Differential sensitivity of osteoblasts and bacterial pathogens to 405-nm light highlighting potential for decontamination applications in orthopedic surgery

Praveen Ramakrishnan
Michelle Maclean
Scott J. MacGregor
John G. Anderson
M. Helen Grant

SPIE.

Differential sensitivity of osteoblasts and bacterial pathogens to 405-nm light highlighting potential for decontamination applications in orthopedic surgery

Praveen Ramakrishnan,[a] Michelle Maclean,[b] Scott J. MacGregor,[b] John G. Anderson,[b] and M. Helen Grant[a,*]
[a]University of Strathclyde, Department of Biomedical Engineering, Wolfson Centre, 106 Rottenrow, Glasgow, Scotland G4 0NW, United Kingdom
[b]University of Strathclyde, The Robertson Trust Laboratory for Electronic Sterilisation Technologies, 204 George Street, Glasgow, Scotland G1 1XW, United Kingdom

Abstract. Healthcare associated infections pose a major threat to patients admitted to hospitals and infection rates following orthopedic arthroplasty surgery are as high as 4%. A 405-nm high-intensity narrow spectrum light has been proven to reduce environmental contamination in hospital isolation rooms, and there is potential to develop this technology for application in arthroplasty surgery. Cultured rat osteoblasts were exposed to varying light intensities and it was found that exposures of up to a dose of 36 J/cm² had no significant effect on cell viability [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay], function (alkaline phosphatase activity), and proliferation rate (BrdU cell proliferation assay). High irradiance exposures (54 J/cm²) significantly affected the cell viability indicating that the effects of 405-nm light on osteoblasts are dose dependent. Additionally, exposure of a variety of clinically related bacteria to a dose of 36 J/cm² resulted in up to 100% kill. These results demonstrating the differential sensitivity of osteoblasts and bacteria to 405-nm light are an essential step toward developing the technique for decontamination in orthopedic surgery. © 2014 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.19.10.105001]

1 Introduction

Healthcare associated infection (HAI) affects approximately 10% of patients admitted to the hospital, and is responsible for over 5000 deaths in the UK annually (Improving Patient Care by Reducing the Risk of Hospital Acquired Infection: A Progress Report, National Audit Office 2004). HAI can be caused by airborne and environmental bacterial contamination which can be transmitted to surgical site wounds either directly from the environment or indirectly via vectors such as healthcare workers' hands.[1,2] A 405-nm high-intensity narrow spectrum (HINS) light has antimicrobial activity against HAI-related bacterial pathogens including methicillin-resistant *Staphylococcus aureus*[3] and, due to the use of visible light wavelengths, this does not pose the same health concerns as those associated with using ultraviolet light. This 405-nm light technology has already been used to develop an environmental decontamination system for the disinfection of air and environmental surfaces, which has been successfully clinically evaluated within isolation rooms at Glasgow Royal Infirmary.[4]

Infection rates following orthopedic arthroplasty surgery are as high as 4%, while the infection rates are even higher after revision surgery,[5] and the environment and surgical devices have been highlighted as sources of bacterial contamination.[6]

The bactericidal properties of 405-nm light suggest that this may aid in reducing the incidence of infections that arise from environmental contamination during arthroplasty surgery. The toxic effect of 405-nm light on bacterial cells is not replicated to the same extent in mammalian cells, and this has been successfully demonstrated in previous studies.[7,8]

If 405-nm light technology was to be promoted for localized disinfection during arthroplasty surgery, the bone would likely be exposed to 405-nm light during the course of surgery and any inhibitory effects on the function of osteoblasts may affect the integration of the orthopedic implant into the bone postsurgery. It is therefore essential to consider the duration of an orthopedic surgical procedure and establish the effects of 405-nm light on osteoblast function during this potential exposure. Discussion with surgeons has revealed that the duration of routine arthroplasty surgeries was between 1 and 2 h, although a complex revision may take up to 4 h. Previous studies on fibroblasts for wound healing have shown that 405-nm light had no significant effect on cell viability when exposed to a dose of 18 J/cm² (5 mW/cm² for 1 h), while a substantial decline in cell viability was observed at a dose of 54 J/cm² (15 mW/cm² for 1 h).[7] It has also been found that exposing osteoblasts to 405-nm light with a dose of 54 J/cm² (5 mW/cm² for 3 h) had a detrimental effect on cell viability.[9] Hence, the aim of this study was to establish whether the adverse effects of 405-nm light seen on mammalian cells were dose dependent, to determine a damage threshold of exposure of osteoblasts to 405-nm light, and to find out whether doses below the damage threshold can exert a bactericidal effect on clinically relevant pathogenic bacteria. Demonstration of these key parameters would be an essential step toward assessing whether this novel technology could be

---

*Address all correspondence to: M. Helen Grant, E-mail: m.h.grant@strath.ac.uk

developed and applied for localized decontamination of the patient environment during arthroplasty surgeries.

