Research Article

Violet-blue 405-nm Light-based Photoinactivation for Pathogen Reduction of Human Plasma Provides Broad Antibacterial Efficacy Without Visible Degradation of Plasma Proteins

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ABSTRACT

In transfusion medicine, bacterial contamination can occur in ex vivo stored blood plasma, and there are continued efforts to improve blood safety and reduce the risk of transfusion-transmitted infections. Visible 405-nm violet-blue light has demonstrated potential for in situ pathogen reduction in ex vivo stored plasma and platelet concentrates. This study investigates the broad-spectrum antibacterial efficacy and compatibility potential of 405-nm light for treatment of blood plasma. Human plasma seeded with bacteria at a range of densities (10¹–10⁷ CFU mL⁻¹) was exposed to 360 J cm⁻² 405-nm light (1 h at 0.1 W cm⁻²), with this fixed dose selected based on the initial analysis of inactivation kinetics. One-dimensional protein mobility analysis and measurement of advanced oxidation protein products (AOPP) was conducted to evaluate compatibility of the antimicrobial dose with plasma proteins and, identify upper levels at which protein degradation can be detected. Broad-spectrum antibacterial efficacy was observed with a fixed treatment of 360 J cm⁻², with 98.9–100% inactivation achieved across all seeding densities for all organisms, except E. coli, which achieved 95.1–100% inactivation. At this dose (360 J cm⁻²), no signs of protein degradation occurred. Overall, 405-nm light shows promise for broad-spectrum bacterial inactivation in blood plasma, while preserving plasma protein integrity.

INTRODUCTION

Since the implementation of blood transfusion, risk reduction measures including donor screening, sample diversion and nucleic acid testing (NAT) for HIV, HBV and HCV have significantly improved the safety of blood components (1). Nevertheless, the low but known risk of bacterial contamination remains the second most common cause of fatal transfusion-associated reactions, after ABO incompatible transfusion-associated fatal reactions due to clerical errors (2). In response to this, three main areas of avoidance, detection and elimination have been explored with the common goal of providing a near zero-risk blood supply (3).

Conventional pathogen reduction techniques for blood plasma, including the use of solvent/detergent, heat treatment and methylene-blue in combination with visible light have been employed throughout Europe and North America for decades (4). Continued efforts to reduce the risk of transfusion-transmitted infections (TTIs) led to the development of ultraviolet (UV) light irradiation technologies including the Intercept (Cereus Corporation) and Mirasol (Terumo BCT) systems. While all demonstrate germicidal efficiency, it is generally accepted that these photoinactivation techniques have limitations as treatment conditions shown to compromise blood product quality and stability, can lead to significant reductions in overall coagulation activity (5). Further, the requirement for addition of photosensitizer increases processing times and, in rare cases, has been associated with adverse reactions in recipients depending on the type of photosensitive agent being used (5,6).

Violet-blue light, in the region of 405 nm, is being investigated as an alternative antimicrobial approach for pathogen reduction of blood transfusion products, without the need for addition of photosensitive agents (7–10). Microbial inactivation by exposure to these wavelengths is accredited to the photoexcitation of endogenous porphyrin molecules within microbes, which results in the generation of reactive oxygen species (ROS), inducing nonspecific oxidative damage and cell death (11–15). As recently reviewed by Tomb et al. (16), this photoinactivation technique has demonstrated efficacy against a wide range of pathogens including vegetative bacteria, bacterial endospores, fungi, yeast (17–21) and, under certain exposure conditions, viruses (22).

Proof-of-concept studies have established the efficacious antimicrobial action of violet-blue light for pathogen reduction in both plasma and platelet concentrates (PCs), using selected bacteria, yeast and most recently a blood-borne parasite.
plasma samples were exposed to a emitting diode array (ENFIS PhotonStar UNO 24; PhotonStar analyzed using UV-V is spectrophotometry (Biomate 5; Thermo

To date, compatibility studies have focused on PCs, therefore further research is required to expand knowledge on the applicability of violet-blue light treatment for blood plasma. The aim of this study is to demonstrate the broad-spectrum antimicrobial treatment of blood plasma, using dose levels that show compatibility with the plasma itself in terms of protein integrity. Initial tests were carried out to identify a germicidal treatment dose that was both effective against selected microbes (Staphylococcus aureus and Escherichia coli), and displayed compatibility with the plasma proteins. Evaluation of this treatment dose against a range of organisms associated with transfusion-transmitted infections was then conducted to assess the broad-spectrum antibacterial efficacy of this light-based technology.

