Inactivation of micro-organisms isolated from infected lower limb arthroplasties using high-intensity narrow-spectrum (HINS) light

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Abstract

High-Intensity Narrow-Spectrum (HINS) light is a novel blue light inactivation technology which kills bacteria through a photodynamic process, and is proven to have bactericidal activity against a wide range of species. Specimens from hip and knee arthroplasty infections were collected over a one year period. A range of these microbial isolates were tested for sensitivity to HINS-light. During testing, suspensions of the pathogens were exposed to increasing doses of HINS-light (of 123mW/cm^2 irradiance). Non-light exposed control samples were also set-up. The samples were then plated onto agar plates and incubated at 37°C for 24 hours before enumeration.

Complete inactivation (greater than 4-log reduction) was achieved for all of the clinical isolates from infected arthroplasty cases. The typical inactivation curve showed a slow initial reaction followed by a period of rapid inactivation. The doses of HINS-light exposure required ranged from 118 – 2214 J/cm^2 respectively. Gram-positive bacteria were generally found to be more susceptible than Gram-negative.

As HINS-light utilises visible-light wavelengths it can be safely used in the presence of patients and staff. This unique feature could lead to possible applications such as use as an infection prevention tool during surgery and post-operative dressing changes.

Introduction

In England and Wales[1] there were over 186,000 hip and knee arthroplasty operations performed in 2013, with a further 14,000 such procedures performed in Scotland the previous year[2]. Prosthetic infection is a major but infrequent complication of the surgery, with a relatively unchanged incidence in recent years of between 0.6% and 2% per joint per year[3-5]. Revision of infected implants is associated with substantial morbidity and has significant economic implications. Any new developments in reducing the burden of prosthetic joint infection are welcome.

The potential of antimicrobial light technologies have previously been considered. The absorption of ultraviolet (UV) light photons (wavelengths 100-400 nm) leads to photobiochemical reactions that cause damage to nucleic acids, with a single photon having the potential to induce a lethal effect[6,7]. However, this effect is often non-discriminatory, and UV light has recognised significant adverse side effects including dermatological (neoplasms) and ophthalmological (cataracts) conditions[8]. As a result of these safety issues, individuals cannot be directly exposed to UV light disinfection technologies.

HINS-light is a narrow band of visible blue light, with peak wavelength of 405 (±5) nm that has been developed by researchers in The Robertson Trust Laboratory for Electronic Sterilisation Technologies (ROLEST) at the University of Strathclyde. The technology induces inactivation of a range of bacterial pathogens via a photodynamic inactivation effect which is triggered by absorption of the light. This leads to photo-excitation of endogenous porphyrins and the production of reactive

oxygen species, primarily singlet oxygen. The subsequent result of this is bacterial cell death[9]. A series of studies by the ROLEST team have proven the germicidal efficacy of HINS-light against a range of bacteria in a variety of laboratory and clinical settings[10-13]. The purpose of this study was to investigate if HINS-light technology could successfully kill microorganisms isolated from cases of infected lower limb arthroplasty.

Materials and methods

Microbiological Methodology

Isolates from clinically relevant arthroplasty infections were prospectively gathered over a one year period. At the Southern General Hospital microbiology lab, when a positive culture was identified, the isolate was transferred onto Microbank™ beads (ProLab Diagnostics) containing cryopreservative solution for storage at -70°C in the hospital freezer. The isolates for testing for susceptibility to HINS-light were then transferred to ROLEST. There were a total of 51 positive cultures (Table I).

For recovery of the microbial isolate, an inoculated Microbank bead was removed under aseptic conditions and streaked onto an agar plate. The agar medium of choice was selected dependent on the organism being cultured (Table II). This streaked plate was then incubated at 37°C for 18 hours. The purity of the isolates was verified by Gram staining and visual identification under the microscope.

In order to culture a microbial strain for experimental use, a loopful of organism from the agar slope (stored at 4°C) was aseptically extracted and inoculated into 100ml broth using a sterile wire loop. The broth was then incubated to provide a population

of approximately 10^9 colony-forming units per millilitre (CFU/ml). The inoculated broth was then centrifuged and serially diluted to the population density required for testing (approximately 10^5 CFU/ml). The diluted solutions produced were clear, with no turbidity.

All clinical isolates were successfully cultured with the exception of the *Streptococcus* isolates. Several attempts to culture for experimental use proved unsuccessful; therefore the effect of HINS-light on streptococcal inactivation could not be observed in this study. In addition, other CNS were not tested for their sensitivity to HINS-light, as the species were not defined. This study aimed to test a microorganism from each genus and/or species highlighted in Table II. The bacterial and yeast strains selected for testing are listed (Table III), along with the respective non-identifiable patient background data.

