A new proof-of-concept in bacterial reduction: Antimicrobial action of violet-blue light (405 nm) in ex vivo stored plasma

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Running Heading: Decontamination of blood plasma using 405nm light

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ABSTRACT

Bacterial contamination of injectable stored biological fluids such as blood plasma and platelet concentrates preserved in plasma at room temperature is a major health-risk. Current pathogen-reduction technologies (PRT) rely on the use of chemicals and/or ultraviolet-light, which affects product quality and can be associated with adverse events in recipients. 405nm violet-blue light is antibacterial without the use of photosensitizers, and can be applied at levels safe for human exposure, making it of potential interest for decontamination of biological fluids such as plasma.

As a pilot study to test whether 405nm light is capable of inactivating bacteria in biological fluids, rabbit and human plasma were seeded with bacteria and treated with a 405nm light emitting diode (LED) exposure system (patent pending). Inactivation was achieved in all tested samples, ranging from low volumes to pre-bagged plasma. 99.9% reduction of low density bacterial populations ($\leq 10^3$ CFUml^{-1}), selected to represent typical 'natural' contamination levels, were achieved using doses of 144 Jcm^{-2}.

The penetrability of 405nm light, permitting decontamination of pre-bagged plasma, and the non-requirement for photosensitizing agents, provides a new proof-of-concept in bacterial reduction in biological fluids, especially injectable fluids relevant to transfusion medicine.

(Word count: 190)

KEY WORDS: Plasma; Decontamination; 405nm light; violet-blue light; transfusion bags; pathogen reduction technology; Bacteria; Contamination

INTRODUCTION

Bacterial contamination of ex vivo stored injectable biological fluids such as blood and blood components preserved in plasma is a major complication for transfusion medicine, resulting in both wasteful discarding of valuable blood products, and more significantly, health risks for recipients of contaminated donor blood [1,2]. Major progress has been made in the provision of a safe supply of blood components, and measures such as more effective donor screening, extensive laboratory testing protocols and the application of bacterial reduction methods have significantly reduced the risk of transfusion-transmitted bacterial infections [1-3]. Nevertheless, the risk of bacterial transmission has not been completely eliminated and there is a recognised need for continued research to improve the efficacy of these methods, and to minimise incidental adverse changes in biological fluids, such as cellular blood components preserved in plasma, that can compromise product quality and safety [4-6].

A number of bacterial reduction methods have been developed for plasma treatment, and pathogen-reduced plasma is routinely used [7], with several of these methods now licensed for use in North America and Europe [5]. The original methods developed for plasma treatment included the use of solvent/detergent, and methylene blue in combination with visible light [8-11]. More recently developed methods have employed ultraviolet (UV) light. Exposure to amotosalen (S-59) plus long-wave ultraviolet (UVA) light [12,13], and treatment with riboflavin and UV light [7,14], have been developed to treat both plasma and platelets. Whilst light-based processes have typically used photosensitive chemicals to generate microbicidal effects, a UVC-based pathogen reduction system without a requirement for photoactive substances has been developed and is undergoing clinical efficacy and safety testing [15-17].

It is generally accepted that all these methods have limitations [5,7], and because the full extent of future microbiological challenges cannot be predicted, pathogen reduction technologies will remain an active area of investigation in transfusion medicine well into the future [1,4].

Here we report the first proof-of-concept results on the use of a novel visible violet-blue light method that does not require the addition of photosensitive chemicals for inactivation of bacterial pathogens in plasma. This method utilises light with a peak wavelength of 405 nm, which causes photoexcitation of endogenous microbial porphyrin molecules and oxidative damage through reactive oxygen species [18]. 405 nm light has previously been shown to inactivate a wide range of bacterial pathogens in laboratory tests [19-28], as well as in hospital settings with use as an environmental disinfection technology [29-31], and also potential for wound decontamination applications in clinical settings [33-35]. An advantage of this technology over UV-light for certain applications is that, even at irradiance values and dose levels that are bactericidal, it can be applied safely for human exposure. Therefore, we envisioned that this feature makes 405 nm light of potential interest for decontamination of injectable stored biological fluids such as blood plasma or plasma containing cellular blood components. Tests on bacterial-seeded plasma were carried out on both small-scale liquid samples and artificially-contaminated pre-bagged plasma. Direct treatment of pre-bagged plasma was facilitated by the highly transmissible properties of 405 nm light, and the bacterial inactivation results obtained using this novel approach are described for the first time in this paper.

MATERIALS AND METHODS

Bacterial Cultures: The organisms used in this study were Staphylococcus aureus NCTC 4135, Staphylococcus epidermidis NCTC 11964 and Escherichia coli NCTC 9001. Cultures were obtained from the National Collection of Type Cultures (NCTC), Collindale, UK. For experimental use, bacteria were cultured in 100 ml nutrient broth at 37°C under rotary conditions (120 rpm) for 18-h. Broths were centrifuged at 3939 × g for 10 minutes and the pellet re-suspended in 100 ml phosphate buffered saline (PBS), and serially diluted to obtain the required cell density (colony-forming units per millilitre, CFU ml^{-1}) for experimental use. All culture media was sourced from Oxoid Ltd (UK).

Plasma: Lyophilised rabbit plasma (LRP020, E&O Laboratories, UK) was reconstituted using sterile distilled water. Fresh frozen human plasma (approximately 300 ml bag volume) was obtained from the Scottish National Blood Transfusion Service (SNBTS, UK), and defrosted before experimental use. Study involving human subjects protocol was approved by FDA Risk Involved in Human Subjects Committee (RIHSC, Exemption Approval # 11-036B) and by the University of Strathclyde Ethics Committee (letter dated February 10, 2011). Rabbit and human plasma suspensions were seeded with known concentrations of bacterial contaminants by adding bacterial-PBS suspension to the plasma.

