405-nm Light for Bacterial Reduction in Blood Plasma:
Preliminary investigations into antimicrobial efficacy and plasma protein integrity

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BACKGROUND & AIM

A major concern in transfusion medicine is bacterial contamination of blood products as it is considered the 2nd most common cause of death from blood transfusion.[1]

Pathogen reduction technologies (PRT) can:
- ✓ **Reduce** the incidence of transfusion-transmitted infections
- ✓ **Reduce** wastage of blood products
- ✓ **Improve overall blood safety**

Violet-blue 405-nm light has shown potential to be developed as a PRT for blood plasma[2]. Violet-blue light offers:

- Broad inactivation spectrum
- Peak antibacterial wavelength in visible light region
- Safer for tissue/material exposure than UV light
- Antibacterial activity in plasma
- No additives required
- *In situ* treatment in blood bag

THIS RESEARCH:
Investigates 405-nm light treatments that provide:
1) Optimal bactericidal activity
2) Compatibility with blood plasma proteins

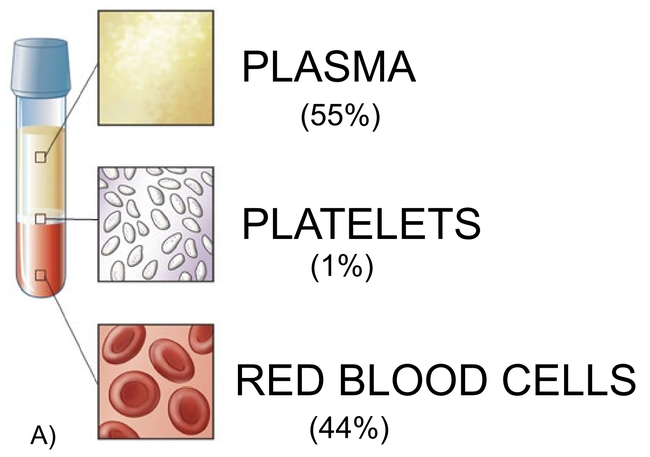


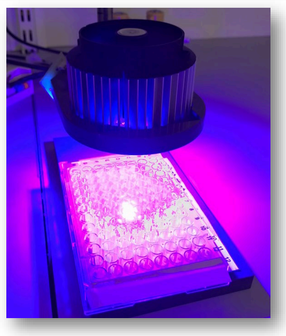
Figure 1- A) Composition of human blood[3] B) Prevalence of blood component transfusion in the United States (%)

Antibacterial Activity

Methodology:

Plasma seeded with *Staphylococcus aureus* (10^3-10^5 CFU/ml) was exposed to fixed doses of 405-nm light using low and high irradiances (10 and 100 mW/cm^2) – Fig-2B. Reductions in viable contamination were compared to non-exposed samples.

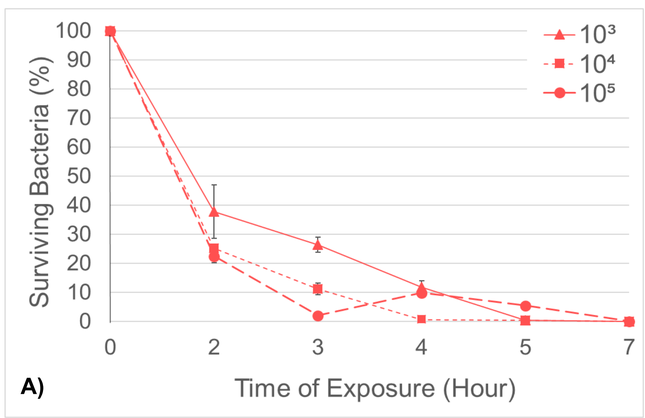
A)

