ANTIBACTERIAL ACTION OF VISIBLE 405 NM LIGHT FOR BACTERIAL REDUCTION IN BLOOD PLASMA

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BACKGROUND

Bacterial contamination of blood products is a major concern in transfusion medicine as it is responsible for approximately two-thirds of all transfusion-transmitted infections (TTIs)[1]. Pathogen reduction technologies (PRTs) have been developed to improve blood product safety by proactively treating to inactivate infectious agents.

PRTs can:
- ✓ **Reduce the rate of TTIs**
- ✓ **Reduce wastage of blood products**
- ✓ **Improve overall blood safety**

GERMICIDAL 405nm VIOLET-BLUE LIGHT

Peak antibacterial wavelength in visible light region at 405 nm

Inactivation by 405nm light is caused by photo-excitation of endogenous porphyrins, which induces production of reactive oxygen species (FIG 1)

Broad spectrum efficacy: bacteria, endospores, fungi, parasites & under certain circumstances, viruses)

Safer for biological material exposure & better penetrability than ultraviolet light

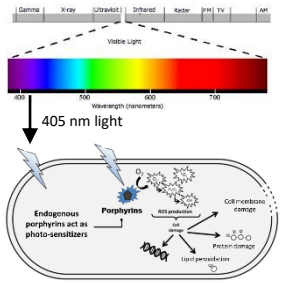


FIG 1. The electromagnetic spectrum, highlighting 405 nm light and it mechanism of action

POTENTIAL PRT FOR BLOOD TRANSFUSION PRODUCTS

405nm violet-blue light has recently demonstrated potential for *in situ* treatment of *ex vivo* stored plasma and platelet products, without the need for additional photosensitizers[2,3,4,5].

Antimicrobial	ability to treat plasma and platelets
No additives	non-requirement for photosensitizers
***In situ* treatment**	405nm light transmits through blood bag

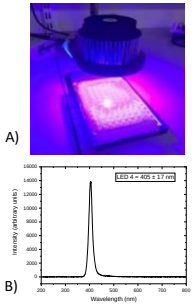


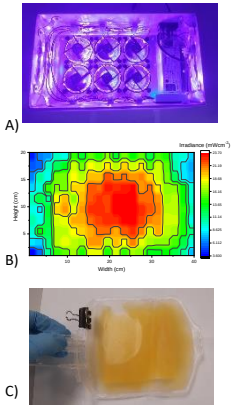
FIG 2. Small scale 405nm light treatment. A) test assembly; B) emission spectrum

SMALL-SCALE EXPOSURE SYSTEM

METHODOLOGY: Broad spectrum efficacy testing

- Plasma was seeded with a range of bacteria commonly associated with TTIs, at low cell densities (10^2-10^3 CFUmL^{-1}).
- Plasma was exposed to 360 Jcm^{-2} (1-hr at 100 mWcm^{-2}) using a small-scale exposure system (FIG 2A).
- Post-exposure, plasma samples were plated, incubated and enumerated. Reductions in viable contamination were compared to non-exposed control samples.

Organisms tested: Gram-positives: *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus.* **Gram-negatives:** *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Yersinia enterocolitica*



LARGE-SCALE EXPOSURE SYSTEM

METHODOLOGY: *T*reatment of pre-bagged plasma

- 100mL plasma in a blood transfusion bag was spiked with *S. aureus* at ~10^3 CFUmL^{-1}, and exposed to 22 mWcm^{-2}, under agitation, for 5-hr (≤396 Jcm^{-2}).
- 10mL control samples were held under identical conditions, but covered to prevent light exposure.
- Samples (3x100µL) were plated at 15-minute intervals, incubated and enumerated.
- Reductions in viable contamination were compared to non-exposed control samples.

FIG 3. Large-scale 405nm light treatment: A) Large-scale 405nm light unit B) irradiance map across the bag surface; C) Prebagged plasma

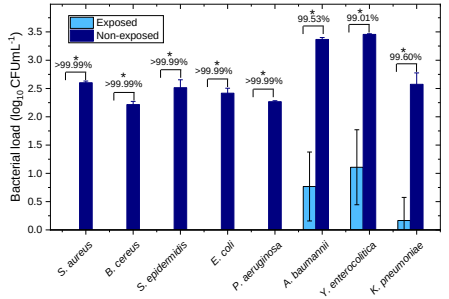


FIG 3. Small-scale exposure system, results: 405 nm light inactivation of bacterial contaminants in blood plasma at 10^2 - 10^3 CFUmL^{-1} using a dose of 360 Jcm^{-2} (n=3 ±SD; * significant difference to non-exposed control, P<0.05).

BROAD SPECTRUM EFFICACY RESULTS

- Broad-spectrum decontamination of plasma seeded at clinically-relevant bacterial contamination levels was successfully achieved.
- Significant >99.01-100% inactivation (≤5 CFUmL^{-1} remaining) was achieved after exposure to a dose of 360 Jcm^{-2} (P≤0.05).
- Minor differences between species are likely due to the slightly varying starting populations due to natural variation in densities after overnight culture of the different organisms.

BACTERIAL INACTIVATION IN PREBAGGED PLASMA BAGS

- Significant bacterial inactivation achieved after 45-mins at 22 mWcm^{-2} (59.4 Jcm^{-2}), with >60% reduction in bacterial load [P=0.033].
- Inactivation kinetics show a steady decrease in bacterial load with complete elimination (3.45-log reduction) of *S. aureus* contamination recorded after 3-hr at 22 mWcm^{-2} (237.6 Jcm^{-2}).
- No bacterial load detected for remainder of exposure period.

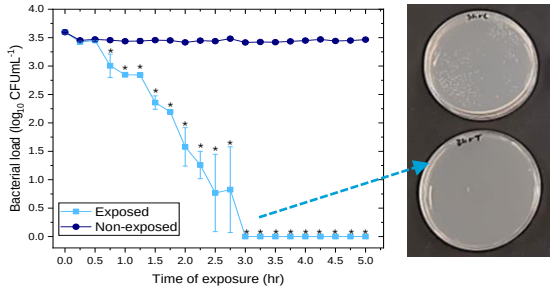


FIG 5. Large-scale exposure system, results: *In situ* treatment of prebagged plasma seeded with *S. aureus* at ~10^3 CFUmL^{-1}, to 22 mWcm^{-2} 405 nm light for 5-hours, under agitation, (≤396 Jcm^{-2}). (n=3 ±SD; *significant difference to non-exposed control, P<0.05)

CONCLUSIONS

- This study has successfully demonstrated the broad spectrum antibacterial efficacy of 405nm light for pathogen reduction of human blood plasma and further, provides evidence for *in situ* treatment of *ex vivo* stored plasma without the need for photo-sensitive agents.
- Results support further development of 405 nm light technology as a pathogen reduction technique for application in transfusion medicine.

NEXT STEPS: Perform protein integrity tests to determine optimal treatment conditions that provide germicidal efficiency without comprising blood quality.

[1] Domanović *et al., Transfusion*, 57:1311 (2017); [2] Maclean *et al., J Blood Transfus*, Article ID 2920514:1-11 (2016); [3] Maclean *et al., Front Med*, 6:331 (2020); [4] Lu *et al., J Biophotonics*,13(1):1-12 (2020); [5] Jankowska *et al., Front Med* 7:1-11 (2020).

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