Photodiagnosis and Photodynamic Therapy 28 (2019) 58–64

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Photodynamic inactivation for *in vitro* decontamination of *Staphylococcus aureus* in whole blood

Thaila Quatrini Corrêa[a,b,*], Kate Cristina Blanco[b], Jennifer Machado Soares[b], Natalia Mayumi Inada[b], Cristina Kurachi[b], Marjorie de Assis Golim[c], Elenice Deffune[c], Vanderlei Salvador Bagnato[b]

[a] PPG Biotec, Federal University of São Carlos, 13565-905, São Carlos, São Paulo, Brazil
[b] São Carlos Institute of Physics, University of São Paulo, PO Box 369, 13560-970, São Carlos, São Paulo, Brazil
[c] Botucatu Medical School, São Paulo State University, 18618-687, Botucatu, São Paulo, Brazil

ARTICLE INFO

Keywords:
Photodynamic inactivation
Blood
Decontamination
Staphylococcus aureus

ABSTRACT

Background: Blood can be the target of microbial cells in the human body. Erythrocytes, platelets, and plasma concentrates in blood bags used in hemotherapy for blood transfusion are contamination targets, which can trigger serious diseases in blood. These infections can cause septicemia that can lead to death if not recognized rapidly and treated adequately. The aim of this study was to evaluate the photodynamic inactivation in the *in vitro* decontamination of *Staphylococcus aureus* in whole blood, erythrocytes and platelet-rich plasma.
Methods: Photodynamic inactivation using light doses of 10, 15 and 30 J/cm^2 at 630 nm and an hematoporphyrin-derivative photosensitizer (Photogem®) solutions at 25 and 50 μg/mL were evaluated. Toxicity of treatment was determined by hemolysis and cell viability assays. *Results:* The *S. aureus* reduction in phosphate buffered saline (PBS), whole blood, erythrocytes and platelet-rich plasma at 15 J/cm^2 and 50 μg/mL were 7.2, 1.0, 1.3 and 0.4 log CFU/mL, respectively. Quantitative and qualitative analyses were performed in whole blood samples, and Photogem® showed a low risk of hemolysis (10.7%) in whole blood. However, 100% of erythrocytes suffered hemolysis in the absence of plasma. The cell viability assay showed 13.9% of apoptosis in erythrocytes, but normal platelet viability.
Conclusion: S. aureus inactivation of whole blood samples using 50 μg/mL Photogem® and 15 J/cm^2 resulted in better outcomes, providing promising indications for treatment of bacterial contamination of blood, and in this work, alternative possibilities to apply the technique for blood decontamination are discussed.

1. Introduction

The presence of bacteria in the bloodstream may lead to sepsis, septic shock, and death [1]. The major contamination occurs by the skin flora at the site of punction, and the most frequently pathogens are Gram-positive bacteria, such as *Staphylococcus aureus*, *Propionibacterium acnes*, *Bacillus cereus* [2,3].

Blood is a present and necessary component in most of the surgical procedures. Its integrity and assurance that it is free of contaminants are one of the most important requirements for its use. The bacterial contamination in the blood components remains the most important infectious risk of blood transfusion [4].

The distribution of bacteria found in cases of transfusion-associated sepsis showed *Staphylococcus spp*. in 42% of cases, *Escherichia coli* in 9%, *Bacillus spp*. in 9%, *Salmonella spp*. in 9%, *Streptococcus spp*. in 12%, *Serratia spp*. in 8%, *Enterobacter spp*. in 7%, and other microorganisms in 4% [5]. Therefore, Gram-negative bacteria appear in a lower percentage, and there are few cases of blood components contamination with resistant bacteria, which are still unusual to be reported [6]. The most prevalent contamination in blood bags is in platelet concentrates because of this biological material is stored at room temperature (20 °C–24 °C), which is the favorable bacterial growth conditions [5].

The conventional treatments used to inactivate microorganisms in blood components were developed principally against viral contamination [7,8]. Heat treatments are not effective against some types of microorganisms in plasma, producing damage to proteins [9]. Ultraviolet light can be used to inactivate microorganisms in blood but damage in components of the blood is observed [10]. Filtration or

* Corresponding author. Present address: São Carlos Institute of Physics, University of São Paulo, Av. Trabalhador São-carlense, 400, PO Box 369, São Carlos, São Paulo, Brazil.
E-mail address: thatrini@gmail.com (T.Q. Corrêa).

https://doi.org/10.1016/j.pdpdt.2019.08.013
Received 22 January 2019; Received in revised form 5 August 2019; Accepted 9 August 2019
Available online 11 August 2019
1572-1000/

cellular lavage are physical methods which aid in the elimination of extracellular microorganisms [7]. The development of compounds with broad spectrum of action has been a problem in blood sterilization. However, the use of broad-spectrum antibiotics may assist in the development of multi-resistant bacterial strains, in addition to being able to develop allergies which are a serious problem for blood transfusion [7,11].