**MATERIALS AND METHODS**

*Preparation of bacterial cultures and blood plasma.* The bacterial strains used in this study were, as Gram positive types: Staphylococcus aureus NCTC 4135, Staphylococcus epidermidis LMG 10273, Bacillus cereus NCTC 11143 and as Gram negative types: Escherichia coli NCTC 9001, Pseudomonas aeruginosa NCTC 9009, Acinetobacter baumannii LMG 1041, Yersinia enterocolitica LMG 7899 and Klebsiella pneumoniae LMG 3081. Bacteria were cultured in nutrient broth (Oxoid Ltd., UK), with the exception of *S. epidermidis* and *Y. enterocolitica*, which were cultured in tryptone soya broth (Oxoid Ltd.). Broths were incubated at 37°C for 18-24 h under rotary conditions (120 rpm), and then centrifuged at 3939 g for 10-min. Cell pellets were re-suspended and serially diluted in phosphate-buffered saline (PBS; Oxoid Ltd.), to the required cell density for experimental use, with the final dilution (1:100) being into human, mixed pool, fresh frozen plasma (FFP; TCS Biosciences Ltd (UK) and the Scottish National Blood Transfusion Service (SNBTS, UK)). The transmissibility of plasma samples (*n* = 8) was analyzed using UV-V is spectrophotometry (Biomate 5; Thermo Spectronic) was found to be 0.08-3.60% at 405 nm (Fig. 1A).

405-nm light treatment system. The light source used was a light emitting diode array (ENFIS PhotonStar UNO 24; PhotonStar Technologies, UK), with a narrow emission (∼14-nm FWHM), and a center wavelength in the region of 405 nm (Fig. 1B). The array was powered by a 40 V Philips Xitanium LED Driver (Philips, the Netherlands) and bonded to a heat sink and fan for thermal management. The LED array was housed in a custom-made stand, which held the array in a fixed position, 5 cm above the plasma samples, providing an irradiance of ∼0.1 W cm−2 at the sample surface (measured using a radiant power meter and photodiode detector (LOT-Oriel Ltd)).

**Determination of an effective antibacterial dose.** To identify effective antibacterial dose levels, the inactivation kinetics of two key problematic organisms—*S. aureus* and *E. coli*—were first established. Plasma samples were seeded with a density of ∼10^7 CFU mL−1, and 250 µL volumes (*n* = 2) were transferred into a 96-well plate, covered with ultra-clear adhesive sealing film and positioned below the light array. Seeded plasma samples were exposed to a fixed irradiance of 0.1 W cm−2 for increasing treatment times, and the temperature checked using a thermocouple.

The applied dose was calculated using the equation:

Dose (J cm−2) = Irradiance (W cm−2) × Exposure time (seconds)

Equivalent control samples were held in identical conditions but shielded from the 405-nm light. Postexposure, the samples were plated onto nutrient agar (Oxoid Ltd.), incubated at 37°C and surviving colony forming units were enumerated, with results recorded as mean (*n* ≥ 3) log_{10} CFU mL−1 ± standard deviation (SD).

**Broad-spectrum antibacterial efficacy testing.** To establish the broad-spectrum antibacterial efficacy of 405-nm light, plasma was seeded with a range of bacteria (see Section ‘Preparation of bacterial cultures and blood plasma’) at three cell densities (“low”: 10^5–10^6 CFU mL−1; “medium”: 10^6–10^7 CFU mL−1 and “high”: 10^7–10^8 CFU mL−1—the range at which each to natural variation in densities after overnight culture of the different organisms) and exposed to a fixed dose of 360 J cm−2.

