions of curcumin on P. acnes. The lethal factors of photolytic curcumin were investigated based on the morphology of P. acnes by SEM and fluorescent images. The membrane disruption of microbes was observed on the PDI against P. acnes. Chromatography and mass spectrometry techniques were also used to identify the photolytic metabolites. Curcumin could be photolysed into vanillin through BL irradiation, which presents a strong linear relationship in quantitation. Because the safety of **blue light** in mammalian cell has been proven, the photolytic curcumin treatment could support non-antibiotic therapy to eradicate P. acnes on clinical dermatology.

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Characterizing the Antimicrobial Properties of 405 nm Light and the Corning® Light-Diffusing Fiber Delivery System.

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Abstract

BACKGROUND AND OBJECTIVES: Hospital-acquired infections (HAIs) and multidrug resistant bacteria pose a significant threat to the U.S. healthcare system. With a dearth of new antibiotic approvals, novel **antimicrobial** strategies are required to help solve this problem. Violet-**blue** visible **light** (400-470 nm) has been shown to elicit strong **antimicrobial** effects toward many pathogens, including representatives of the ESKAPE bacterial pathogens, which have a high propensity to cause HAIs. However, phototherapeutic solutions to prevention or treating infections are currently limited by efficient and nonobtrusive **light**-delivery mechanisms.

STUDY DESIGN/MATERIALS AND METHODS: Here, we investigate the in vitro **antimicrobial** properties of flexible Corning® **light**-diffusing fiber (LDF) toward members of the ESKAPE pathogens in a variety of growth states and in the context of biological materials. Bacteria were grown on agar surfaces, in liquid culture and on abiotic surfaces. We also explored the effects of 405 nm **light** within the presence of lung surfactant, human serum, and on eukaryotic cells. Pathogens tested include Enterococcus spp, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp., Staphylococcus epidermidis, Streptococcus pyogenes, Candida albicans, and Escherichia coli.

RESULTS: Overall, the LDF delivery of 405 nm violet-**blue light** exerted a significant degree of microbicidal activity against a wide range of pathogens under diverse experimental conditions.

CONCLUSIONS: The results exemplify the fiber's promise as a non-traditional approach for the prevention and/or therapeutic intervention of HAIs. Lasers Surg. Med. © 2019 The Authors. Lasers

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Pulsed 450nm blue light suppresses MRSA and Propionibacterium acnes in planktonic cultures and bacterial biofilms.

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Abstract

In our recent study, we showed that pulsed **blue light** (PBL) suppresses the growth of Propionibacterium acnes more than continuous wave (CW) **blue light** in vitro, but it is not known that other bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA), respond similarly to PBL. The high potency of PBL relative to CW **blue light** makes it a suitable **antimicrobial** for suppressing bacterial growth in biofilms as well. Therefore, we determined if MRSA-a deadly bacterium of global concern-is susceptible to 450nm PBL irradiation in vitro, and ascertained whether the bactericidal effect of PBL on planktonic P. acnes culture can be replicated in biofilms of P. acnes and MRSA. In three series of experiments, we irradiated P. acnes and MRSA respectively, either in planktonic cultures, forming biofilms or formed biofilms. Compared to controls, the results showed 100% bacterial suppression in planktonic cultures of MRSA irradiated with 3mW/cm^2 irradiance and 7.6J/cm^2 radiant exposure three times at 30-minute intervals, and also in P. acnes cultures irradiated with 2mW/cm^2 irradiance 5J/cm^2 radiant exposure thrice daily during each of 3days. Irradiation of biofilms with the same irradiances and radiant exposures that gave 100% bacterial suppression in planktonic cultures resulted in disruption and disassembly of the architecture of MRSA and P. acnes biofilms, more so in forming biofilms than formed biofilms. The **antimicrobial** effect on each bacterium was minimal in forming biofilms, and even less in formed biofilms. Increasing radiant exposure slightly from 7.6J/cm^2 to 10.8J/cm^2 without changing any other parameter, yielded more disruption of the biofilm and fewer live MRSA and P. acnes,

suggesting that 100% bacterial suppression is possible with further refinement of the protocol. In both planktonic cultures and biofilms, PBL suppressed MRSA more than P. acnes.

KEYWORDS: Antimicrobial therapy; Biofilm; Methicillin-resistant Staphylococcus aureus; PBM; Printed LEDs; Propionibacterium acnes

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Sci Transl Med. 2014 May 28;6(238):238ra69. doi: 10.1126/scitranslmed.3008234.