The search for techniques that allow blood decontamination, or keeping it decontaminated during storage, is a constant concern. The use of photonic techniques in this circumstance can be a possibility. Photodynamic inactivation (PDI) consists of the combined action of three elements: photosensitizer (PS), that is a non-toxic photosensitive molecule; source of light at a specific spectral region to activate the PS; and molecular oxygen. The combination of these three elements promotes the generation of reactive oxygen species (ROS) as free radicals and singlet oxygen that cause cytotoxic damages [12–14]. PDI has demonstrated significant inactivation effects on hepatitis B, C and influenza viruses in addition to bacteria and protozoa in the blood [8,11,15–24]. However, most of these studies concerning the inactivation of viruses or parasites in blood components, *i.e.*, in red blood cell concentrates, platelet concentrates and in fresh frozen plasma, with few studies investigating the PDI of bacteria in the whole blood.

In this study, we performed a complete analysis of PDI for *in vitro* decontamination of *Staphylococcus aureus* in whole blood, erythrocytes and platelet-rich plasma. The study aimed mainly to analyze the PDI effects that can occur in blood cells and this is certainly a first step in the direction of making photodynamic action an attractive technique for whole blood decontamination.

2. Materials and methods

2.1. Blood samples

Blood samples were provided by healthy volunteers in blood collection tubes (BD Vacutainer) containing 7.2 mg EDTA K2 anticoagulant. The tubes were homogenized immediately after collection by inversion of 5 to 8 times to avoid hemolysis and blood coagulation. Whole blood, erythrocyte and plasma samples were used in PDI assays. Erythrocytes and platelet-rich plasma were obtained from whole blood samples centrifuged at 1000 × g for 5 min at 25 °C.

2.2. Microorganism and growth conditions

Staphylococcus aureus (ATCC 25923) were cultivated in Brain Heart Infusion (BHI) at 37 °C for 16 h under shaking. After this period, the suspension was homogenized, centrifuged at 1000 × g for 15 min and resuspended in phosphate buffered saline (PBS) solution. The inoculum suspension was quantified through the optical density (Cary 50 Bio UV–vis Spectrophotometer, Varian, Australia) at 600 nm and adjusted for 10^8 CFU/mL, approximately.

2.3. Photosensitizer (PS)

The PS used was a porphyrin derivative of the first generation of PDT photosensitizers, Photogem® (Moscow, Russia). The stock solution was prepared by dissolving 5 mg of PS in 1 mL saline solution (0.9%, w/v, NaCl). From this solution, and just before the beginning of the assays, new dilutions were carried out to obtained concentrations of 25 and 50 μg/mL for PDI assays. The incubation time in the dark before samples irradiation was 30 min at 37 °C.

2.4. Irradiation

Irradiation was performed with a light-emitting diode (LED)-based device that consists of 24 emitting centers, with wavelength at 630 nm. The irradiance obtained with this device was 30 mW/cm^2. The light doses applied were 10, 15 and 30 J/cm^2, at exposure times of 5.56, 8.34 and 16.68 min, respectively.

2.5. PDI assays

PDI assays consisted of illuminating *S. aureus* suspensions incubated with Photogem® in four different media: PBS, whole blood, erythrocytes, and platelet-rich plasma. In each experiment, there were three control groups and one treatment group: [1] control ($L_{(-)}PS_{(-)}$); [2] light control ($L_{(+)}PS_{(-)}$); [3] PS control ($L_{(-)}PS_{(+)}$); and [4] PDI ($L_{(+)}PS_{(+)}$), in which (–) represents light and/or PS absence and (+) represents light and/or PS presence.

All experiments were performed in 24-well plates containing 200 μL of bacterial inoculum in 200 μL of PS solution or saline solution (0.9%, w v, NaCl), depending on the group evaluated. The final volume of each well was 400 μL. In the $L_{(-)}PS_{(-)}$ group, the samples without PS were protected from light during the experiment. In the $L_{(+)}PS_{(-)}$ group, the samples without PS were exposed to 630 nm wavelength with light doses of 10, 15 and 30 J/cm^2. In the $L_{(-)}PS_{(+)}$ group, the samples with PS at final concentrations of 25 and 50 μg/mL were protected from light during the experiment. And, in the $L_{(+)}PS_{(+)}$ group, the samples with PS were exposed to 630 nm wavelength at the same conditions described above.