Two hundred and fifty microliter volumes of seeded plasma (*n* = 3) were exposed to 0.1 W cm−1 for 1 h (360 J cm−2), with control samples shielded from the light treatment. Postexposure, samples were spread plated in duplicate onto nutrient agar (or tryptone soya agar (Oxoid Ltd.) for *S. epidermidis* and *Y. enterocolitica*), incubated at 37°C overnight and enumerated. Results were recorded as mean log_{10} CFU mL−1 ± SD. Means for exposed samples are taken from triplicate samples plated in duplicate (*n* = 6); control samples were plated in duplicate (*n* = 2).

**Plasma protein integrity.** To assess whether the antimicrobial light dose of 360 J cm−2 (0.1 W cm−2 for 1 h) had degradative effects on the plasma proteins, protein integrity was examined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Doses greater than 360 J cm−2 were also investigated in order to establish the threshold energy level for damage to occur. Unseeded plasma samples were exposed to a fixed irradiance of 0.1 W cm−2 for 1–5; 24, 48, 72, 96, 120, 144 h (360 J cm−2–51.8 kJ cm−2), as described in Section ‘Determination of an effective antibacterial dose’. Post-exposure, samples were diluted in tris-buffered saline (1:100; Sigma-Aldrich) and

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combined with deionized water, reducing agent (Bolt™ 10x) and LDS sample buffer (Bolt™ 4x) at a ratio of 10:3:2:5, respectively. The samples were then reduced in a thermocycler (Prime Thermal Cycler, Techne, UK) at 70°C for 10 min. Positive controls were prepared by mixing 0.1 mg mL⁻¹ of Proteinase K (Sigma-Aldrich, UK) with nonexposed plasma at a 1:1 ratio, and reducing in a thermocycler at 65°C for 3 h. Negative controls were reduced, protein-free Tris-buffered saline (TBS) and freshly thawed plasma. An additional control sample—plasma exposed to thermal treatment—was also prepared by holding plasma in a water bath at 43°C, and the maximum temperature was recorded during light exposure for 72 h. Plasma proteins were separated on a 4–12% Bis-tris Gel (Invitrogen™, NuPAGE), submerged in running buffer (Life Technologies, MES SDS (20x)) in a Mini Gel Tank (Invitrogen™) at 200 V and 120 mA for ~25 min. Protein bands were stained using Coomassie blue (Fisher Scientific, UK), and the resulting bands were visually analyzed, alongside a protein standard (Invitrogen™, SeeBlue™ Plus2 Pre-Stained). Semi-quantitative analysis of gels was also performed using ImageJ software (http://rsb.info.nih.gov/ij) to establish protein intensity levels as an indicator of protein damage.

Measurement of advanced oxidation protein products. To quantitatively assess potential protein damage in the exposed plasma, an measurement of advanced oxidation protein products (AOPP) assay, which uses Chloramine-T as a marker of oxidative damage, was conducted. The AOPP Assay Kit (ab242295; Abcam) was used in accordance with manufacturer’s instructions. In brief, exposed and non-exposed plasma samples were diluted in PBS (1:10). Two hundred microliter volumes of the diluted plasma (n = 3), and standards of Chloramine-T (0-100 µM), were transferred to a 96-well plate and 10 µL of Chloramine Reaction Initiator was added to all plates. The wells were shaken for 5-min (120 rpm, room temperature) and 20 µL of Stop Solution was then added. Absorbance at 340 nm was then measured, and the AOPP levels expressed as µM of Chloramine-T equivalents, with values corrected for dilution factor. AOPP data is presented as mean (n = 3) ± SD.

Statistical analysis. Significant differences between exposed and nonexposed plasma samples were calculated using two-sample t tests at the 95% confidence interval (Minitab Statistical Software v18).

RESULTS

Bacterial inactivation kinetics in plasma

Figure 2 illustrates the efficacy of 0.1 Wcm⁻² 405-nm light for inactivation of low-level (~10² CFU mL⁻¹) S. aureus and E. coli contamination in plasma samples. A significant 0.8 log₁₀ reduction of S. aureus was achieved after exposure to the lowest dose of 72 J cm⁻² (82% reduction, P = 0.041), and a 2.4 log₁₀ reduction was recorded after exposure to 144 J cm⁻² (24 min exposure). The reduction of E. coli contamination required increased doses (Fig. 2B), with a significant 0.3 log₁₀ reduction not observed until exposure to 144 J cm⁻² (P = 0.008). Inactivation was more gradual, with 0.6 log₁₀ reduction observed by 180 J cm⁻², followed by a sharp decrease and 2.4 log₁₀ reduction by 360 J cm⁻². Bacterial levels in control samples remained relatively constant over the exposure period.