405-nm light source: The 405 nm light sources used were rectangular arrays of 99-LEDs in an 11 × 9 matrix (Optodiode Corp, USA). The array had a centre wavelength close to 405 nm, with a bandwidth of approximately 10 nm at full-width half maximum (FWHM). The LED array was powered by a direct current supply, and for thermal management, the LED array was bonded to a heat sink and fan, thus ensuring that heating had no effect on the test samples exposed to the 405-nm light (Device patent pending [36]).

405 nm antimicrobial light treatment: Three arrangements were employed for exposure of three different sample volumes: 3 ml, 30 ml and approximately 300 ml (whole plasma transfusion bags). For exposure of 3 ml sample volumes, the samples were held in the well of a 12-well microplate (without the lid), and the LED array was mounted in a polyvinyl chloride (PVC) housing which positioned the array approx. 3 cm directly above the sample. Irradiance at the sample surface was measured to be approximately 100 $mWcm^{-2}$ (measured by using a radiant power meter and photodiode detector; L.O.T.-Oriel Ltd.).

For exposure of 30 ml sample volumes, the human plasma was held in a sterile 90 mm Petri dish with the lid on. The LED array was positioned 8 cm directly above the closed sample dish, providing an irradiance of approximately 8 $mWcm^{-2}$, through the lid, at the centre of the sample dish.

For exposure of plasma bags, a test rig was constructed which held two 405 nm LED arrays at a distance of 12 cm above the horizontally-positioned plasma bag. This arrangement provided an irradiance of approximately 5 $mWcm^{-2}$ at the centre position of the plasma bag, taking into account a 20% reduction in irradiance as the light transmits through the bag layer. In order to investigate the influence of higher irradiances on bacterial inactivation, plasma bags were also exposed using irradiances of 16 and 48 $mWcm^{-2}$. These higher irradiances were achieved by using two high-power 405 nm LED arrays (PhotonStar Technologies, UK), with 14 nm FWHM.

All experimental systems were held in a shaking incubator (72 rpm; 25°C) to allow continuous sample agitation and maintain exposure conditions. Samples seeded with bacterial contamination were treated with increasing exposures of 405 nm light. Control samples were held in identical conditions, but shielded from the 405 nm light.

The optical profiles of the light distribution across the Petri dishes and transfusion bags (plotted using MATLAB R2012b software) demonstrate the non-uniform irradiance of the plasma (Figs 2a, 3a), however, continuous agitation of the plasma samples during treatment ensure uniform mixing of the plasma contaminants. Negligible variation was recorded across the 22 mm Ø of the 3 ml samples.

Determination if Light Induces Toxicity within Human Plasma: To ensure that bacterial inactivation was not the result of the plasma becoming toxic upon exposure to 405 nm light, S. aureus (1×10^3 CFUml^{-1}) was seeded into 3 ml plasma that had been pre-exposed to 1.08 kJcm^{-2} 405 nm light at an irradiance of 100 mWcm^{-2} (the highest dose employed in the present study) and samples were taken at 30-min intervals for up to 3-hr.

Bacterial Enumeration: Following 405 nm light exposure, samples were plated onto nutrient agar using either an automatic spiral plater (Don Whitley Scientific, UK) or manually spread by using sterile L-shaped spreaders, depending on the expected population density of the samples. Sample plates were incubated at 37°C for 24 hours, and then enumerated with the surviving bacterial load reported as colony-forming units per mililitre (CFUml^{-1}).

Inactivation Data Analysis: Results are reported as surviving bacterial load ($\log_{10}$ CFU ml^{-1}) as a function of dose, and are presented as mean values from triplicate independent experiments (n=3). Dose (J cm^{-2}) is calculated as the product of the irradiance (W cm^{-2}) multiplied by the exposure time (sec), with the irradiance value being the maximum measured at the centre position of the sample dish/bag. Significant differences in the results were identified using 95% confidence intervals and one-way analysis of variance (ANOVA) with Minitab software Release 16. For dose response curves the dose that reduces the $\log_{10}$ CFU count at 0-dose by 50% was estimated. This 50% $\log_{10}$ reduction was estimated using

curve fitting software (GraphPad Prism V6) and quadratic or 5PL variable slope sigmoidal curves with R-squared fits in excess of 90%.

Optical Analysis of Plasma: The transmission values for rabbit and human plasma, PBS, and the blood bag material, were measured by using a Biomate 5 UV-Visible Spectrophotometer (Thermo Spectronic). Analysis was carried out in the wavelength range 220-700 nm. Fluorescence spectrophotometry (RF-5301 PC spectrofluorophotometre; Shimadzu, US) was used to determine whether plasma or PBS contained photosensitive components which could be excited by 405 nm light. Excitation was carried out at 405 nm and emission spectra were recorded between 500-700 nm.

RESULTS

Inactivation of Microbial Contaminants in 3 ml PBS and Plasma: Results from the exposure of PBS, rabbit plasma and human plasma seeded with bacterial contamination (10^5 CFUml^{-1}) to 100 mWcm^{-2} 405nm light, are presented in Figure 1. Results demonstrate that bacterial inactivation in PBS is achieved using the lowest dose. Data for S. aureus (Fig.1a) shows that near complete inactivation (<10 CFUml^{-1} surviving) of the organism in PBS was achieved after exposure to a dose of 60 Jcm^{-2}. To achieve a comparable reduction in rabbit and human plasma, 4.5 times the dose was required (270 Jcm^{-2} compared to 60 Jcm^{-2}). 50% $\log_{10}$ reductions were estimated to occur at doses 23, 224 and 181 Jcm^{-2} for PBS, rabbit and human plasma, respectively.