The efficiency of PDI was evaluated through the quantification of viable microorganisms by colony-forming unit (CFU) before and after treatment. For this, 100 μL of each sample were transferred into microtubes containing 900 μL of PBS to carry out the serial dilution until 10^{-5}. Aliquots of 25 μL of all dilutions of samples were uniformly spread to Petri dishes with BHI agar in triplicate. Plates were maintained at 37 °C for 48 h to carry out the counts of CFU. Three independent experiments were performed for each experimental condition.

2.6. Hemolysis assays

For the hemolysis assays, the whole blood was centrifuged (1000 × g for 5 min) to remove platelet-rich plasma and the leukocyte layer, washed three times with PBS solution and centrifuged again. Erythrocytes were resuspended in saline solution (0.9%, w/v, NaCl) to be used in PDI assays as described in the previous section. After treatments, the erythrocytes were maintained at 4 °C in the dark for 24 h. Thereon, the samples were centrifuged (1000 × g for 5 min) and 50 μL of supernatant were diluted in 10 mL of distilled water. The hemoglobin content was determined by measuring the absorbance at 413 nm. Results were expressed as percentage of hemolysis based on absorbance obtained from lysed cells in distilled water (100% hemolysis) [24]. Besides the absorbance measurements performed on the supernatants of erythrocytes in absence of plasma, absorbance measurements were also performed on the supernatants of erythrocytes in presence of plasma, *i.e.*, in whole blood, following the same described protocol.

2.7. Quantitative and qualitative analysis of whole blood

Assays were performed to analyze quantitatively and qualitatively the whole blood after treatments, following the same PDI assays experimental design. For this analysis, Photogem® concentration and light dose chosen were 50 μg/mL and 15 J/cm^2, respectively. After treatment, blood samples from all groups were taken to an automatic counter of the Hematology Department of Maricondi Laboratory (São Carlos/SP) for quantitative evaluation of cells by blood counts. Results were compared to the reference values of the cells and blood constituents for women, according to the donor volunteers of this study. Qualitative and morphological evaluation of erythrocytes and leukocytes cells were performed by blood smear blades stained with May Grünwald-Giemsa using optical microscopy.

2.8. Cell viability analysis by flow cytometry

For this analysis, erythrocytes and platelets were obtained from whole blood by centrifugation (1000 × g for 5 min) to be used in PDI assays. Photogem® concentration and light dose were 50 μg/mL and 15 J/cm^2, respectively. After treatment, cells were labeled with annexin-V and propidium iodide following manufacturer instructions. Erythrocytes and platelets were diluted in saline solution (0.9%, w/v, NaCl) to obtain 1 × 10^6 cells. Samples were resuspended in 100 μL of buffer (Pharmingen BD Kit) after centrifugation. All samples, except the auto-fluorescence control, received 2.5 μL of annexin-V and 3 μL of propidium iodide. After 15 min incubation at 25 °C, in the dark, 400 μL of buffer were added to all samples.

The cells were analyzed in a FACSCalibur flow cytometer equipped with an argon laser and PAINT-A-GATE BD software (Becton Dickinson, San Jose, CA, USA), at the Cellular Engineering Laboratory of the Botucatu Medical School, Blood Transfusion Center, (UNESP, Botucatu/SP, Brazil). Ten thousand events were collected for each sample. The populations of erythrocytes and platelets were identified by their light-scattering characteristics, and analyzed for the intensity of the fluorescent probe signal.

2.9. Statistical analysis

Results were expressed as mean ± standard deviation of measurement. The normality of the data was tested by the Kolmogorov-Smirnov normality test before analysis. Statistical significance was assigned at a value of $p < 0.05$. The comparisons between all experimental groups were performed by ANOVA followed by the Tukey post hoc test.

3. Results

3.1. PDI assays

The results shown in Fig. 1 demonstrated that photoinactivation pattern of *S. aureus* in the presence of Photogem® is different for experiments were performed in PBS, whole blood, erythrocytes and platelet-rich plasma. In PBS, the combination of light and PS resulted in a relevant decrease of the microorganism viability. The absence of either light or PS resulted in no variation of microorganism. On the opposite, in whole blood or erythrocytes, the decreases were only more sensitive at the larger concentrations of PS and high light dose.