Inactivation kinetics identified that a 405-nm light dose of 360 J cm⁻² was an effective antibacterial dose that achieved inactivation of both S. aureus and E. coli low-level contamination in plasma. This dose was therefore selected as the fixed light treatment for use in subsequent bacterial inactivation tests.

Broad-spectrum antibacterial efficacy testing

Results from the exposure of human plasma spiked with a range of bacteria, to the 360 J cm⁻² 405-nm light treatment are presented in Fig. 3. The results demonstrate that 405-nm light achieved significant inactivation in all exposed plasma samples spiked with low, medium and high bacterial cell densities (P ≤ 0.05).

Bacterial loads in exposed human plasma seeded at a low-level (10¹–10³ CFU mL⁻¹), were reduced by up to 2.6 log₁₀ for S. aureus, S. epidermidis, B. cereus, E. coli and P. aeruginosa (Fig. 3A–E). A. baumannii, Y. enterocolitica and K. pneumoniae were successfully reduced by 2.55 (P = 0.036), 2.35 (P ≤ 0.001) and 2.41 log₁₀ (P = 0.032), respectively, after exposure which resulted in a bacterial load of ≤ 5 CFU mL⁻¹ in exposed plasma samples (≥ 99.01% reduction).

Results for plasma spiked at medium contamination levels (10³–10⁶ CFU mL⁻¹) demonstrate that a dose of 360 J cm⁻² can significantly reduce all bacterial species, with >4.5 log₁₀ reductions achieved for Y. enterocolitica (Fig. 3G) and K. pneumoniae

Figure 2. Inactivation kinetics for the 405 nm light treatment of plasma seeded with low level bacterial contamination. *Staphylococcus aureus, (A) and Escherichia coli, (B) were used to seed small volume plasma samples at ~10² CFU mL⁻¹, and were exposed to 405-nm light at an irradiance of 0.1 W cm⁻². Data represent mean (n ≥ 3) ± SD, with asterisks (*) representing a significant decrease in the bacterial load in treated plasma compared to the equivalent nontreated control [P ≤ 0.05; 2-sample t test (Minitab v18)].
Similar results were observed for highly contaminated plasma ($10^{7}$–$10^{8}$ CFU mL$^{-1}$) with significant reduction of all organisms following light treatment ($P < 0.05$). Antibacterial efficacy varied the most between organisms at high contamination levels. The highest reductions were observed with the Gram-negative organisms—with the exception of E. coli. Data for P. aeruginosa, A. baumannii, Y. enterocolitica and K. pneumoniae (Fig. 3E–H) show near complete inactivation ($\leq 5$ CFU mL$^{-1}$ remaining; $P \leq 0.03$) was achieved following exposure to a 360 J cm$^{-2}$ dose. In comparison, a 1.32 log$_{10}$ (95.05%; $P = 0.005$) reduction was achieved for E. coli (Fig. 3D). More than 99.97% (>3.6 log$_{10}$; $P \leq 0.04$) reductions were observed with the Gram-positive organisms (Fig. 3A–C).

**Plasma protein compatibility**

Figure 4A is an image of the master gel used to assess plasma protein integrity and compare electrophoretic patterns of plasma exposed to 0.1 W cm$^{-2}$ 405-nm light for 1–5 h (360 J cm$^{-2}$–1.8 kJ cm$^{-2}$). The electrophoretic patterns of plasma exposed to the effective antibacterial dose of 360 J cm$^{-2}$ did not demonstrate visually detectable differences between the exposed and nonexposed plasma samples (lanes 2 and 3), indicating that the antibacterial effect can be achieved with no obvious damage to the plasma proteins. When exposed to an increased dose of 720 J cm$^{-2}$ (double the effective antibacterial dose), the plasma banding appeared to show slight changes in the region of the high-molecular-weight proteins (HMWPs; 80–198 kDa); however, as highlighted in Fig. 4B, the intensity level of the plasma proteins exposed to 720 J cm$^{-2}$ was similar to that of some of the other nonexposed controls, therefore these changes may indicate sample-to-sample variation. However, further in-depth proteomic analysis will be required to identify possible early indicators of damage, and at what dose level between 360 and 720 J cm$^{-2}$ this phenomenon may begin to become evident.