2 Methods and Materials

2.1 Mammalian Cell Study

Immortalized osteoblast (OST 5) cells isolated by SV40 transfection of neonatal rat calvarial osteoblasts were chosen for use in the experiments. The cells were cultured as monolayers using Dulbecco's modified Eagle's medium (DMEM) containing 10% v/v fetal calf serum in an atmosphere of 5% CO_2 in air at 37°C.

The 405-nm light system used in this study consisted of an array of nine 405-nm light-emitting diodes (LEDs) (GE Illumination), with a full-width half-maximum of 20 nm. The LEDs were arranged in a 3×3 grid pattern (6 cm × 6 cm), and attached to a heat sink for thermal management, thus preventing sample heating during light exposure. The heat sink was supported by two pillars above a molded base designed to fit a multiplate in order to fix the position of the treatment plates directly below the LEDs. The light source was set at a height of 8 cm above the multiwell plate. The middle 4 wells in a 24-well plate and the middle 16 wells in a 96-well plate were used for cell exposures. Cells were seeded at a seeding density of 5×10^3 cells/cm^2 in a 24-well plate and 2×10^4 cells/cm^2 in a 96-well plate and left in the incubator overnight at 37°C.

To investigate the dose-dependent effects of 405-nm light on the osteoblasts, the samples were exposed to light in 1 ml Dulbecco's phosphate-buffered saline (DPBS), inside an incubator at 37°C and 5% CO_2, at a low dose of 18J/cm^2 and a high dose of 54 J/cm^2 using varying irradiance/exposure regimes (Table 1). Dose (J/cm^2) is calculated according to the equation:

$$E' = P't, \tag{1}$$

where E' is the dose (energy density) in J/cm^2, P' is the irradiance (power density) in W/cm^2, and t is the time in seconds.

Unexposed controls were treated in the same way. After the stipulated exposure time, the DPBS solution was removed, 1 ml

Table 1 Irradiance levels and exposure times used to apply low dose (18 J/cm^2) and high dose (54 J/cm^2) 405-nm light to osteoblasts.

Period of exposure (minutes)	Irradiances (mW/cm^2)	Dose (J/cm^2)
Low Dose exposures		
60	5	18
120	2.5	18
180	1.7	18
240	1.25	18
High Dose exposures		
60	15	54
120	7.5	54
180	5	54
240	3.75	54

of DMEM was added to the wells, and the cells were incubated for 48 or 72 h before assessing the cell population using crystal violet staining and Lowry protein assay.[10]

To establish a damage threshold for osteoblast cells, samples were exposed to 405-nm light at an irradiance of 5 mW/cm^2 in 1 ml DPBS for increasing time periods (60, 90, 120, and 150 min corresponding to doses of 18, 27, 36, and 45 J/cm^2) inside an incubator at 37°C and 5% CO_2. Unexposed controls were treated in the same way. After the stipulated exposure time, the DPBS was removed, 1 ml DMEM was added to wells, and the cells were incubated for 48 or 72 h before carrying out the following assays.

To measure cell viability, the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was carried out as described by Ho et al.[11] Cell function was measured using the alkaline phosphatase (ALP) assay as described by McDonald et al.[7] The bromodeoxyuridine (BrdU) assay was carried out to measure the cell proliferation rate postlight exposure using QIA 58 BrdU cell proliferation assay kit (Calbiochem—Merckmillipore) as described by the manufacturer.

Live/dead staining of the cells was carried out using acridine orange (AO; live cells stain green) and propidium iodide (PI; dead cells stain red). The cells were stained with phalloidin-fluorescein isothiocyanate (FITC) (green stain for actin; Sigma-Aldrich Company Ltd., UK)/4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) (blue nuclear stain; Life technologies, Paisley, Scotland, UK) to study the cytoskeletal actin structures of cells.

For microscopy, cells were seeded on glass coverslips (13-mm diameter) at 5×10^3/cm^2 and viewed under a Zeiss AxioImager Z1 fluorescent microscope using a 20× wet lens (NA = 0.5).

2.2 Bacterial Study

The bacteria used in this study were *S. aureus* NCTC 4135, *Escherichia coli* NCTC 9001, *Pseudomonas aeruginosa* LMG 9009, *Klebsiella pneumoniae* NCTC 09633, *Staphylococcus epidermidis* LMG 10474, and *Acinetobacter baumannii* LMG 1041 (NCTC, National Collection of Type Cultures, Collindale, UK; LMG, Laboratorium voor Microbiologie, Universiteit Gent, Belgium). Bacteria were cultured in nutrient broth (Oxoid, UK) at 37°C for 18 h under rotary conditions (120 rpm). Broths were centrifuged at 4300 rpm for 10 min, and the cell pellet was resuspended in PBS. The cell suspension was then diluted in PBS to obtain a concentration of 10^3 colony forming units (CFU)/ml. Samples (100 μl) were then spread onto 55 mm agar plates, with approximately 150 to 300 CFU/plate, and seeded agar plates were then exposed to 405-nm light at an irradiance of 5 mW/cm^2 for increasing time periods (15, 30, 60, 90, and 120 min corresponding to doses of 4.5, 9, 18, 27, and 36 J/cm^2). Postexposure, plates were incubated at 37°C for 24 h before enumeration of the viable CFU/plate. Results are reported as % surviving CFU/plate as compared to nonexposed control samples.