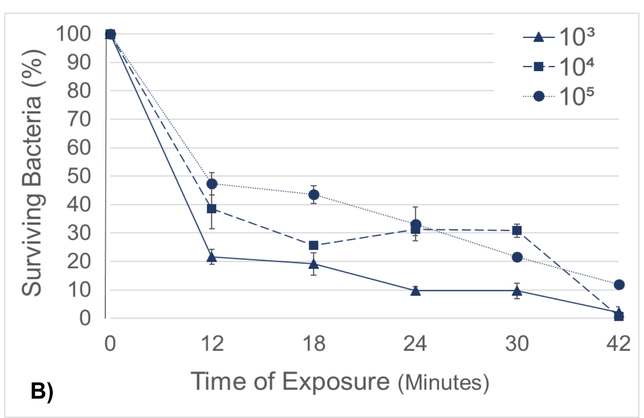


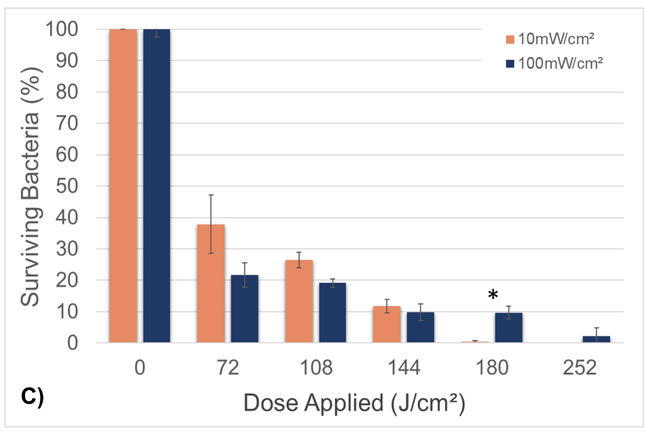
B)

Dose (J/cm^2)	Exposure time @ 10 mW/cm^2	Exposure time @ 100 mW/cm^2
72	2hr	12m
108	3hr	18m
144	4hr	24m
180	5hr	30m
252	7hr	42m

Figure 2 – 405-nm light test set up A) Exposure of seeded plasma samples to 405-nm LED array (±10nm); B) Fixed doses and treatment regimes for plasma exposure using 10 and 100 mW/cm^2







- Significant bacterial inactivation achieved after 2-hr at 10 mW/cm^2 ($P<0.05$). **>99.97% reduction** achieved for all seeding densities after 7-hr.
- A 12-min exposure to 100 mW/cm^2 **halved** the number of surviving bacteria at all levels of bacterial contamination.

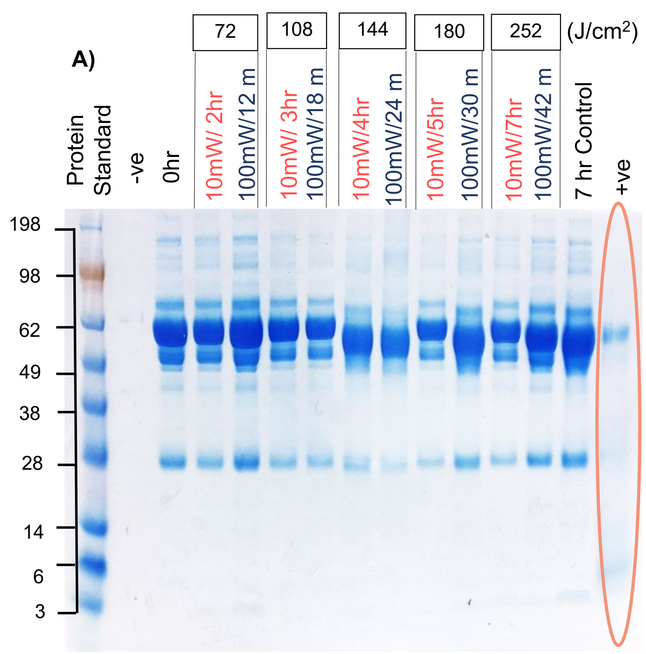
Figure 3 – Inactivation of *S. aureus* contamination *(10^3-10^5 CFU/ml)* in human plasma following exposure to A) 10 mW/cm^2 and B) 100 mW/cm^2 violet-blue light irradiances. C) Dose delivery effects on plasma seeded at 10^3 CFU/ml (*significant difference between 10 and 100 mW/cm^2 irradiances $p<0.05$). Data points represent the percentage of surviving bacteria in exposed and non-exposed plasma samples (n=3 ± SD).