Photoactivation of endogenous latent transforming growth factor-β1 directs dental stem cell differentiation for regeneration.

Arany PR[1], Cho A[2], Hunt TD[3], Sidhu G[3], Shin K[4], Hahm E[3], Huang GX[3], Weaver J[5], Chen AC[6], Padwa BL[7], Hamblin MR[8], Barcellos-Hoff MH[9], Kulkarni AB[2], J Mooney D[10].

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Abstract

Rapid advancements in the field of stem cell biology have led to many current efforts to exploit stem cells as therapeutic agents in regenerative **medicine**. However, current ex vivo cell manipulations common to most regenerative approaches create a variety of technical and regulatory hurdles to their clinical translation, and even simpler approaches that use exogenous factors to differentiate tissue-resident stem cells carry significant off-target side effects. We show that non-ionizing, low-power **laser** (LPL) treatment can instead be used as a minimally invasive tool to activate an endogenous latent growth factor complex, transforming growth factor-β1 (TGF-β1), that subsequently differentiates host stem cells to promote tissue regeneration. LPL treatment induced reactive oxygen species (ROS) in a dose-dependent manner, which, in turn, activated latent TGF-β1 (LTGF-β1) via a specific methionine residue (at position 253 on LAP). **Laser**-activated TGF-β1 was capable of differentiating human dental stem cells in vitro. Further, an in vivo pulp capping model in rat teeth demonstrated significant increase in dentin regeneration after LPL treatment. These in vivo effects were abrogated in TGF-β receptor II (TGF-βRII) conditional knockout (DSPP(Cre)TGF-βRII(fl/fl)) mice or when wild-type mice were given a TGF-βRI inhibitor. These findings indicate a pivotal role for TGF-β in mediating LPL-induced dental tissue regeneration. More broadly, this work outlines a mechanistic basis for harnessing resident stem cells with a light-activated endogenous cue for clinical regenerative applications.

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Mesenchymal Stem Cells Synergize with 635, 532, and 405 nm Laser Wavelengths in Renal Fibrosis: A Pilot Study.

O'Connor M[1], **Patil R**[1], Yu J[1], Hickey R[1], Premanand K[1], Kajdacsy-Balla A[2], Benedetti E[1,3], Bartholomew A[1].

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Abstract

OBJECTIVE: To address whether a single treatment of one of three visible light wavelengths, 635, 532, and 405 nm (constant wave, energy density 2.9 J/m^2), could affect the hallmarks of established renal fibrosis and whether these wavelengths could facilitate mesenchymal stem cell (MSC) beneficence.

BACKGROUND DATA: Chronic kidney disease is a global health problem with only 20% receiving care worldwide. Kidneys with compromised function have ongoing inflammation, including increased oxidative stress and apoptosis, peritubular capillary loss, tubular atrophy, and tubulointerstitial fibrosis. Promising studies have highlighted the significant potential of MSC-based strategies to mitigate fibrosis; however, reversal of established fibrosis has been problematic, suggesting that methods to potentiate MSC effects require further development. Laser treatments at visible wavelengths have been reported to enhance mitochondrial potential and available cellular ATP, facilitate proliferation, and inhibit apoptosis. We hypothesized that laser-delivered energy might provide wavelength-specific effects in the fibrotic kidney and enhance MSC responses.

MATERIALS AND METHODS: Renal fibrosis, established in C57BL6 mice following 21 days of unilateral ureter obstruction (UUO), was treated with one of three wavelengths alone or with autologous MSC. Mitochondrial activity, cell proliferation, apoptosis, and cytokines were measured 24 h later.

RESULTS: Wavelengths 405, 532, and 635 nm all significantly synergized with MSC to enhance mitochondrial activity and reduce apoptosis. Proliferative activity was observed in the renal cortices following combined treatment with the 532 nm laser and MSC; endothelial proliferation increased in

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J Hosp Infect. 2014 Sep;88(1):1-11. doi: 10.1016/j.jhin.2014.06.004. Epub 2014 Jul 3.

405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infection control.

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Abstract

BACKGROUND: Although the germicidal properties of ultraviolet (UV) light have long been known, it is only comparatively recently that the antimicrobial properties of visible violet-blue 405 nm light have been discovered and used for environmental disinfection and infection control applications.

AIM: To review the antimicrobial properties of 405 nm light and to describe its application as an environmental decontamination technology with particular reference to disinfection of the hospital environment.