Similar inactivation kinetics were observed for S. epidermidis (Fig.1b), although this species showed comparatively greater susceptibility to 405 nm light when exposed in plasma. The 50% $\log_{10}$ reductions were obtained in PBS, rabbit and human plasma at 36, 121 and 174

183 Jcm^{-2} respectively. Reduction of E. coli contamination required markedly increased doses
184 (Fig.1c). The 50% log_{10} reductions required doses of 328, 585 and 742 Jcm^{-2} for PBS, rabbit
185 and human serum respectively. For inactivation in PBS, 450 Jcm^{-2} was required for near
186 complete inactivation (<10 $CFUml^{-1}$ surviving): 7.5 times more than observed with the
187 staphylococci. Inactivation of E. coli contamination in plasma again required increased doses
188 compared to when suspended in PBS, with a 5-log_{10} reduction in human plasma achieved
189 after a dose of 1.08 $kJcm^{-2}$.

190

191 **Determination of Light Induced Toxicity within Human Plasma:** No significant change in
192 the seeded 10^3 $CFUml^{-1}$ population [P=0.663] was evident in the bacterial contamination
193 added to plasma post-exposure, thus indicating no residual toxicity in 405-nm light exposed
194 plasma that could induce the inactivation of microbial contaminants.

195

196 **Inactivation of Contaminants in Larger Volumes of Human Plasma:**

197 **30 ml Volume in Covered Sample Dish:** Figure 2 demonstrates the inactivation of
198 low density S. aureus contamination in 30 ml plasma in a closed Petri dish using an
199 irradiance of ~8 $mWcm^{-2}$. Results for a seeding density of 10^3 $CFUml^{-1}$ (Fig 2b) demonstrate
200 that exposure to doses of greater than 100.8 Jcm^{-2} caused significant inactivation of the
201 contamination [P=0.030], with near-complete inactivation achieved with 230.4 Jcm^{-2}.
202 Control contamination levels rose significantly by approximately 1-log_{10} over the course of
203 the experiment [P=<0.001]. Similar results were observed for inactivation of the 10^2 $CFUml^{-1}$
204 contamination levels (Fig 2c): significant inactivation became evident after exposure to a
205 dose of 115.2 Jcm^{-2} [P=0.009], with near complete inactivation achieved with 187.2–

230.4 Jcm^{-2}. Control contamination levels remained relatively unchanged [P=0.255]. Significant inactivation of a 10^1 $CFUml^{-1}$ seeding population was shown after a dose of 115.2 Jcm^{-2} [P=0.031], with near complete inactivation achieved by exposure to doses of 201.6-230.4 Jcm^{-2} (Fig 2d). Control contamination levels showed no significant change compared to the exposed samples [P=0.054].

Decontamination of Plasma in a Blood Bag: Inactivation of low density (10^1-10^2 CFU ml^{-1}) bacterial contaminants within plasma transfusion bags was achieved using irradiances as low as 5 $mWcm^{-2}$ (Fig.3b) A notable downward trend in contamination was observed after exposure to 108 Jcm^{-2}, with a significant 0.6 log_{10} reduction in contamination [P=<0.001]. Complete/near complete inactivation was achieved after exposure to 144 Jcm^{-2} [P=0.017] This slightly reduced inactivation rate, compared to that found within the sample dishes, is due to the lower irradiance light being used for exposure. Contamination levels in the control plasma bags rose by approximately 0.5-log_{10} [P=0.052]. Similar inactivation kinetics were obtained for seeded transfusion bags exposed to irradiances of 16 and 48 $mWcm^{-2}$, with contamination levels decreasing upon exposure to increasing treatment. Comparison of the results for the three irradiance levels used, demonstrated that, when looking at exposure time (Fig. 4a), the decontamination effect observed with 16 and 48 $mWcm^{-2}$ is relatively comparable, with inactivation being slightly slower when using the lowest irradiance of 5 $mWcm^{-2}$. However, when looking at the actual dose levels applied (Figure 4b), it is apparent that the germicidal efficiency (defined as the log_{10} reduction of a bacterial population [$log_{10}(N/N_0)$] by inactivation per unit dose in Jcm^{-2} [23]) of the 5 $mWcm^{-2}$ irradiance is greater than that of the 16 and 48 $mWcm^{-2}$ irradiances (P=0.007 and 0.013, respectively). Comparison of exposure to doses in the region of 140-180 Jcm^{-2} highlights this difference in efficacy, with a 1.91 log_{10} reduction being achieved after exposure to 5 $mWcm^{-2}$

for 8h (144 Jcm^{-2}), a 1.14 log_{10} reduction with 16 $mWcm^{-2}$ for 3h (172.8 Jcm^{-2}), but only 0.08 log_{10} reduction observed after 1h exposure to 48 $mWcm^{-2}$ (172.8 Jcm^{-2}).

Optical Analysis of Plasma: Spectrophotometric analysis shows that transmission of 405 nm light through plasma is low (1-2%) compared with transparent PBS (99%), and this correlates with the longer exposure times/increased doses required for comparative microbial inactivation in plasma compared to PBS. Figure 5a highlights the transmissibility of the Petri dish material and the blood bag, with results showing that 405 nm light can transmit through these materials, thus permitting decontamination of the blood plasma whilst contained in the sample dish and blood bag. The fluorescence emission spectra of rabbit and human plasma, and PBS demonstrated that excitation of the suspensions at 405 nm produced no prominent fluorescence emission peaks between 500 and 700 nm (Fig 5b).