3 Results and Discussion

Data in Fig. 1 show that exposure of cells to 18 J/cm^2 405-nm light using different irradiance/exposure period regimes caused no significant difference in the crystal violet staining or protein concentration between the control and the treated samples after 48- and 72-h posttreatment periods. In contrast, a significant difference was evident between the control and the 405 nm
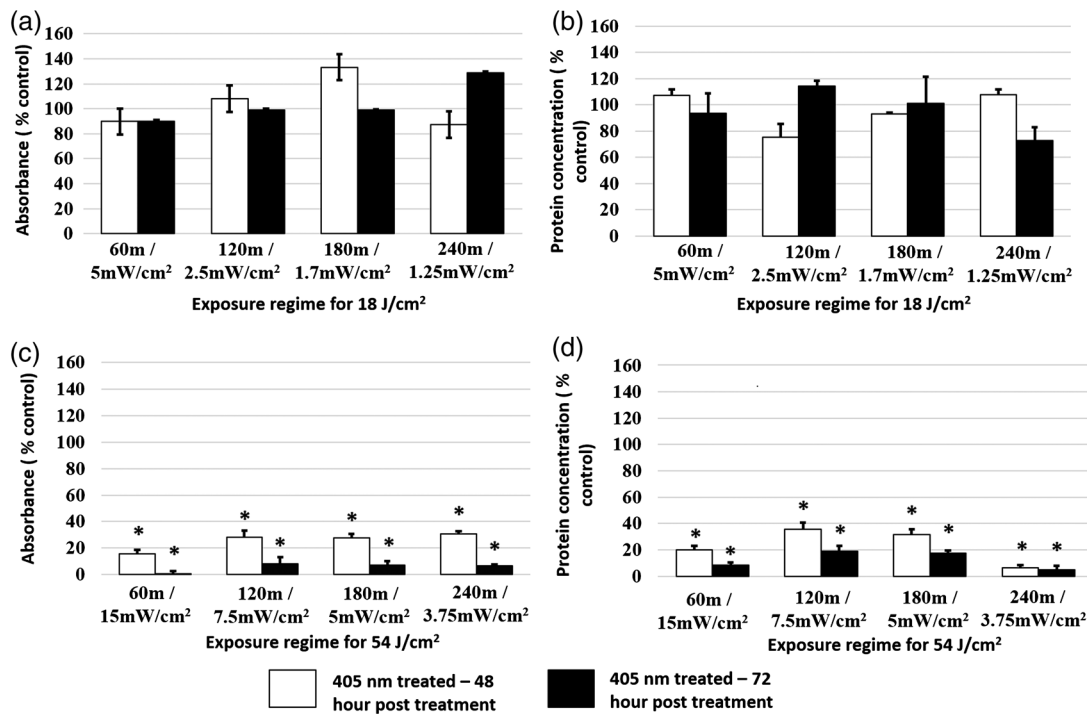
Fig. 1 Crystal violet staining and protein content of osteoblasts exposed to different doses of 405-nm light. (a) Crystal violet absorbance (% control), (b) protein concentration (% control) of rat osteoblasts exposed to 405-nm light at a low dose of 18 J/cm^2 and (c) crystal violet absorbance (% control), (d) protein concentration (% control) of rat osteoblasts exposed to a high dose of 54 J/cm^2. Doses were achieved using different irradiance/exposure time regimes, and were followed by either 48 or 72-h posttreatment periods. Results are means of $n = 8 \pm$ SEM. *Statistically different using unpaired Student's t-test comparing control and light treated samples, $p < 0.05$.

treated samples exposed to higher doses of 54 J/cm^2 irrespective of how the dose was administered. These results confirm that the effect of 405-nm light on mammalian cells is dose dependent.

Data in Fig. 2 show that cells exposed to doses of 18, 27, and 36 J/cm^2 (5 mW/cm^2 for 60, 90, and 120 min, respectively) exhibited no significant effect on the MTT reduction, the ALP activity or the cell proliferation rate as measured by the BrdU method. In contrast, cells exposed to doses above 36 J/cm^2 (5 mW/cm^2 for more than 120 min) showed a significant decrease in all the parameters of cell function.