Protein Stability Assessment

Methodology:

Plasma samples were exposed to 405nm light regimes (Fig-2B). Samples were then reduced and SDS-PAGE was used to analyse plasma protein integrity.

- *No major changes* to protein integrity following any of the treatment regimes.
- For comparison, complete plasma protein breakdown is highlighted in Fig-4A (+ve control; Proteinase K).
- Densitometry using ImageJ (Fig-4B, Fig-4C) suggests that plasma proteins have been retained after exposure to 252 J/cm^2 405-nm light.



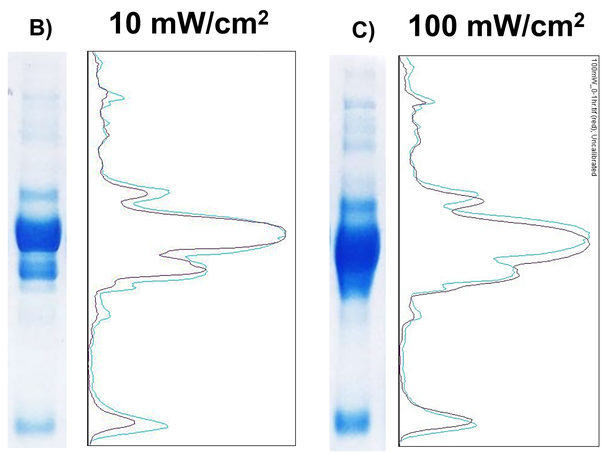
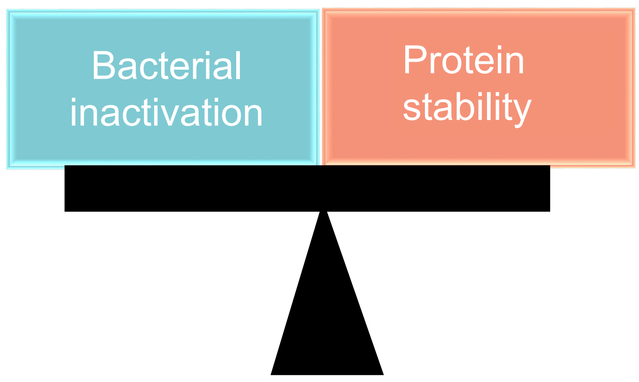


Figure 4 – A) SDS PAGE analysis of 405-nm light treated plasma samples. Quantification of protein spots observed in the 'zero hour' plasma control *versus* plasma irradiated with 252 J/cm^2 of violet-blue light via B) 10 mW/cm^2 (7hr) and C) 100 mW/cm^2 (42 min) irradiances.

Conclusions

- Both 10 mW and 100 mW/cm^2 irradiances provide significant bacterial inactivation following application of the lowest light dose used (72 J/cm^2).
- Slightly enhanced **germicidal efficiency** was evident with use of 10 mW/cm^2 irradiance (Fig 3C)
- Preliminary protein analysis *suggests* that doses ≤252 J/cm^2 do not induce major plasma protein damage.
- The method of dose delivery does not appear to effect plasma protein stability → *various in vitro characterisation tests must be conducted before safe compatibility can be confirmed.*
- This research has provided further evidence supporting the potential for 405nm-light to be developed as a pathogen reduction tool for human blood plasma.



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[b] Disclaimer: Views expressed in this article are an informal communication and represent the authors own best judgment. These comments do not bind or obligate FDA

[1] Hillyer *et al.*, Society of Heamtology, p.p 575-589 (2003)
[2] Maclean *et al., J Blood Transfus.*, Article ID 2920514, 11 pages (2016)
[3] http://healthlibrary.uchospitals.edu/Content/healthsheets-v1/when-your-child-needs-a-blood-transfusion/ [DA:01/11/19]
[4] https://www.redcrossblood.org/donate-blood/how-to-donate/how-blood-donations-help/blood-needs-blood-supply.html {DA: 31/10/19]