DISCUSSION

In order to assess the potential of 405 nm light for decontamination of blood plasma, the penetrability and antimicrobial efficacy of 405 nm light in plasma required evaluation, and the aim of this study was to determine the antibacterial effects of 405 nm light at varying irradiances on bacteria seeded in blood plasma ranging from small volume samples up to pre-bagged plasma.

Initial investigation of the inactivation of bacterial contaminants in low volume (3 ml) plasma samples using 100 $mWcm^{-2}$ 405 nm light demonstrated that successful inactivation could be achieved in both rabbit and human plasma. Significantly greater doses were required for inactivation of bacterial contaminants when suspended in plasma compared to PBS, and this is accredited to the differing optical properties of these suspending media. The opacity, and

consequent low transmissibility of plasma (Fig.5a), reduces photon penetration through the suspension, resulting in the requirement for greater doses, compared with suspension in clear, transparent liquids such as PBS. Despite this, these proof-of-principle results demonstrate that significant inactivation of bacterial contaminants in human plasma can be achieved, with the higher the irradiance of light applied, the shorter the exposure time required for successful inactivation.

Despite the optical transmission properties of rabbit and human plasma being relatively similar, slight differences were recorded between the susceptibility of the bacterial contaminants when seeded in these media. This is likely due to the batch-to-batch variation in color and opacity of the rabbit and, in particular, the human plasma. Indeed, optical analysis of a number of human plasma bag samples (n=30) demonstrated variation in transmission at 405 nm of between 0.2-25% (Maclean, Anderson, MacGregor, Atreya; unpublished data). This is likely the reason for the large standard deviation in some of the data points in the inactivation kinetics for the pre-bagged plasma.

The bacterial species used in this study were selected to represent significant contaminants associated with blood components [3]. Although only three organisms were utilized, the wide antimicrobial efficacy of 405 nm light has been reported in a number of publications [20,22,23,25,37]. It is therefore expected that these organisms would also be successfully inactivated by 405 nm light when suspended in plasma. Typically, Gram-positive bacteria tend to have greater susceptibility to 405 nm light than Gram-negative bacteria [23], and this is consistent with the results reported here, with approximately 4-times greater dose required to inactivate E. coli in plasma, compared to the staphylococci. Interestingly, the difference between the susceptibilities of the staphylococci and E. coli was less pronounced when suspended in plasma compared to in PBS (4 versus 7.5 times the dose required).

The initial exposure tests in this study to establish proof-of-principle, utilised low volumes of plasma seeded with high population densities of bacterial contaminants at a level of 10^5 CFUml^{-1}. A more realistic scenario involves larger volumes of plasma contaminated with low microbial densities. Indeed, it has been reported that the levels of naturally-occurring bacterial contamination in plasma are likely to be as low as 10-100 bacterial cells per product at the beginning of storage [38]. Accordingly, experiments were scaled up 10 and 100-fold using larger plasma volumes seeded with bacterial contamination levels down to 10^1 CFUml^{-1}, using S. aureus as the model organism. Results demonstrated that bacterial contamination levels, even when less than 10 CFUml^{-1}, can be significantly reduced in larger volumes of plasma by exposure to 405 nm light. It was interesting to note that, when using similar irradiances, the bacterial inactivation rates in the 30 ml and 300 ml samples were very similar (~1.5 $\log_{10}$ reductions with a dose of 144 Jcm^{-2} – Fig.2c, Fig.3b) despite the 10-fold difference in sample volume. Although the sample volumes were different, the depth of plasma was similar (~1-2 cm in both cases) thus indicating that, when using similar irradiances, it is the depth of plasma that is likely to influence the light inactivation efficacy, rather than the overall sample volume. Also, results demonstrated that use of lower irradiances is likely to be more efficient, both in terms of optical energy and antimicrobial activity, than higher irradiances. This is possibly due to there being a critical level of photons that can be involved in the photo-excitation of the bacterial porphyrin molecules, and above this irradiance level, there is provision of excess photons which, although exposing the cells, are unable to contribute to the reaction due to there being a limit on the free porphyrin to photon ratio.

In addition to demonstrating efficacy when applied to larger volumes of plasma, these experiments highlighted that the 405 nm light disinfection effect can be achieved through transparent packaging. A similar effect was reported in a recent study which highlighted the

ability of 405 nm light to decontaminate biofilms on the underside of transparent materials [39]. The ability of 405 nm light to transmit through the PVC bag layer to treat the plasma is particularly advantageous as it opens up the possibility for pre-bagged plasma to be treated immediately prior to storage, without the need for addition of photosensitizers, and/or passing the plasma through external decontamination systems, which can potentially introduce new contamination into the plasma products [6]. The transmissibility of 405 nm light is also a significant advantage over UVC-light, which is blocked by the PVC bag material (Fig.5a). Measurements in the present study demonstrated that transmission of 405 nm light though the blood component bag material resulted in an approximate 20% loss in irradiance, however light irradiance can be increased through the use of higher power light sources in order to compensate for this loss if required. Future developments would also look to improve the uniformity of the light systems used to treat the plasma.

Published studies have identified microbial endogenous porphyrin molecules as the key photosensitive targets which initiate the lethal oxidative damage exerted by 405 nm and other violet light wavelengths [19,33]. Since human blood also contains porphyrins and porphyrin derivatives, it was important to establish that inactivation by 405 nm light in our study was a result of the photoexcitation reaction within the microbial contaminants, and not a consequence of excitation of any photosensitive molecules within the plasma, and this was evidenced by the absence of antimicrobial toxicity to bacterial contaminants seeded into the 405 nm light-exposed plasma. Qualitative analysis of the rabbit and human plasma also detected no notable fluorescence emission peaks between 500-700 nm when excited at 405 nm, thus indicating no significant levels of free porphyrins or other photoexcitation sources within the plasma that might have acted as exogenous photosensitizers for the inactivation of the microbial contaminants.