AO and PI dyes were used in this study to differentiate live cells, apoptotic and necrotic cells and study the viability of osteoblasts after 48- and 72-h postlight exposure periods. All live nucleated cells fluoresce green, early apoptotic cells fluoresce bright green, late apoptotic cells fluoresce orange, and all dead nucleated cells fluoresce red. After both 48- and 72-h culture postexposure to light, more apoptotic and dead cells were observed following 150 min (45 J/cm^2) exposure compared to 120 min (36 J/cm^2) exposure to 5 mW/cm^2 405-nm light (Fig. 3), confirming the findings with MTT. Staining of the actin with phalloidin-FITC showed that after exposure to 45 J/cm^2 light, the actin formed a ring around the cell membrane, and more cells rearranged themselves into a circular shape, when compared with the samples exposed to 36 J/cm^2 for both 48- and 72-h posttreatment period (Fig. 4). Accumulation of actin around the outer-cell perimeter is indicative of membranes being strengthened to prevent leakage of the cell contents and is a sign of apoptosis. At 48 h in culture postexposure to light, the cells show early apoptosis [Fig. 3(b)], but

this damage appears to be repaired at 72 h in culture postexposure to light [Fig. 3(f)]. Exposure to 405-nm light may indeed cause sublethal damage to the cells, and further studies such as assessment of deoxyribose nucleic acid (DNA) integrity will be required.

The mammalian cell studies suggest that cell exposures to 405-nm HINS light for up to a dose of 36 J/cm^2 cause no observable effects on the cell viability, function, proliferation rate, and morphology using the techniques employed in this study. When investigating the comparative effects of 405-nm light exposure on bacterial cells, results demonstrate that doses up to 36 J/cm^2, which were found to have no impact on the mammalian cells, induce significant bactericidal effects. Results in Fig. 5 highlight the inactivation kinetics of a range of Gram-positive (*S. aureus, S. epidermidis*) and Gram-negative (*P. aeruginosa, A. baumannii, E. coli, K. pneumoniae*) bacteria. The susceptibility of the organisms varied with *A. baumannii* proving most susceptible with >98% kill achieved after exposure to 4.5 J/cm^2 ($p < 0.001$). Significant inactivation of all organisms was achieved by exposure to 18 J/cm^2 and between 99.5% to 100% inactivation of all species was shown after application of 36 J/cm^2. A previously published study reported the antibacterial effects of 405-nm light against these clinically relevant organisms, demonstrating their susceptibility at population densities of up to 9-$\log_{10}$ CFU/ml in liquid suspension;[3] however, the present study uses light doses and surface-seeded bacterial contaminants at population densities (~10^2 CFU/plate) that are more relevant to practical clinical environmental decontamination applications,[4] as has been demonstrated in a previous work.[9] The present study confirms, by
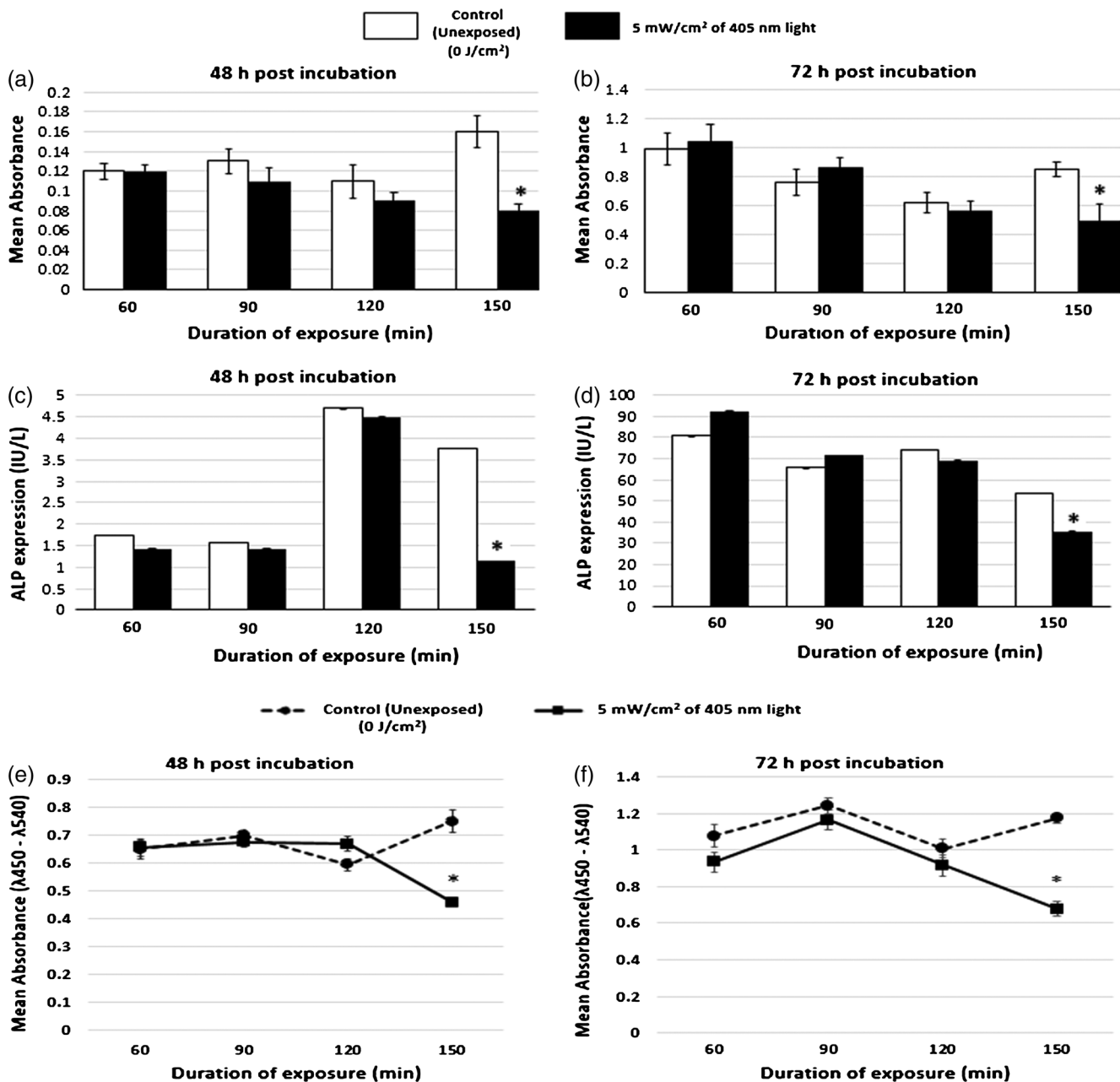
Fig. 2 MTT reduction, ALP activity, and cell proliferation in osteoblasts exposed to different doses of 405-nm light. (a) and (b) MTT reduction (mean absorbance $\pm$ SEM, $n = 8$), (c) and (d) ALP activity (IU/L $\pm$ SEM, $n = 8$), and (e) and (f) BrdU absorbance (mean $\pm$ SEM, $n = 8$) of rat osteoblasts exposed to 405-nm light doses of 18, 27, 36, and 45 J/cm^2 (5 mW/cm^2 for 60, 90, 120, and 150 min, respectively) followed by 48- (left) and 72-h (right) posttreatment period. *Statistically different, using unpaired Student's t-test comparing control and light treated samples, $p < 0.05$.