To be quantitative, the *S. aureus* viability in PBS was reduced below the detection limit of the method after a light dose of 30 J/cm^2 in the presence of Photogem® at 50 μg/mL. The same Photogem® concentration reduced the *S. aureus* by 7.2 log CFU/mL after light dose of 15 J/cm^2. This inactivation condition was not achieved when the experiments were performed in blood components. The same conditions used in the assays conducted in whole blood, erythrocytes, and platelet-rich plasma were not effective to photo-inactivate *S. aureus*. Photogem® at 50 μg/mL reduced the *S. aureus* viability in whole blood by 1.0 log CFU/mL after light dose of 15 J/cm^2. In erythrocytes and platelet-rich plasma, *S. aureus* reductions were 1.3 and 0.4 log CFU/mL, respectively, after the same conditions applied in whole blood. Although PDI was not so effective to photo-inactivate the microorganism in blood components, these reduction results presented statistical differences from their control groups. The light and PS controls showed that the *S. aureus* viability was affected neither by light alone (10, 15 and 30 J/cm^2) nor by Photogem® alone (25 and 50 μg/mL) in PBS, whole blood and platelet-rich plasma.

3.2. Hemolysis assays

Fig. 2 shows the percentage of hemolysis in treated blood samples in whole blood (A) and in absence of plasma (B). For erythrocytes in whole blood, the percentage of hemolysis in control, light control and PS control groups were low, with values from 1 to 1.5%. PDI group presented values from 1.6 to 10.7%, but still, they are considered low values (Fig. 2A). For erythrocytes in absence of plasma, there was a significant difference between control and PS control group with 50 μg/mL. Moreover, hemolysis in PDI group increased with increasing light dose and PS concentration and the hemolysis was 100% with 15 J/cm^2 and 50 μg/mL (Fig. 2B).

3.3. Quantitative and qualitative analysis of whole blood

Table 1 shows the quantitative results of blood cells before and after treatment with light dose of 15 J/cm^2 and Photogem® at 50 μg/mL. In this condition, PDI did not cause damage to the cellular constituents of whole blood. Morphological changes of erythrocytes, white blood cells, and platelets were not observed (data not shown). Accordingly, qualitative analysis confirmed the results obtained by the blood count.

3.4. Cell viability analysis by flow cytometry

The results of viability of erythrocytes isolated from the whole blood are shown in Fig. 3. For control group (Fig. 3A) and PS control group (Fig. 3B) the viability were 99.9% and 99.7%, respectively. After PDI using 50 μg/mL Photogem® and light dose of 15 J/cm^2, a significant displacement of the cell population into two distinct groups was observed in a dot plot graph: one with normal size (FSC-H) and granularity (SSC-H), and another with both lower size and granularity (data not shown). Both populations were gated and analyzed separately (Fig. 3C and D).

At Fig. 3C, corresponding to the cellular population gated without reduced size and granularity, it was observed an increase in the fluorescence detected by the FL-3 channel, corresponding to the fluorescence detection channel of the propidium iodide. However, this fact occurred only due to the Photogem® fluorescence interference. This phenomenon was also observed for another gated population corresponding to the cells with reduced size and granularity (Fig. 3D). The level of apoptosis detected in these cell populations was 13.9%.

For platelets, the results of viability are shown in Fig. 4. For control group (Fig. 4A) and PS control group (Fig. 4B) the viability were 92.6% and 96.5%, respectively. After PDI, the viability was 95.9% (Fig. 4C). Under the conditions evaluated, PDI did not cause damage to these cell fragments.

4. Discussion

This study aimed to evaluate Photogem® to photo-inactivate the bacteria *S. aureus* in whole blood, erythrocytes and platelet-rich plasma. Many classes of PSs have been tested to inactivate microorganisms by PDI, which has been considered an alternative technique to decontaminate some blood components. Photogem® was chosen because of its effectiveness in inactivating different types microorganisms [25–29]. Moreover, it has a range of applications in biological systems, such as cancer treatment and microbial inactivation due to the absence of toxicity without light, ability to absorb various wavelengths and generation of good amounts of ROS [30].