The 405 nm light doses required in this study for the decontamination of blood plasma have been in the region of 158 Jcm^{-2} and above. These doses are relatively high compared to those typically required for other light-based methods, and this is due to the higher germicidal efficacy of UV-light compared to 405 nm light [40], and the involvement of photosensitizing compounds such as riboflavin, methylene blue and amotosalen, also accelerates the antimicrobial effects of light, with doses as low as 6.24 Jml^{-1} being reported as sufficient for use [7,41]; significantly lower than the 83 Jml^{-1} used in the present study (calculated based on the 158 Jcm^{-2} dose, transfusion bag dimensions and volume). This benefit however is counterbalanced by the fact that photosensitizers are added to the blood products, and significant care must be taken to ensure there is no toxicity to the blood components or the recipient due to the presence of residual photosensitizers [6]. Methods utilizing UV-C light are currently under development and also demonstrate efficient microbial inactivation [16]. Although not requiring photosensitizers, UV-C is naturally more germicidal than 405 nm light, however, as mentioned, the limited penetrability of shortwave UV-C radiation means it is unable to decontaminate plasma packed in blood bags, as evidenced in the present study using 405 nm light (Fig.4a). The longer wavelength of 405 nm light also confers other benefits when compared to UV-light, including reduced polymer degradation and increased human safety [42,43].

Due to the absence of cells, solvent/detergent treatment, methylene blue and visible light, amotosalen and UV-A light, riboflavin and UV, and UV-C light are generally accepted as suitable for plasma decontamination. This study has generated significant evidence of the efficacy of 405 nm light for decontamination of blood plasma as a model system to study injectable biological fluids. Since person-to person variation in the activity of plasma proteins in healthy individuals is known to be significant, any loss in plasma integrity due to 405 nm light treatment is unlikely to have noticeable clinical impact. Further, since violet-

blue light (405 nm) is relatively safer compared to already accepted UV-light based methods [40], its impact on plasma integrity has the potential to be reduced. Nonetheless, it is important in future studies to establish what effects are imparted onto plasma proteins when exposed to antimicrobial levels of 405 nm light relative to UV light exposure.

CONCLUSIONS

Overall, this study provides the first evidence that 405 nm light has the ability to inactivate bacterial contamination within biological fluids such as blood plasma. Significant inactivation of microbial contaminants was achieved in plasma samples of varying volumes held in different containers including pre-bagged plasma. The penetrability of 405 nm light and the non-requirement for photosensitizing agents, provide this antimicrobial method with unique benefits that could support its further development as a potential alternative to UV light-based systems. Further work is however required, not only to extend the microbiological data, but also to investigate the compatibility of 405 nm light with plasma components before its potential for plasma decontamination can be fully assessed. Although this study has focused on the antimicrobial effects of 405 nm light for the decontamination of plasma, it will also be of interest to establish whether bacterial reductions can be achieved in platelets stored ex vivo in plasma-based suspensions, which have a significantly greater risk of contamination due to the limitations of their storage conditions.

ACKNOWLEDGEMENTS

This work was supported by US FDA funding to CDA, with experimental work conducted at ROLEST through a collaborative research contract. The authors would like to thank J

374 Gillespie and HVT technical staff (Department of Electronic & Electrical Engineering (EEE),
375 University of Strathclyde) for technical support with Matlab software and system
376 construction, respectively, and Prof MH Grant, K Henderson and K McKenzie for access and
377 technical support with the fluorescence spectrophotometry (Departments of Biomedical
378 Engineering and EEE, University of Strathclyde). The authors would also like to thank the
379 Scottish National Blood Transfusion Service (SNBTS) for provision of blood components.

380

381 **CONFLICT OF INTEREST**

382 The authors declare that there is no conflict of interest regarding the publication of this paper.
383 The authors have filed a joint US device patent application.

384

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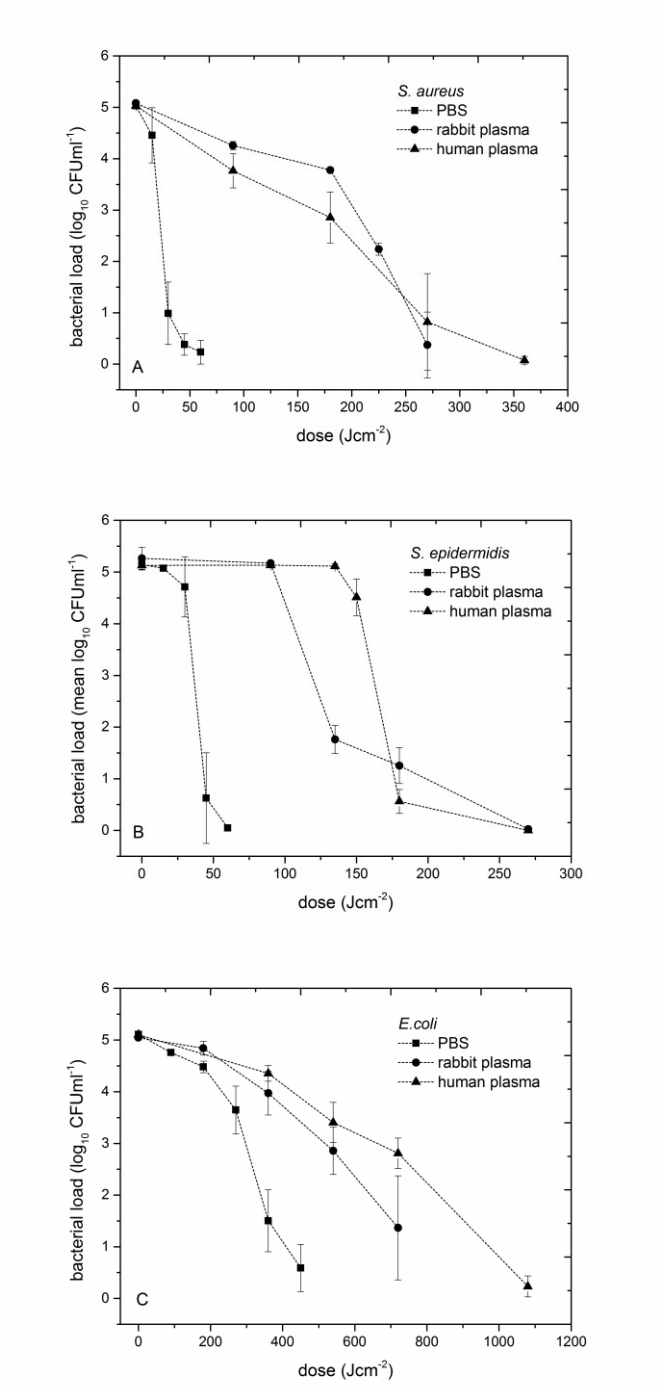