direct comparison, the selective detrimental effect that light of this wavelength can have on infection-causing organisms in comparison to osteoblasts.

The antibacterial effect of 405-nm light is not thought to be mediated through direct DNA crosslinking, unlike that of UV light.[12] Instead, the 405 nm violet-blue light inactivation effect is considered to be caused by the excitation of intracellular photosensitive porphyrin molecules, which results in the production of reactive oxygen species (ROS), inducing oxidative damage and, consequently, cell death.[3,13–15] Indeed, previous studies have detected endogenous porphyrins within *S. aureus*,[15] *P. aeruginosa*,[14] and *A. baumannii*,[16] and implicated these violet-blue

light sensitive molecules in the inactivation mechanism. In addition, a study by Lipovsky et al.[15] compared the sensitivity of two strains of *S. aureus* and found that the more light-sensitive strain contained 10-fold more endogenous porphyrins than the more resilient strain. Further evidence of the involvement of porphyrins in the inactivation process was provided by Nitzan et al., who demonstrated that bacterial susceptibility to violet-blue light inactivation can be increased when exogenous δ-aminolevulinic acid (ALA), the precursor of the porphyrin biosynthesis pathway, is added in order to amplify the levels of endogenous porphyrins within the bacterial cells.[15–18] Since ROS are considered to cause widespread oxidative damage to bacterial cells and the
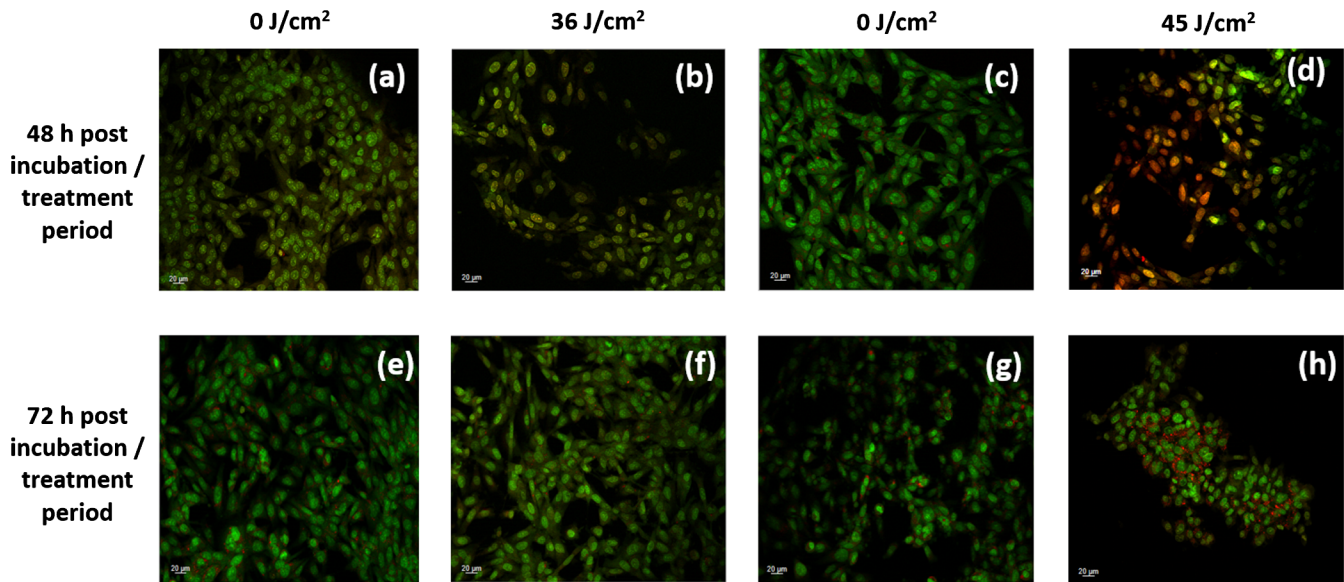
0 J/cm² 36 J/cm² 0 J/cm² 45 J/cm²