HINS-light source

Light-emitting diodes (LED) provide a much higher intensity light emission when used as arrays rather than single units. A 405 nm 99-DIE LED array (Opto Diode Corp., California, USA) was used in this study. This is composed of 99 LEDs (9 x 11 rectangle) closely packed. This array has an area of $3.2cm^2$ (2 x 1.6cm) and is powered by a DC supply (HQ POWER) with a controllable output in the range 0 – 3 A and 0 – 15 V. It emits violet light across a narrow spectral region and is made from indium-gallium-nitride. The 405 nm LED array has a centre wavelength (maximum emission) close to 405 nm, and the bandwidth is ~10 nm at full-width half-maximum (Fig. 1). Though LEDs are known to have minimal heat dissipation, a heatsink and cooling fan were attached to the LED array as a precautionary measure. This ensured

that a stable temperature around the LED array was maintained throughout testing. The LED array unit was mounted in PVC housing, and this unit was used for all microbial suspension experiments.

Experimental arrangement for exposure of microbial suspensions

A Gilson pipette and sterile tip was used to transfer 3ml of the liquid bacterial sample to the central well within a 12-well multidish (Nunc, Denmark). A 7mm x 2mm magnetic follower (Fisher Scientific) was added to the sample. The multidish was then placed onto a magnetic stirrer (Yellowline MSH Basic), ensuring continuous mechanical agitation of the sample during light exposure. The 99-DIE LED array, fan and PVC housing unit were then placed directly over the well containing the suspension to be exposed. The underside of the housing unit had an outer edge that fitted around the multidish, ensuring it was firmly held in place. The HINS light input current was set at 1A (+/- 0.05), with a voltage of 11.5V (+/- 0.25) leading to irradiance from the LED array of 123 mW/cm^2. The distance between the sample and the LED array was approximately 2cm, thus keeping the light intensity constant for all exposures. The complete HINS-light exposure set-up with all components can be seen in Figure 2.

A control sample was set-up for each test. This was a bacterial suspension held in the well of a multidish which was mechanically agitated for the same period of time as the test sample under normal laboratory light conditions, but not exposed to 405nm light. Following exposure at the various time settings, samples were plated onto agar plates (the type dependent on the organism being tested – Table II) using a WASP 2

spiral plater (Don Whitley Scientific). The plates were then incubated at 37°C for 24 hours before enumeration. All of the experiments were repeated at least once.

Results

Complete inactivation was achieved for all of the clinical isolates from infected arthroplasty cases. A graph comparing the inactivation kinetics for all the Gram-positive bacteria exposed to 405nm HINS-light is shown in Figure 3. With the exception of *E. faecalis,* all of the Gram positive bacteria were successfully inactivated in under an hour, with an approximate 5 $\log_{10}$ reduction achieved in each case. *S. epidermidis* was the quickest to be completely inactivated in 16 minutes, followed closely by *S. aureus* which took 20 minutes. *E. faecalis* was the slowest to be inactivated, taking around 120 minutes.

The inactivation times for the Gram-negative organisms were clearly longer than those for the Gram-positive bacteria (Fig. 4). The longest time required for inactivation was 5 hours of 405 nm light exposure; and this was for *E.coli*. The other enterobacteriaceae, namely *K. pneumoniae* and *S. marcescens*, required slightly shorter exposure times of 3 and 4 hours respectively for complete inactivation. *P. aeruginosa* was the last Gram-negative organism tested, and was found to be inactivated quickest, over the course of 90 minutes. *C. albicans* was the sole yeast isolate exposed to HINS-light, and a clear 4 $\log_{10}$ reduction was apparent after 45 minutes (Fig. 5). The control line was unremarkable and flat throughout, similar to what was seen with all of the other control samples.

When the irradiance and exposure time of the HINS-light applied to the microorganisms is known, the dose applied for complete inactivation may be calculated based on the formula:

E (energy or dose) = **P** (power or irradiance) x **t** (time, in seconds)

Based on the experimental results for the HINS-light exposure of microbial suspensions, the inactivation capability of the 405nm HINS-light can be quantified. This is known as the germicidal efficiency (GE). This can be defined as the $\log_{10}$ reduction of a given microbial population by inactivation per unit of light energy density in J/cm^2, also known as the dose[14]. This calculation can be shown as:

Germicidal Efficiency, $\eta = \log_{10}(N/N_0)$ per J/cm^2

Table IV provides a summary of the GE of the 405 nm light emitted from the 99-DIE LED array for all the pathogens tested.