Upon exposure to further increased dose levels, there were clearer indications of protein modification of HMWPs. As shown in Fig. 4B, the intensity levels of HMWPs in plasma exposed to $\geq 1.08$ kJ cm$^{-2}$ were considerably higher than their nonexposed paired control. Nevertheless, comparison with the positive control (+ve, lane 13), showing complete plasma protein degradation, suggests that the degree of modification observed in the above cases, where plasma was exposed to doses $\leq 1.8$ kJ cm$^{-2}$, should not be regarded as major damage.

To determine the light dose at which major protein damage becomes evident, plasma was exposed to higher doses, up to 25.92 kJ cm$^{-2}$. Figure 5 shows SDS-PAGE analysis of plasma samples treated with 0.36, 1.80, 8.64, 17.28 and 25.92 kJ cm$^{-2}$ (0.1 Wcm$^{-2}$ at 1, 5, 24, 48 and 72 h, respectively). The electrophoretic pattern of plasma exposed to a dose of 1.80 kJ cm$^{-2}$ (lane 4) supports the results in Fig. 4 as it also provides early evidence of modification of HMWPs. Changes in HMWP banding became more visually detectable after exposure to a dose of...
8.64 kJ cm\(^{-2}\), with major protein modifications visible by 17.28 and 25.92 kJ cm\(^{-2}\) exposures (lane 6 and 7), likely due to protein cross-linking. As shown in Fig. 5B, the level of HMWPs exposed to $\geq 17.28$ kJ cm\(^{-2}\) increased five-fold compared to plasma exposed to an effective antibacterial dose of 360 J cm\(^{-2}\). Additionally highlighted in Fig. 5, is a loss of protein banding at approximately 45 kDa by exposure to 25.92 kJ cm\(^{-2}\) (lane 7).

Importantly, there were no visually detectable differences between the heat-treated plasma sample (lane 8)—held at the maximum exposure temperature of 43°C for 72 h—and the non-exposed control sample (lane 2). This indicates that the protein modifications observed in exposed plasma electrophoretic patterns occurred as a direct result of high-dose 405-nm light exposure and not by any residual heating.

To quantitatively assess potential oxidative damage in exposed plasma, an AOPP assay was performed. Figure 6 demonstrates that there was no significant difference in the AOPP levels of exposed and nonexposed plasma samples ($P > 0.05$) until a dose of 1.8 kJ cm\(^{-2}\), where levels increased from 18–21 µM ($P = 0.011$). AOPP levels continued to increase with exposure to higher doses (8.64, 17.28 and 25.92 kJ cm\(^{-2}\), with a maximum of 53 µM after 25.95 kJ cm\(^{-2}\) (compared to 25 µM in the nonexposed control sample; $P = <0.001$). AOPP levels in nonexposed control samples showed no significant change ($P = 0.134$).

**DISCUSSION**

This study has assessed the broad-spectrum inactivation efficacy of 405-nm violet-blue light for the pathogen reduction of blood plasma and established an effective antibacterial dose against a panel of bacteria, commonly associated with TTI’s. Single dimension protein gel analysis was conducted to assess the compatibility of the effective antibacterial dose with plasma proteins, and further, to determine an upper threshold limit of violet-blue light treatment below which there is no evidence of observable protein degradation.

![Figure 5](image-url)  
*Figure 5.* Plasma protein damage observed upon exposure to high dose violet-blue 405-nm light ($\geq 17.28$ kJ cm\(^{-2}\)). (A) SDS-PAGE master gel, and (B) Semi-quantitative analysis (using Image J software) of plasma exposed to 405-nm light at 0.1 W cm\(^{-2}\) for 1–72 h (0.36–25.92 kJ cm\(^{-2}\)). Lane 3–7 represent plasma samples exposed to doses of 0.36 (1 h), 1.80 (5 h), 8.64 (24 h), 17.28 (48 h) and 25.92 (72 h) kJ cm\(^{-2}\), respectively. Lane 8 represents a heat-treated plasma sample (43°C for 72 h), representative of the maximum temperature of the plasma samples over the extended treatment periods. Lanes 1, 2 and 9 represent negative, 0 h and positive control samples, respectively.