Fig. 3 The cell viability of osteoblast cells after exposure to 5 mW/cm² of 405-nm light. [(a) and (c) Control (0 J/cm² for 120 and 150 min, respectively for a 48-h postincubation period), (e) and (g) control (0 J/cm² for 120 and 150 min, respectively for a 72-h postincubation period). (b) and (d) Exposure for 120 min (36 J/cm²) and 150 min (45 J/cm²), respectively, for a 48-h posttreatment period and (f) and (h) exposure for 120 min (36 J/cm²) and 150 min (45 J/cm²), respectively, for a 72-h posttreatment period).] Acridine orange stains live cells green, early and late apoptotic cells bright green and orange, respectively, and propidium iodide stains dead cells red.

mechanism of action is not site specific, as is the case with many antibiotics, this explains not only the broad spectrum antimicrobial efficacy of 405-nm light but also why highly antibiotic resistant species such as *Acinetobacter baumanii* show no greater resistance to 405-nm light than nonantibiotic resistant bacteria. Bacterial cells have also been shown to have much greater sensitivity than mammalian cells to violet-blue light

in the region of 405 nm.[9,14] Low levels of antioxidant defence enzymes in certain bacteria have been implicated as a possible reason for this,[19] and as such are more susceptible to ROS mediated oxidative damage. In contrast, most mammalian cells are well equipped to survive in high oxygen conditions, and contain ample cytoprotective mechanisms such as superoxide dismutase, catalase, and glutathione peroxidise.[19]