METHODS: Extensive literature searches for relevant scientific papers and reports.

FINDINGS: A large body of scientific evidence is now available that provides underpinning knowledge of the 405 nm light-induced photodynamic inactivation process involved in the destruction of a wide range of prokaryotic and eukaryotic microbial species, including resistant forms such as bacterial and fungal spores. For practical application, a high-intensity narrow-spectrum light environmental disinfection system (HINS-light EDS) has been developed and tested in hospital isolation rooms. The trial results have demonstrated that this 405 nm light system can provide continuous disinfection of air and exposed surfaces in occupied areas of the hospital, thereby substantially enhancing standard cleaning and infection control procedures.

CONCLUSION: Violet-blue light, particularly 405 nm light, has significant antimicrobial properties against a wide range of bacterial and fungal pathogens and, although germicidal efficacy is lower than UV light, this limitation is offset by its facility for safe, continuous use in occupied

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Toxicol In Vitro. 2016 Jun;33:54-62. doi: 10.1016/j.tiv.2016.02.011. Epub 2016 Feb 23.

Cytotoxic responses to 405nm light exposure in mammalian and bacterial cells: Involvement of reactive oxygen species.

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Abstract

Light at wavelength 405 nm is an effective bactericide. Previous studies showed that exposing mammalian cells to 405 nm light at 36 J/cm(2) (a bactericidal dose) had no significant effect on normal cell function, although at higher doses (54 J/cm(2)), mammalian cell death became evident. This research demonstrates that mammalian and bacterial cell toxicity induced by 405 nm light exposure is accompanied by reactive oxygen species production, as detected by generation of fluorescence from 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate. As indicators of the resulting oxidative stress in mammalian cells, a decrease in intracellular reduced glutathione content and a corresponding increase in the efflux of oxidised glutathione were observed from 405 nm light treated cells. The mammalian cells were significantly protected from dying at 54 J/cm(2) in the presence of catalase, which detoxifies H2O2. Bacterial cells were significantly protected by sodium pyruvate (H2O2 scavenger) and by a combination of free radical scavengers (sodium pyruvate, dimethyl thiourea (OH scavenger) and catalase) at 162 and 324 J/cm(2). Results therefore suggested that the cytotoxic mechanism of 405 nm light in mammalian cells and bacteria could be oxidative stress involving predominantly H2O2 generation, with other ROS contributing to

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ELSEVIER FULL-TEXT ARTICLE

Drug Resist Updat. 2017 Nov;33-35:1-22. doi: 10.1016/j.drup.2017.10.002. Epub 2017 Oct 13.

Antimicrobial blue light inactivation of pathogenic microbes: State of the art.

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Abstract

As an innovative non-antibiotic approach, **antimicrobial blue light** in the spectrum of 400-470nm has demonstrated its intrinsic **antimicrobial** properties resulting from the presence of endogenous photosensitizing chromophores in pathogenic microbes and, subsequently, its promise as a counteracter of antibiotic resistance. Since we published our last review of **antimicrobial blue light** in 2012, there have been a substantial number of new studies reported in this area. Here we provide an updated overview of the findings from the new studies over the past 5 years, including the efficacy of **antimicrobial blue light** inactivation of different microbes, its mechanism of action,

Mitochondrial Medicine
Mitochondrial Metabolism, Diseases, Diagnosis and Therapy

Anna Gvozijakova, editor
Medical Faculty, Comenius University,Bratislava, Slovakia

Springer
2008

"Polarized Light"

Chapter 22
Jan Palinkas and Alfonz Smola

"The actual development of polarized light application started after implementation of laser therapy in the 1960s of the 20th century,"

"Energy of photons as the smallest parts of light waves depends indirectly on the wavelength.

Photons with lower wavelengths have more energy than those with longer wavelengths.

So, photons of UV radiation are richer in energy than photons of visible light.

Photons of blue light have more energy than those of red light."

Lasers work through a "biostimulation effect."

The energy of red color "has the greatest ability of biostimulation."

"Red stimulates when there is lack of energy."

"With monochromatic polarized light of red color we can irradiate inflammations from the very beginning."

"Blue color: 400-490 nm. The light of this color has calming effects—blue is considered to be a cold color. It slows down pulse frequencies, helps the overloaded vessels get into normal state, acts as an antiseptic, kills pain and cools."

"For children, it is one of the best healing colors."

"Blue is very effective in combination with red."