![Figure 6](image-url)  
*Figure 6.* Levels of advanced oxidation protein product (AOPP) detected in plasma following exposure to violet-blue 405-nm light (0.36–25.92 kJ cm\(^{-2}\)). The concentration of AOPPs is expressed as µM of Chloramine-T equivalents, with values corrected for dilution factor. Data represent mean ($n = 3$) ± SD, with asterisks (*) representing a significant increase in AOPP level detected in exposed plasma when compared to the equivalent nonexposed control ($P \leq 0.05$; 2-sample $t$ test (Minitab v18)).
Initial microbial inactivation tests determined that a violet-blue light dose of 360 J cm\(^{-2}\) (1 h at 0.1 W cm\(^{-2}\)) was required for complete inactivation of both *S. aureus* and *E. coli* seeded at low-level contamination levels. Inactivation kinetics demonstrated the increased susceptibility of *S. aureus*, with approximately 2.5 times less dose required for complete inactivation, compared to *E. coli* (144 vs 360 J cm\(^{-2}\)). This is consistent with other studies which have highlighted that *S. aureus* typically demonstrates greater susceptibility to violet-blue light inactivation than *E. coli* when exposed in both nonbiological and biological media, including plasma (7,18). The mechanism of violet-blue light inactivation is accredited to the photoexcitation of endogenous porphyrin molecules within the bacterial cells, leading to generation of reactive oxygen species (ROS), and subsequent oxidative damage, and differences in susceptibility between organisms is thought to be due to the levels and distributions of endogenous porphyrin types present in the bacterial species and/or differences in cell structure, as has been discussed in other violet-blue light and broader photodynamic inactivation studies (7,18,23).

For effective application of 405-nm light for pathogen reduction of blood plasma, it is important that the antimicrobial treatment dose used is capable of significantly reducing bacterial contamination, regardless of the contaminating species. Therefore, the fixed dose of 360 J cm\(^{-2}\), which achieved complete/near complete reduction of both *S. aureus* and the more resilient *E. coli*, was selected for assessing efficacy against a range of key organisms associated with TTIs (1–4) to establish potential for broad-spectrum antibacterial efficacy (Fig. 3). Results demonstrated successful reductions of all species, not only at low contamination levels (<10\(^{3}\) CFU mL\(^{-1}\)), but at contamination levels as high as 10\(^{9}\) CFU mL\(^{-1}\). Naturally occurring levels of bacterial contamination in blood products are typically in the region of 10–100 bacterial cells per unit prior to storage (3) therefore the low-level seeding densities used in this study are the most clinically representative. As shown in Fig. 3, successful broad-spectrum decontamination of plasma seeded at these levels was achieved, with $\geq 99.01$–100% inactivation ($\leq 5$ CFU mL\(^{-1}\) remaining) after exposure to a dose of 360 J cm\(^{-2}\), with minor differences likely due to the slightly varying starting populations between the different species. As demonstrated by the results shown in Fig. 2, and in previous studies (8,9) the kinetics for each organism can vary, but the fixed dose of 360 J cm\(^{-2}\) proved sufficient to achieve broad-spectrum bacterial inactivation across the species tested.

Successful inactivation was also achieved using the higher seeding densities; however, some differences in susceptibility were observed. In general, the Gram-negative organisms—except for *E. coli*—appeared to be most sensitive, with complete/near complete inactivation achieved in all cases. Similar results were also found by Lu et al. (9) who demonstrated increased susceptibility of *P. aeruginosa* compared to *S. epidermidis* and *B. cereus* in exposed platelet concentrates. Previous studies investigating the fundamental antimicrobial efficacy of 405-nm light have shown that Gram-positive organisms tend to be more susceptible, but these studies used simple buffer solution as the suspending fluid to establish direct interactions between the light photons and the cells (18,24). In this study, the bacteria are suspended in human plasma, and this may have an influence on microbial susceptibility. Previous work investigating the antiviral efficacy of 405-nm light demonstrated the enhanced susceptibility of Feline Calicivirus (FCV) when suspended in blood plasma, and accredited this to the excitation of photosensitive components, such as flavins and chromophores, within the plasma, which could then act as exogenous photosensitizers contributing to ROS production (22). The increased susceptibility of the Gram-negatives could potentially be attributed to a similar process, with the enhanced antimicrobial effects caused by extracellular oxidative damage to the outer membrane of these organisms. The reason for the resilience of high-density *E. coli* contamination compared to the other Gram-negative species is unclear and requires further investigation.