Discussion

New advances to aid in the prevention or management of prosthetic joint infection are welcome. HINS-light is a novel visible blue light technology which achieves the inactivation of pathogens via a photodynamic inactivation effect, which is triggered by absorption of the light and is mediated by porphyrins[12]. In this study, the sensitivity of clinical isolates from hip and knee arthroplasty infections to HINS-light

was examined. Pathogens that could be cultured and identified were exposed to HINS-light, with all inactivated using 405 nm light. *S. epidermidis* was the bacteria inactivated in the shortest period of time, 16 minutes. This Gram-positive bacterium required the lowest dose of all those tested, and as a result had the highest GE. *E. coli* took the longest period of time to achieve complete inactivation, 300 minutes. This Gram-negative bacterium therefore required the highest dose of all those tested, and in turn had the lowest GE. Murdoch et al[11] found the GE of *E. coli* O157:H7 exposed to 405 nm light to be 0.02, ten times greater efficiency than what we noted. This may be due to differences in the exact nature of the light source used in each study, leading to variations in the emission and peak wavelengths from each source. Alternatively, this may simply be a reflection on the different bacterial strains examined in the separate studies.

The Gram-positive bacteria were generally noted to require lower doses of HINS-light exposure to achieve inactivation in comparison to the Gram-negative bacteria. This was similar to what has previously been published in 2009 by Maclean et al[10]. The authors of that paper suggested that Gram-positive bacteria produce greater quantities of porphyrins, and specifically coproporphyrin, making them more readily inactivated by visible light than Gram-negative bacteria. The enteric bacteria were noted to be the least responsive to HINS-light. This may represent inherent resilience from their natural gut habitat. The only yeast tested was *C. albicans*, and it was seen to behave in a manner more similar to the Gram-positive bacteria than Gram-negative, with respect to inactivation dosages and GE.

The suspension inactivation curves for the bacteria followed a similar shape. There was an initial period of inactivity, followed by a rapid acceleration until complete inactivation. The distinct inactivation times observed may be explained by the fact that different bacteria produce different porphyrins, the peak absorption wavelengths are therefore likely to vary, and in turn different wavelengths may be required for optimum photostimulation[10].

Differing mechanisms of bacterial inactivation between blue light (porphyrins mediated) and UV light (DNA damage) are emphasised when our work is compared to the landmark publication of Chang et al[15]. The authors conducted a series of experiments using a collimated beam of UV light set at 254 nm and found that Gram-negative bacteria were more susceptible than Gram-positive bacteria. This is the opposite of the results with HINS-light thus far. Furthermore, the study[15] reports that the dosage of UV light required for a 5-$\log_{10}$ reduction of both *S. aureus* and *E. coli* was approximately 10 mW-sec/cm^2. Not only is this considerably less than any of the dosages required involving 405 nm blue light, but the dosage of UV light required for inactivation of both bacteria was similar. We acknowledge that visible light inactivation is much less efficient than UV-inactivation, but highlight the greater operational safety it offers. Furthermore, recent studies have shown that therapeutic doses of HINS-light does not adversely affect *in vitro* models of wound healing[16] and that the technology is potentially effective against biofilm[17].

This study provides further support for the anti-microbial properties of HINS-light, specifically addressing isolates from infected arthroplasty specimens. Future work assessing the efficacy of 405 nm light in the presence of blood and pus, as well as

further work on biofilm decontamination are planned. This would potentially help differentiate whether the light is useful for the treatment of infection, or only in prevention. Potential intraoperative roles for HINS-light include direct application to the wound or to the prosthesis prior to insertion, minimising implant associated infection. At present the technology is being used for environmental decontamination of isolation rooms in the Canniesburn Plastic Surgery Unit[13]. Pending further development of the technology, HINS-light may help prevent infection in orthopaedic operating theatres and beyond.