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531 **Fig 1:** Inactivation of bacterial contamination, (a) S. aureus, (b) S. epidermidis (c) E. coli, in
532 phosphate buffered saline (PBS) rabbit plasma, and human plasma, by exposure to 405 nm
533 light with an irradiance of approximately 100 mWcm^{-2} (n=3 ±SD). Non-exposed control
534 samples for all experiments demonstrated no significant change in population over the
535 exposure period [P=>0.05].

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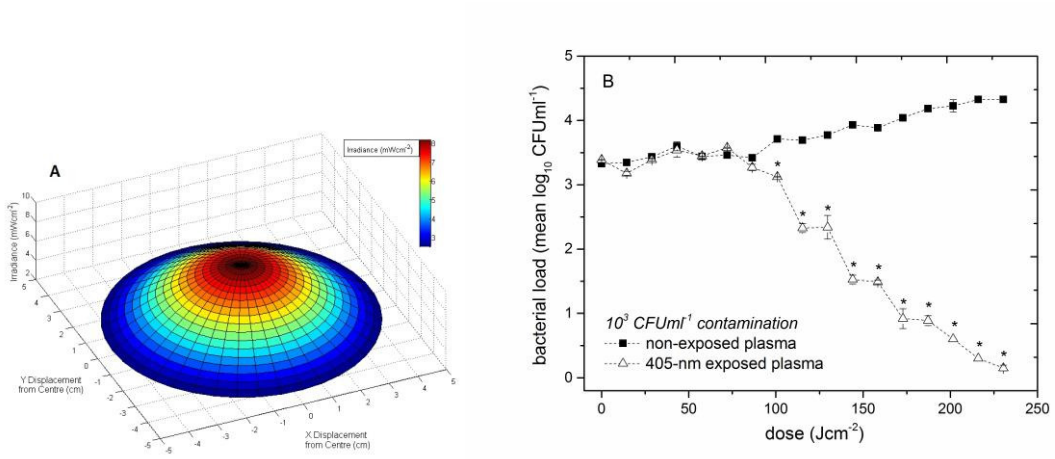

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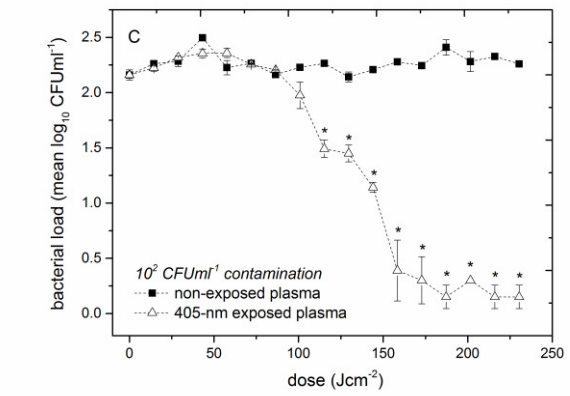

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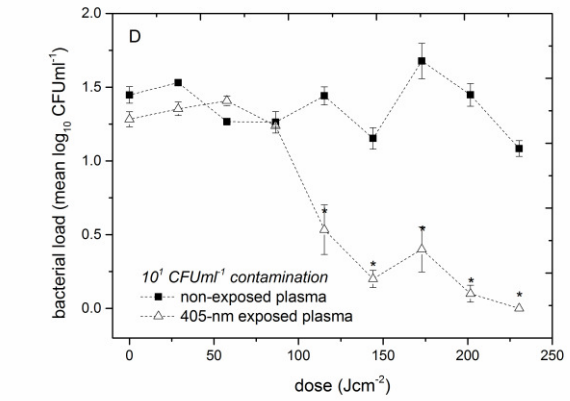
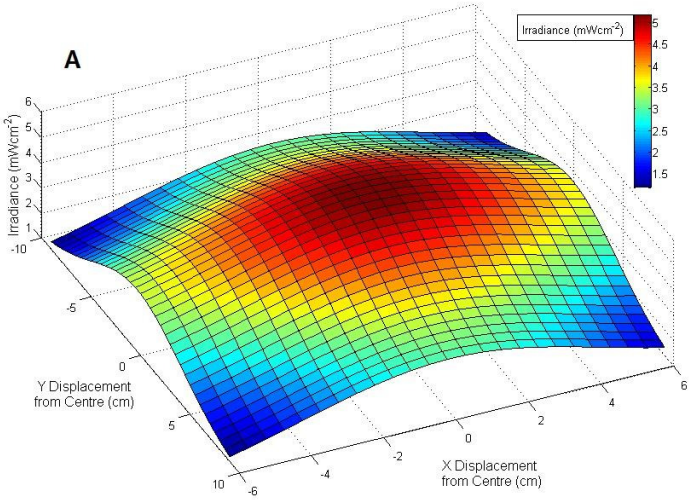