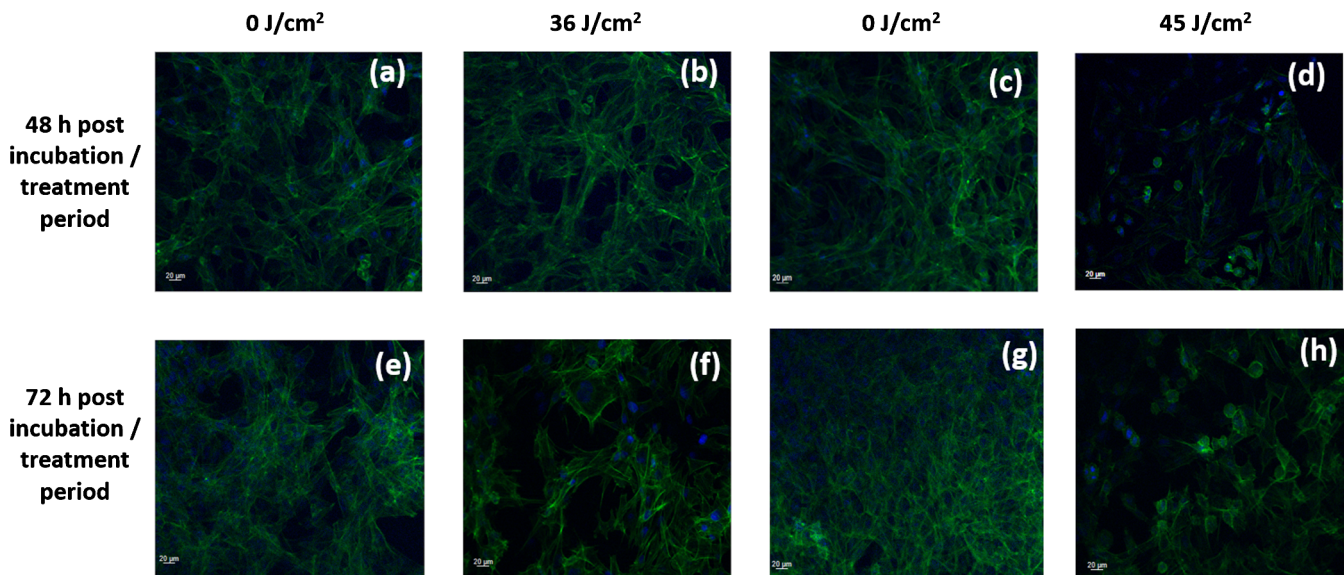0 J/cm² 36 J/cm² 0 J/cm² 45 J/cm²

Fig. 4 The cell morphology of osteoblast cells after exposure to 5 mW/cm² of 405-nm light. [(a) and (c) Control (0 J/cm² for 120 and 150 min, respectively, for a 48-h postincubation period), (e) and (g) control (0 J/cm² for 120 and 150 min, respectively, for a 72-h postincubation period). (b) and (d) exposure for 120 min (36 J/cm²) and 150 min (45 J/cm²), respectively, for a 48-h posttreatment period and (f) and (h) exposure for 120 min (36 J/cm²) and 150 min (45 J/cm²), respectively, for a 72-h posttreatment period).] Phalloidin-FITC stains the cytoskeleton green and DAPI stains the nucleus blue.
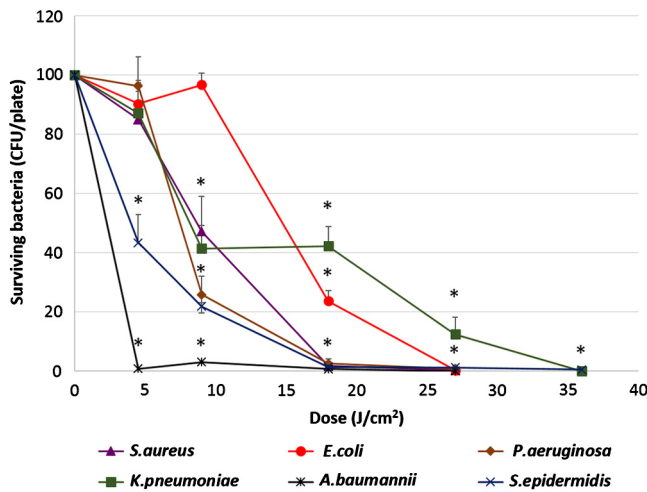
Fig. 5 Survival of a range of surface seeded clinically relevant bacterial pathogens exposed to 405-nm HINS light at doses ranging from 4.5 to 36 J/cm^2. Results are means of $n = 4 \pm$ SEM. *Statistically different, using unpaired Student's t-test comparing control and light treated samples, $p < 0.05$.

These findings thus highlight the potential for development of 405-nm light for inactivation of bacterial contaminants around surgical sites. Exposure to 405-nm light has also been proven to cause no adverse effects on wound healing,[7] thus providing further evidence for potential clinical application of this disinfection technology.

Importantly, the dose-dependent effects of 405-nm light on osteoblast cells suggest that the dose of 36 J/cm^2, which may be achieved by applying different irradiances for varying time periods (e.g., 10 mW/cm^2 for 1 h, 5 mW/cm^2 for 2 h, 3.3 mW/cm^2 for 3 h, and 2.5 mW/cm^2 for 4 h), does not cause detectable damage to mammalian cells, regardless of how the dose is applied. These findings are significant, and should provide a basis for the development of light sources which could be applied practically within operating theaters at levels appropriate for the typical operating times relevant to arthroplasty surgery.

Further safety advantages of light of this wavelength relate to the photon energy of 405-nm light. The depth of penetration of visible light into tissue increases with wavelength[20] and the penetrability of 405-nm light, which although greater than that of UV light, is low and will not penetrate deeply into bone; particularly when compared to light of a longer wavelength, e.g., 690-nm laser light.[21] Also, the 405-nm intensities suggested for use during surgical exposures would be low; therefore, theater personnel are unlikely to require protection from these visible violet-blue light wavelengths. This is unlike UV light which, due to the high photon energy and associated detrimental health effects, is normally only used in the absence of people. There have been limited attempts to utilize UV light during surgical procedures but these have required operating staff to wear protective clothing during exposure.[22,23]

4 Summary

A 405-nm HINS light has proven to have strong antimicrobial effects against a variety of medically significant bacteria at a dose level of 36 J/cm^2. Conversely, mammalian cell studies found that the osteoblasts appear healthy when exposed to doses up to 36 J/cm^2; at higher doses, there is a negative impact

on cell viability, function, and proliferation rate. The results obtained from the application of low dose and high dose light using a range of irradiance/exposure time regimes have demonstrated that the effects of 405-nm light on osteoblast cells are dose dependent. This study has established a dose-dependent level at which there is a differential sensitivity to the effects of 405-nm light between osteoblasts and bacteria; exposure of mammalian cells up to a dose of 36 J/cm^2 does not cause any observable effect on osteoblast viability. At this dose, the 405-nm light is still bactericidal indicating that there is potential to develop the technology for decontamination of the environment around patients during arthroplasty surgery.