The inactivation of *S. aureus* in whole blood was greatly reduced in comparison to inactivation of bacteria in PBS (Fig. 1A). The difference between the results can be explained by the variety of molecules present in the blood. Blood is a complex matrix that contains biomolecules such as proteins and different cell types (erythrocytes, lymphocytes, and platelets). The formation of aggregates between the PS molecules is a problem and can damage the photodynamic action, since it drastically reduces the PS's ability to generate ROS. The aggregates decrease the lifetime of the PS singlet and triplet states, reducing their quantum yield with consequent loss of their efficiency. These aggregates are also formed when the PS concentration increases [31]. Another problem that may explain the reduced results of inactivation in whole blood is

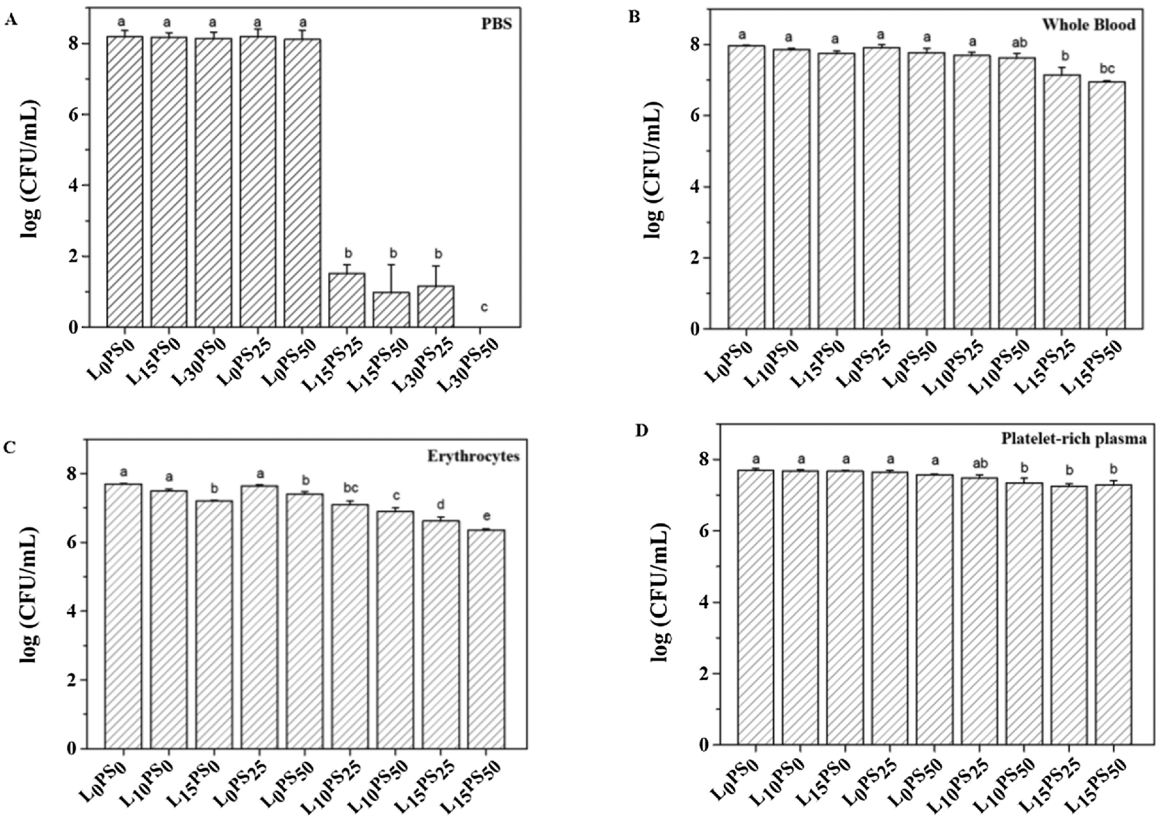

Fig. 1. Log (CFU/mL) of *S. aureus* photoinactivation in PBS (A), whole blood (B), erythrocytes (C) and platelet-rich plasma (D). Photogem® (PS) at 25 and 50 μg/mL; red light (L) at 15 and 30 J/cm^2 (PBS conditions), 10 and 15 J/cm^2 (blood conditions). Irradiance: 30 mW/cm^2. All results represent the mean ± standard deviation of three independent assays. Different letters above the bars (*a, b, c, d, e, ab, bc*) represent the significant difference compared between each experimental group ($p < 0.05$; ANOVA and Tukey test).

the nonspecific binding of PS to proteins and cells. The nonspecific binding of PS may also decrease the efficiency of the photodynamic inactivation process, and our hypothesis is that the PS molecules bound with plasma proteins.