539 **Fig 2:** Inactivation of S. aureus contamination in 30 ml volumes of human plasma held in a
540 closed sample dish by exposure to 405 nm light. (a) Three-dimensional model demonstrating
541 the irradiance profile across the sample dish, with an irradiance of ~8 $mWcm^{-2}$ at the centre.
542 Populations of (b) 10^3, (c) 10^2 and (d) 10^1 $CFUml^{-1}$ were used as the seeding densities (n=3
543 ±SE). Asterisks (*) represent data points where the bacterial levels in light-exposed plasma
544 were significantly different to the equivalent non-exposed control [P=<0.05].



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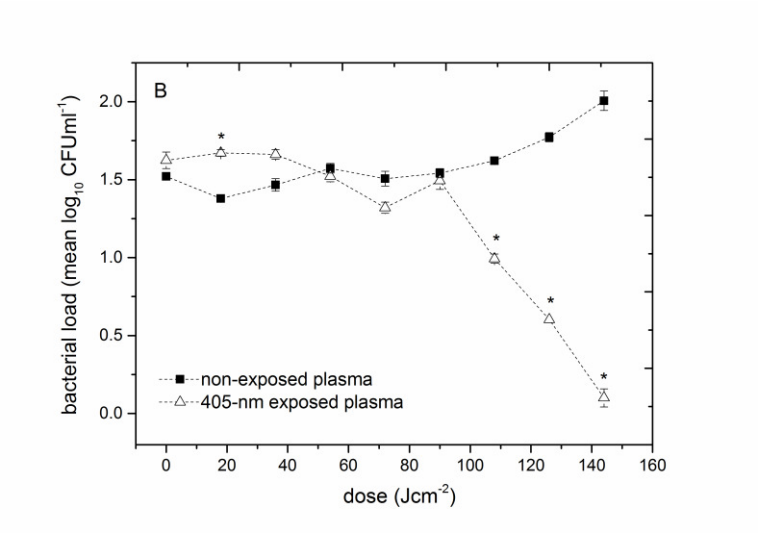

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548 **Fig 3:** 405 nm light exposure of contaminated human plasma transfusion bags (a) Three-
549 dimensional model demonstrating the irradiance profile across the plasma bag, with an
550 irradiance of ~5 mWcm^{-2} at the centre (b) Inactivation of S. aureus contamination in 300 ml
551 bags of human plasma by exposure to 405 nm light (n=3 ±SE). Asterisks (*) represent data
552 points where the bacterial levels in light-exposed plasma were significantly different to the
553 equivalent non-exposed control [P=<0.05].

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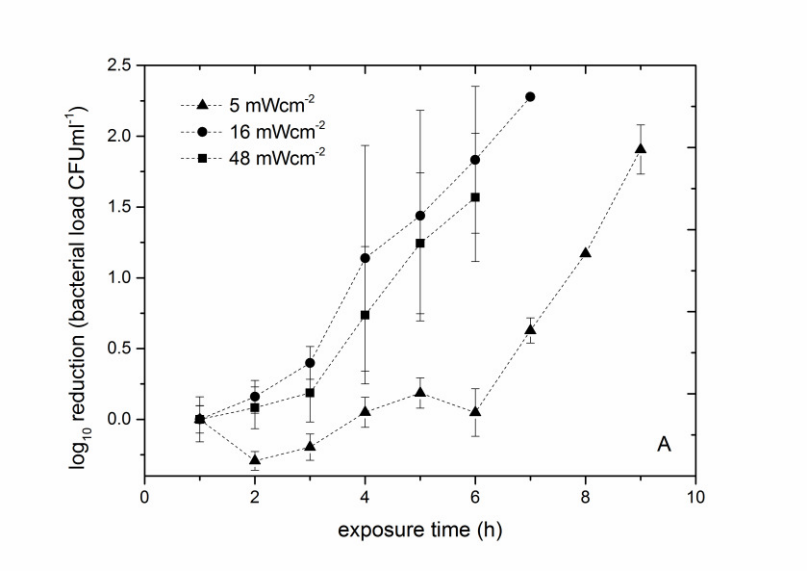

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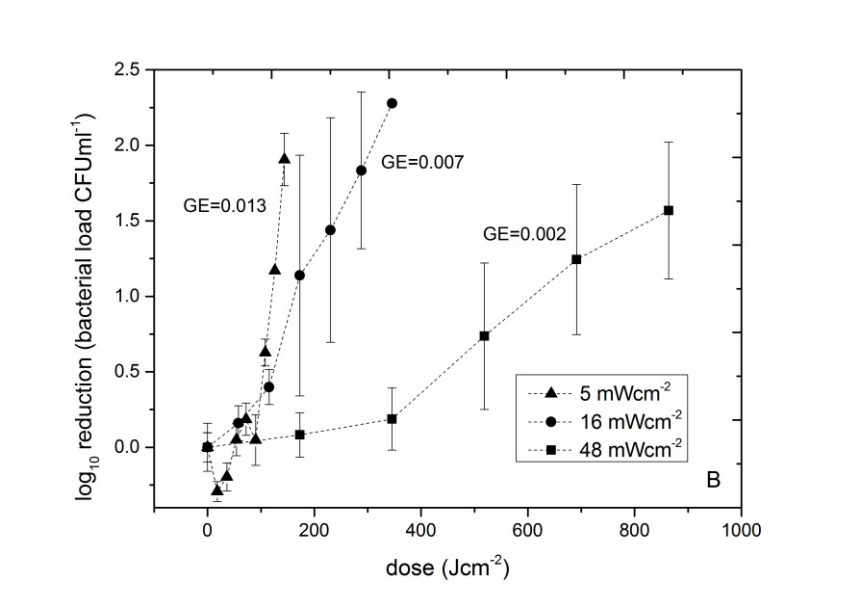