References

1. M. Albrecht, R. Gauthier, and D. Leaper, "Forced-air warming: a source of airborne contamination in the operating room?," *Orthop. Rev. (Pavia)* **1**(2), 85–89 (2009).
2. R. W. Loftus et al., "Hand contamination of anesthesia providers is an important risk factor for intraoperative bacterial transmission," *Anesth. Analg.* **112**(1), 98–105 (2011).
3. M. Maclean et al., "Inactivation of bacterial pathogens following exposure to light from a 405-nm LED array," *Appl. Environ. Microbiol.* **75**(7), 1932–1937 (2009).
4. M. Maclean et al., "Environmental decontamination of a hospital isolation room using high-intensity narrow-spectrum light," *J. Hosp. Infect.* **76**(3), 247–251 (2010).
5. H. Hamilton and J. Jamieson, "Deep infection in total hip arthroplasty," *Can. J. Surg.* **51**(2), 111–117 (2008).
6. N. Davis et al., "Intraoperative bacterial contamination in operations for joint replacement," *J. Bone Joint Surg. Br.* **81**(5), 886–889 (1999).
7. R. McDonald et al., "Effect of 405 nm high-intensity narrow-spectrum light on fibroblast populated collagen lattices: an in vitro model of wound healing," *J. Biomed. Opt.* **16**(4), 048003 (2011).
8. S. Smith et al., "Exposure of 3T3 mouse fibroblasts and collagen to high intensity blue light," in *IFMBE Proc. 13th Int. Conf. on Biomedical Engineering*, Vol. 23, pp. 1352–1355, Springer, Berlin Heidelberg (2009).
9. R. McDonald et al., "405 nm light exposure of osteoblasts and inactivation of bacterial isolates from arthroplasty patients: potential for new disinfection applications?," *Eur. Cell Mater.* **25**, 204–214 (2013).
10. O. H. Lowry et al., "Protein measurement with the folin phenol reagent," *J. Biol. Chem.* **193**(1), 265–275 (1951).
11. G. Ho et al., "Low-level laser therapy on tissue-engineered skin substitutes: effect on the proliferation rate of 3T3 mouse fibroblast cells," *Proc. SPIE* **5610**, 124–134 (2004).
12. K. Oguma et al., "Determination of pyrimidine dimmers in Escherichia coli and Cryptosporidium parvum during UV light inactivation, photoreactivation, and dark repair," *Appl. Environ. Microbiol.* **67**(10), 4630–4637 (2001).
13. R. Lubart et al., "A possible mechanism for the bactericidal effect of visible light," *Laser Ther.* **20**(1), 17–22 (2011).
14. T. Dai et al., "Blue light rescues mice from potentially fatal Pseudomonas aeruginosa burn infection: efficacy, safety, and mechanism of action," *Antimicrob. Agents Chemother.* **57**(3), 1238–1245 (2013).
15. A. Lipovsky et al., "Visible light-induced killing of bacteria as a function of wavelength: implication for wound healing," *Lasers Surg. Med.* **42**(6), 467–472 (2010).
16. Y. Zhang et al., "Antimicrobial blue light therapy for multidrug-resistant Acinetobacter baumannii infection in a mouse burn model: implications for prophylaxis and treatment of combat-related wound infections," *J. Infect. Dis.* **209**(12), 1963–1971 (2014).

17. Y. Nitzan and M. Kauffman, "Endogenous porphyrin production in bacteria by δ-aminolaevulinic acid and subsequent bacterial photoeradication," *Lasers Med. Sci.* **14**(4), 269–277 (1999).
18. Y. Nitzan et al., "ALA induced photodynamic effects on Gram positive and negative bacteria," *Photochem. Photobiol. Sci.* **3**(5), 430–435 (2004).
19. B. Halliwell and J. M. C. Gutteridge, "Antioxidant defences," Chapter 3 in *Free Radicals in Biology and Medicine*, pp. 105–245, Oxford University Press, Oxford (2001).
20. E. Sternberg and D. Dolphin, "Porphyrin-based photosensitizers for use in photodynamic therapy," *Tetrahedron* **54**(17), 4151–4202 (1998).
21. S. K. Bisland and S. Burch, "Photodynamic therapy of diseased bone," *Photodiagn. Photodyn. Therapy* **3**(3), 147–155 (2006).
22. P. E. Gosden, A. P. MacGowan, and G. C. Bannister, "Importance of air quality and related factors in the prevention of infection in orthopaedic implant surgery," *J. Hosp. Infect.* **39**(3), 173–180 (1998).
23. G. J. S. Taylor, G. C. Bannister, and J. P. Leeming, "Wound disinfection with ultraviolet radiation," *J. Hosp. Infect.* **30**(2), 85–93 (1995).

Biographies of the authors are not available.