The inactivation data presented in this study has provided the first evidence of the broad-spectrum antibacterial efficacy of 405-nm violet-blue light for treatment of blood plasma. As mentioned above, promising results published by the authors also indicate anti-viral potential, with successful inactivation (4.8 log\(_{10}\) reduction) of FCV demonstrated in small volume plasma samples with a dose of 561 J cm\(^{-2}\) (22). Work has also been conducted investigating antimicrobial efficacy of violet-blue light for pathogen reduction of human platelets using other microbial species. Anti-fungal efficacy was demonstrated in a study by Lu et al. (9), which observed an approximate 5 log\(_{10}\) reduction of the yeast *Candida albicans* after exposure to a dose of 75 J cm\(^{-2}\) ($P \leq 0.01$). More recently, successful pathogen reduction treatment of platelet concentrates has demonstrated anti-parasitic activity against *Trypanosoma cruzi*, with reductions of over 9 log\(_{10}\) achieved with a 270 J cm\(^{-2}\) dose (10). Since the transmissibility of violet-blue light in platelet concentrates is in the same region as (if not slightly lower than) plasma (0.1–0.3% compared to 0.08–3.6%, respectively), based on data from this study and Maclean et al. (8), it would be expected that similar pathogen inactivation would be observed in human plasma. Nevertheless, future work is required to fully assess the inactivation of a range of pathogens associated with transfusion transmitted infections including bacterial spores, viruses and protozoa (1–4).

Preliminary protein compatibility evaluation indicates that the effective antibacterial dose of 360 J cm\(^{-2}\) had no visible effect on plasma protein integrity (Fig. 4), highlighting its potential compatibility for pathogen reduction of human blood plasma. The detection of minor but clinically important proteins, such as clotting factors, is limited as highly abundant proteins dominate the electrophoretic patterns of plasma (25). Nevertheless, the SDS-PAGE analysis reported here acts as a useful indicator for future, more in-depth proteomic studies to assess the functionality of violet-blue light treated plasma proteins, such as fibrinogen and essential clotting factors, via practical clot-based assays.

To establish what levels of exposure can be tolerated by the plasma before negative effects on the protein integrity are observed, extended high dose light treatments were also conducted. These tests provided clear evidence of plasma protein modification (supported by ImageJ analysis in Fig. 4B) after exposure to a high-dose of 1.08 kJ cm\(^{-2}\), where protein levels in the exposed sample were greater than that of all nonexposed control samples. Exposure to this dose resulted in detection of modifications in the electrophoretic patterns of HMWPs: a region associated with macroglobulin chains and complement proteins (25). This increased level of HMWPs could indicate protein damage, as a major pathway of redox protein modification is protein-to-protein crosslinking (26). After exposure to a further increased dose of 17.28 kJ cm\(^{-2}\), major protein modifications
were visible, with protein bands of all molecular weights affected (Fig. 5A). Since the electrophoretic pattern of the thermal control showed no detectable differences with the nonexposed plasma sample (Fig. 5; lanes 8 and 2, respectively), this confirms that the protein modifications observed with exposure to doses \( \geq 1.08 \, \text{kJ cm}^{-2} \) (shown in Figs. 4 and 5), were a result of these high-dose light exposures.