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Table I *Positive cultures grown from infected lower limb arthroplasty over one year from the Southern General Hospital Department of Microbiology*

Gram Stain Result	Microorganism	Positive cultures n = 51
+	*Staphylococcus aureus*	23
+	*Coagulase negative Staphylococcus*	6
+	*Staphylococcus epidermidis*	2
+	*Corynebacterium striatum*	2
+	*Enterococcus* species	1
+	*Enterococcus faecalis*	1
+	*Micrococcus* species	1
+	*Streptococcus* species	1
+	Group B *Streptococcus*	1
+	*Streptococcus pneumoniae*	1
-	*Escherichia coli*	4
-	*Klebsiella pneumoniae*	4
-	*Pseudomonas aeruginosa*	2
-	*Serratia marcescens*	1
N/A	*Candida albicans*	1

Table II *The bacterial and yeast strains selected for experimental use listed along with the appropriate culture medium*

Microorganism	**Growth Media**
Staphylococcus aureus	Nutrient Broth & Agar
Staphylococcus epidermidis	Tryptone soya Broth & Agar
Corynebacterium striatum	Brain Heart Infusion Broth & Blood Agar
Enterococcus faecalis	Nutrient Broth & Agar
Micrococcus species	Nutrient Broth & Agar
Streptococcus species	Brain Heart Infusion Broth & Blood Agar
Escherichia coli	Nutrient Broth & Agar
Klebsiella pneumoniae	Nutrient Broth & Agar
Serratia marcescens	Nutrient Broth & Agar
Pseudomonas aeruginosa	Nutrient Broth & Agar
Candida albicans	Malt extract Broth & Agar

Table III *Details of the bacterial and yeast isolates from infected THR/TKR selected for experimental use*

Microbial Isolate	**Patient Age (Years)**	**Gender**	**Source**
Staphylococcus aureus	67	Male	Right knee tissue
Staphylococcus epidermidis	68	Male	Right knee swab
Corynebacterium striatum	75	Female	Right hip tissue
Enterococcus faecalis	68	Male	Right knee tissue
Micrococcus species	80	Female	Left hip tissue
Streptococcus species	57	Female	Left knee tissue
Escherichia coli	71	Male	Right knee swab
Klebsiella pneumoniae	81	Male	Left hip swab
Serratia marcescens	64	Male	Right hip aspiration
Pseudomonas aeruginosa	62	Male	Right knee swab
Candida albicans	79	Female	Left hip swab

Table IV *Germicidal efficiency of 99-DIE LED array against pathogens isolated*

Microorganism	Exposure Time (s)	Power Density (mW/cm^2)	Dose (J/cm^2)	Log_{10} Reduction	Germicidal Efficiency ($log_{10}(N/N_0)$ / J/cm^2)
S. aureus	1200	123	147.6	5.16	0.0350
S. epidermidis	960	123	118.1	5.12	0.0434
C. striatum	1800	123	221.4	5.02	0.0227
E. faecalis	7200	123	885.6	4.72	0.0053
Micrococcus	3600	123	442.8	4.85	0.0110
E. coli	18000	123	2214	5.01	0.0023
K. pneumoniae	10800	123	1328.4	5.05	0.0038
S. marcescens	14400	123	1771.2	5.26	0.0030
P. aeruginosa	5400	123	664.2	5.00	0.0075
C. albicans	2700	123	332.1	4.52	0.0136