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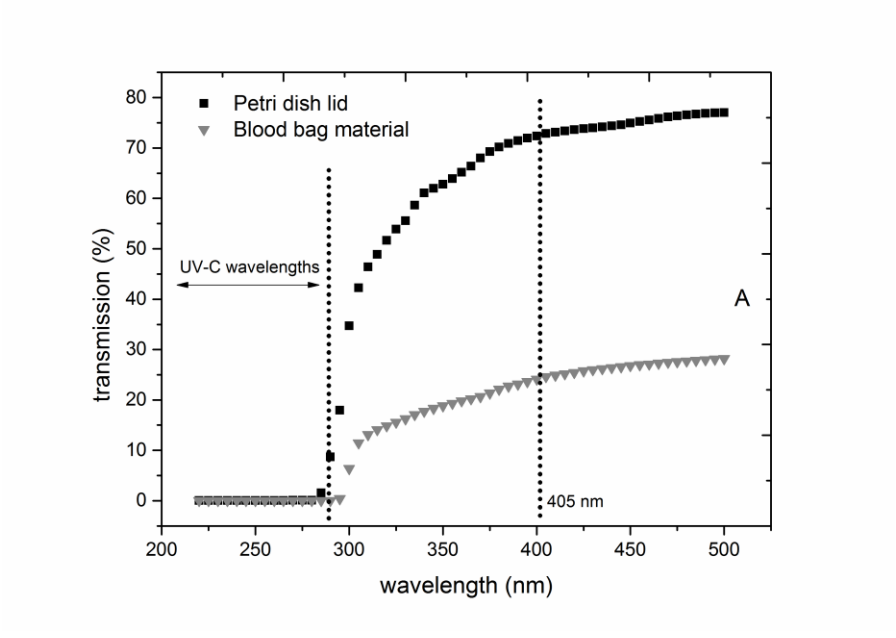
557 **Fig 4:** Comparison of the exposure times (a) and doses (b) required for inactivation of
558 S. aureus contamination in human plasma transfusion bags. (a) Inactivation kinetics were
559 achieved utilising irradiances of 5, 16 and 48 $mWcm^{-2}$ at the centre of the bags. Results are
560 presented as log_{10} reduction ($CFUml^{-1}$) as compared to the equivalent non-exposed control
561 samples (n=3 ±SD). Germicidal efficiency (GE) values for each of the irradiances are shown
562 on (b). (GE is defined as the log_{10} reduction of a bacterial population [$log_{10}(N/N0)$] by
563 inactivation per unit dose in Jcm^{-2}).



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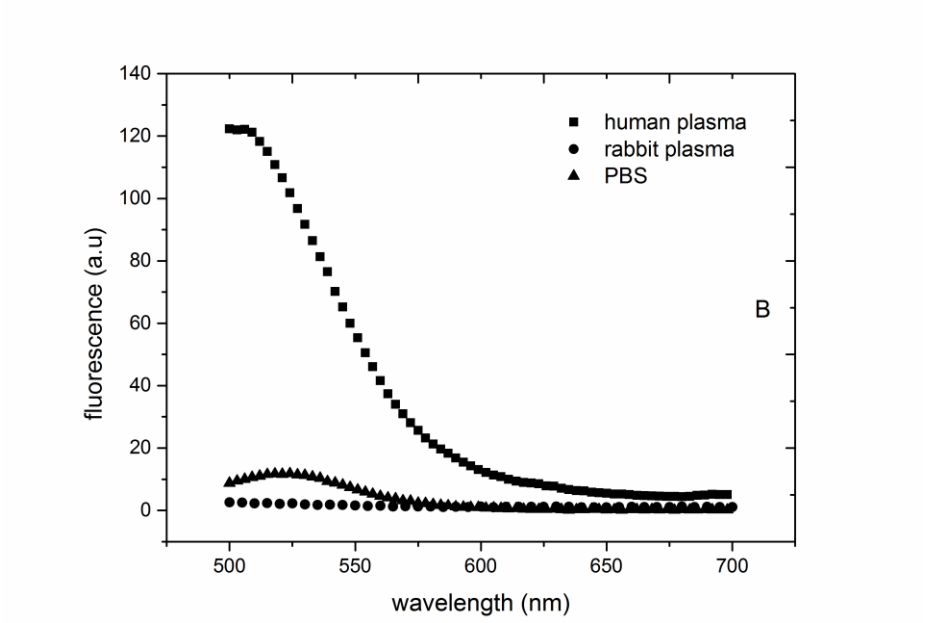

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566 **Fig 5:** Optical analysis (a) Transmission properties of the Petri dish and blood bag material,
567 highlighting 405 nm and UV-C light wavelengths for reference; (b) Fluorescence emission
568 spectra of PBS and plasma (500-700 nm), detected using an excitation wavelength of 405 nm.

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