To quantitatively measure any potential oxidative damage in exposed plasma, an AOPP assay was performed (27–29). Results from the AOPP assay support findings from the 1D protein mobility analysis, indicating that no significant oxidative protein damage was detected in plasma exposed to a dose of 360 J cm\(^{-2}\)—the effective antibacterial dose used to achieve broad-spectrum bacterial inactivation in this study. Indeed, AOPP testing indicates that no significant plasma protein oxidation was detected following exposure to doses of up to 1.44 kJ cm\(^{-2}\)—four times greater than the effective antibacterial dose. The slight protein modifications observed in electrophoretic pattern of plasma exposed to 1.8 kJ cm\(^{-2}\), shown in Fig. 4B, are reflected in the significant increase of AOPP levels reported in Fig. 6. Recent research suggests that the key protein responsible for this oxidation reaction is oxidized fibrinogen, an essential plasma protein involved in coagulation; however, the assay is a useful indicator representative for oxidative damage of all plasma proteins (27,30).

It is worth noting that the work of this study, to establish broad-spectrum antimicrobial efficacy of 405-nm light for pathogens present in plasma, was conducted using low sample volumes and high irradiance light treatments to generate proof-of-concept data. As demonstrated in earlier studies by the authors, practical application of the technology would involve utilizing lower irradiance for whole bag treatment. This was demonstrated in the study by Maclean et al. (7), which utilized irradiances as low as 5 mW cm\(^{-2}\) for successful pathogen reduction of prebagged human blood plasma seeded with low-density \( S. aureus \) contamination. Comparison of the results between these studies demonstrates similar levels of antimicrobial efficacy against \( S. aureus \) despite the differing irradiance levels used: approximate 2 log\(_{10}\) reductions achieved by 144 J cm\(^{-2}\) using 0.1 W cm\(^{-2}\) and low sample volumes in this study (Fig. 2A); and 5 mW cm\(^{-2}\) with prebagged plasma in the previous study (7). This highlights the potential for the dose regime to be manipulated in order to establish the optimal treatment conditions for both effective pathogen inactivation and compatibility with the plasma.

It is recognized that the effective antibacterial dose of 360 J cm\(^{-2}\) used for pathogen reduction in this study is considerably higher than doses employed by other optical pathogen reduction technologies currently in use which involve UV-light and the use of photosensitive additives (5). This is due to the higher germicidal efficacy of UV light compared to visible blue light and the involvement of additives, such as amotosalen, methylene-blue and riboflavin that enhance the antimicrobial effects of light. Although UV light-based technologies in combination with external photosensitizers can inactivate pathogens with short exposure times using doses as low as 3 J cm\(^{-2}\), changes in product quality have been reported (5,31–34), and subsequent removal of the residual photosensitizer is required to avoid rare but severe allergic reactions (35,36). Additionally, the increased penetrability of violet-blue light compared to UV enables light-treatment of prebagged plasma without the need to transfer contents to an external decontamination system (7). This closed loop system will reduce the risk of new contaminants being introduced to the blood bag. As this study focused on small-scale testing using an early-stage prototype, and previous studies from other groups have assessed UV light-based antibacterial efficacy in large volume plasma and platelet concentrates (5,6), it is not yet possible to assess relative costs of pathogen reduction using 405-nm light compared to UV light. However, advantages such as use of light in the visible range and the longevity and efficiency of LED sources, as well as the capacity for \textit{in situ} treatment of components within blood bags without the processing required for addition and subsequent removal of photosensitizers, are likely to make this a competitive technology.

In conclusion, this study has successfully demonstrated the broad-spectrum antibacterial efficacy of violet-blue light for treatment of blood plasma. Significant inactivation of a panel of key TTI-related bacterial species was achieved in plasma samples at low, medium and high contamination levels. Protein stability tests also demonstrated the potential compatibility of the antibacterial dose of 360 J cm\(^{-2}\) for treatment of the plasma; however, further work will be required to assess the posttreatment functionality of key plasma proteins to fully establish the suitability of this technology for pathogen reduction treatment of human blood plasma.

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DISCLOSURES

The views expressed in this article are an informal communication and represent the authors own best judgment. These comments do not bind or obligate FDA. The authors declare that there are no competing interests regarding the publication of this paper. CDA, MM, JGA and SJM have filed a joint US device patent application.

DATA AVAILABILITY

Data supporting this publication are stored by the University of Strathclyde. Details of the data and how it can be accessed are available from the University of Strathclyde KnowledgeBase at https://doi.org/10.15129/41755c8d-06c8-4f93-a460-6118ebdc6757.

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