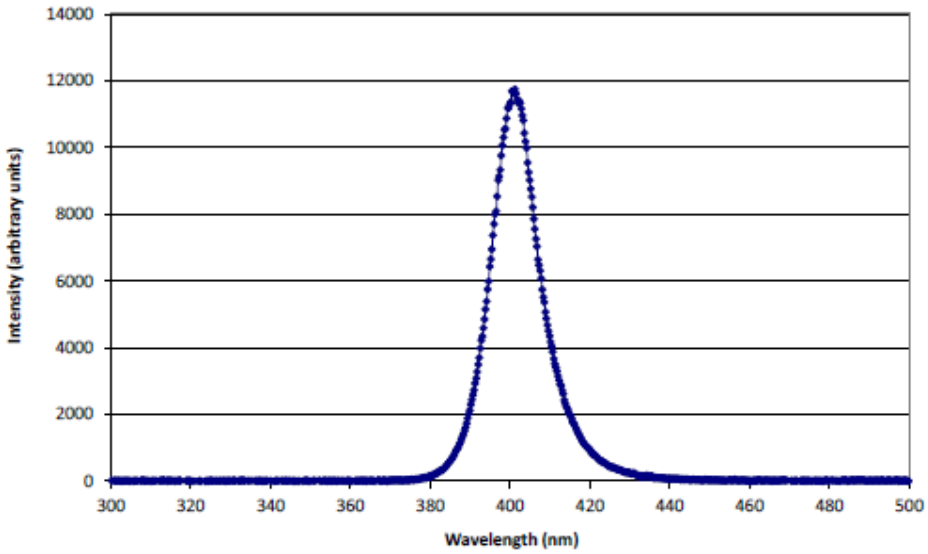

Figure 1 Emission spectrum of the 99-DIE 405 nm light emitting diode array

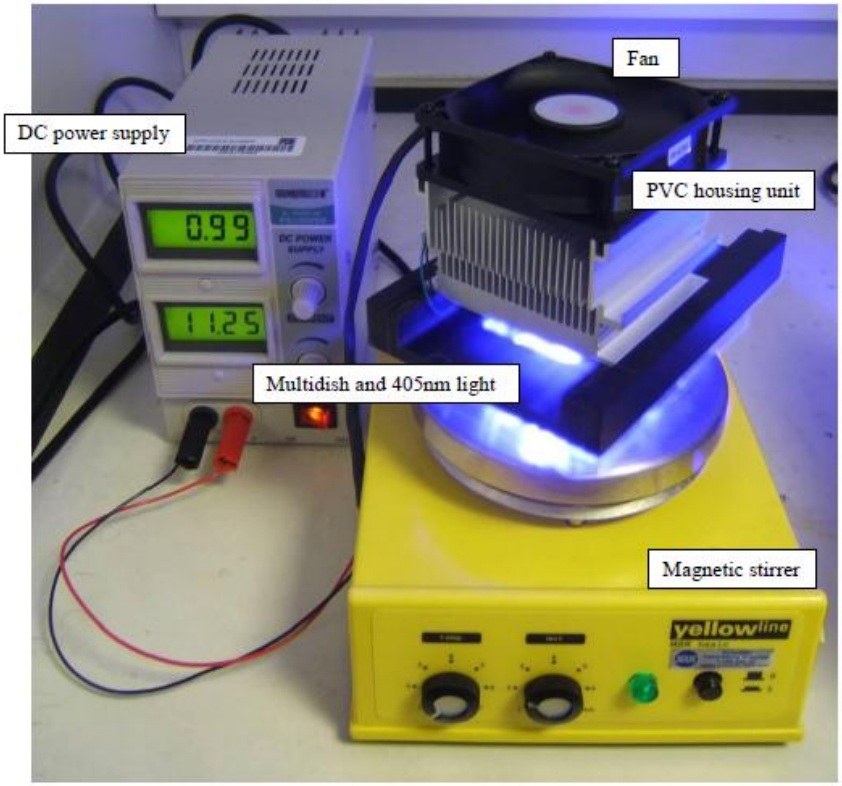

Figure 2 High-intensity narrow-spectrum light treatment system for exposure of bacterial suspensions

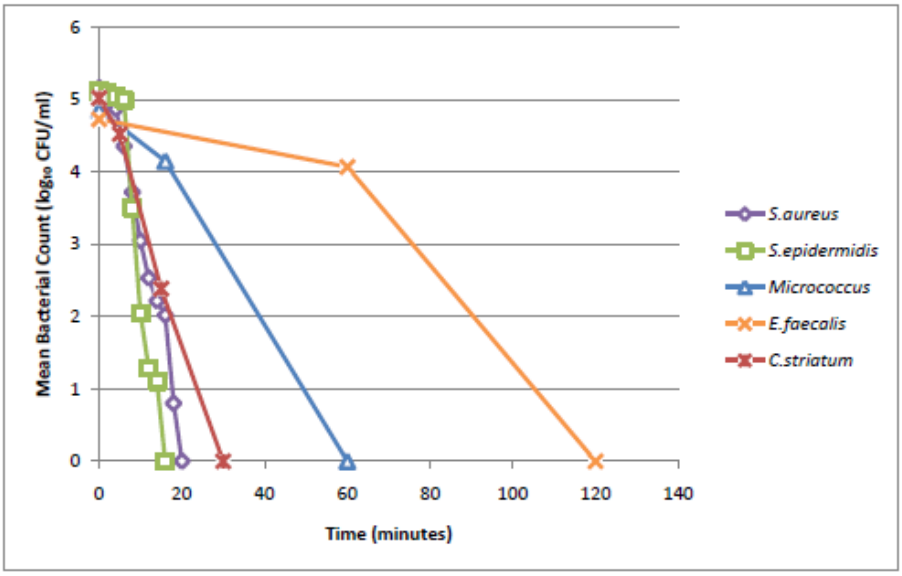

Figure 3 Comparison of the kinetics for High-intensity narrow-spectrum light inactivation of the Gram-positive bacterial isolates in suspension (123 mW/cm^2 irradiance).

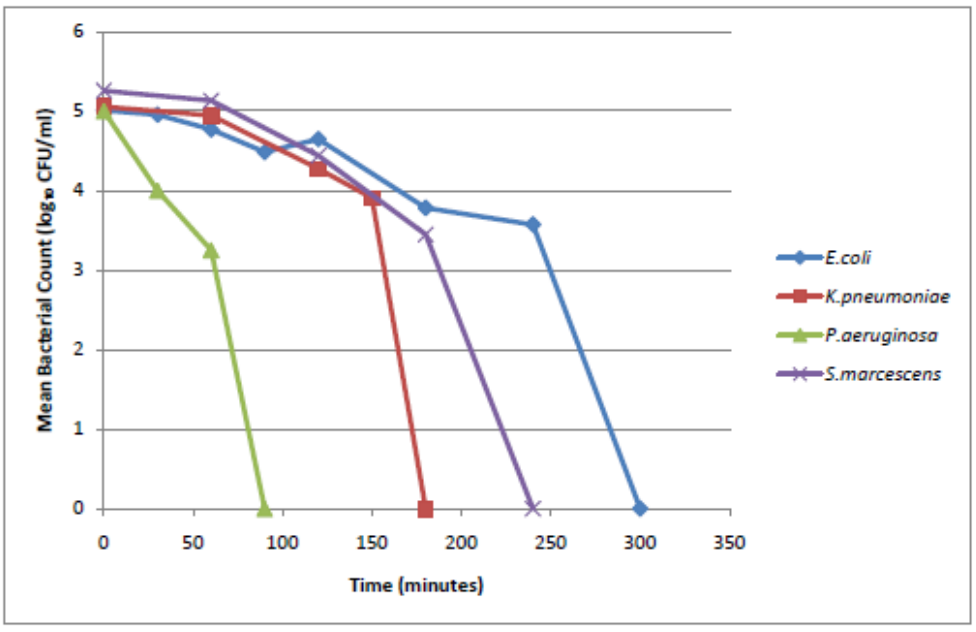

Figure 4 Comparison of the kinetics for High-intensity narrow-spectrum light inactivation of the Gram-negative bacterial isolates in suspension (123 mW/cm^2 irradiance).

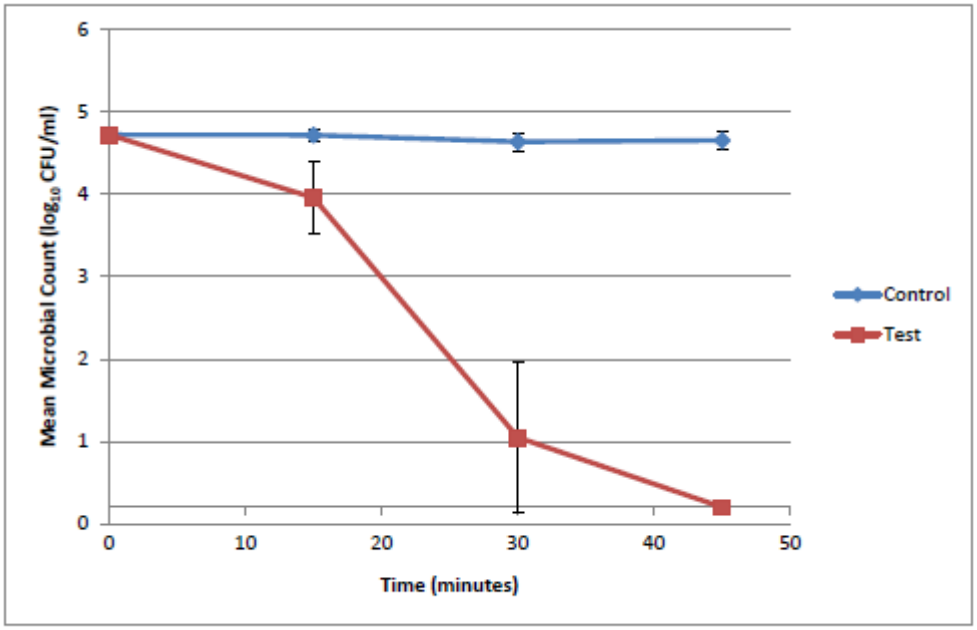

Figure 5 Inactivation of *Candida albicans* in suspension by High-intensity narrow-spectrum light exposure (123 mW/cm^2 irradiance).