Although the reduction of 1.0 log CFU/mL of *S. aureus* seems to be no significative when are discussed bacteria inactivation (Fig. 1B), studies have shown that the number of bacteria present in the blood of adult patients with significant bloodstream infections ranges from 1 and 10 CFU/mL to 1×10^3 and 1×10^4 CFU/mL. The magnitude of bacteremia is generally higher in children than in adults, reaching 1×10^3 CFU/mL of blood [32–34]. Based on this, PDI could be an alternative for blood controlling microorganisms until the patient's immune system recruits defense cells to help decrease the infection.

PDI of *S. aureus* in erythrocytes (1.3 log CFU/mL, 15 J/cm^2, 50 μg/mL) was more efficient than in plasma (0.4 log CFU/mL, 15 J/cm^2, 50 μg/mL) (Fig. 1C and D). According to these results, plasma appears to be the major blood component responsible for hindering and preventing the inactivation process of bacteria in the blood. Plasma is essentially an aqueous solution with proteins and several molecules in suspension. These molecules can be the main impediment to the PDI success since the PS may first interact with them, preventing its bioavailability to the photodynamic reaction and reducing the effectiveness of the technique. In addition, the PS molecules binding to plasma proteins makes them unable to be exposed to the microorganisms.

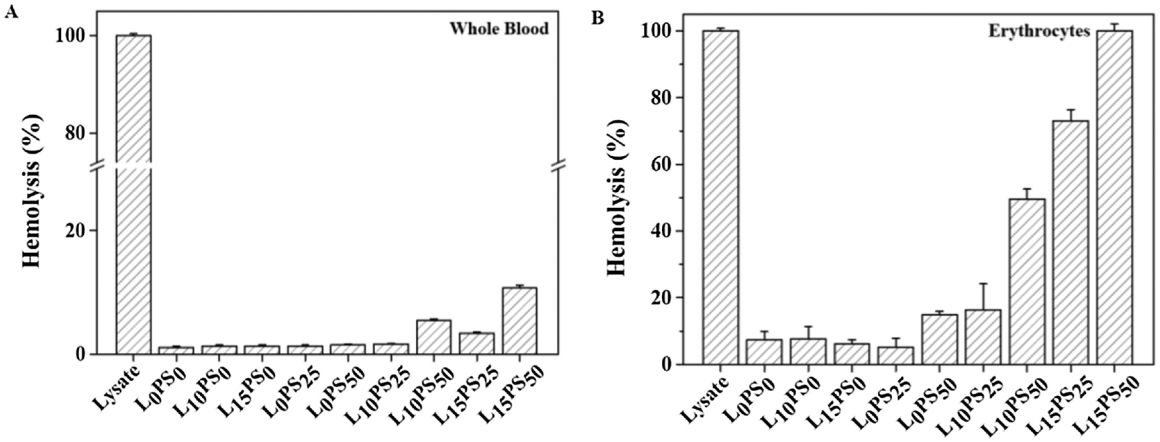

Fig. 2. Percentage of hemolysis of erythrocytes in whole blood (A) and in the absence of plasma (B) with Photogem® (PS) at 25 and 50 μg/mL and red light (L) at 10 and 15 J/cm^2. All results represent the mean ± standard deviation of three independent assays.

Table 1
Quantitative analysis of whole blood with Photogem® (PS) at 50 μg/mL and red light (L) at 15 J/cm^2. The reference values shown in the table are for women. All results represent the mean ± standard deviation of three independent assays.

	Reference values	L_0PS_0	$L_{15}PS_0$	L_0PS_{50}	$L_{15}PS_{50}$
Erythrocytes	4.2 a 5.4 millions/mm^3	4.2 ± 0.1	4.0 ± 0.5	4.7 ± 0.6	4.6 ± 0.4
Hemoglobin	12 a 16 g/dL	11.9 ± 0.4	11 ± 2	14 ± 2	13 ± 1
Hematocrit	35 a 47%	35 ± 1	34 ± 5	40 ± 5	39 ± 3
Leukocytes	4,000 a 10,000/mm^3	5,600 ± 800	6,000 ± 500	5,500 ± 200	5,400 ± 900
Eosinophils	50 a 400/mm^3	200 ± 50	220 ± 60	200 ± 30	240 ± 50
Neutrophils	1,800 a 7000/mm^3	3,700 ± 500	3,400 ± 400	3,600 ± 200	3,400 ± 400
Lymphocytes	1,500 a 4,000/mm^3	1,400 ± 200	1,600 ± 300	1,300 ± 300	1,400 ± 400
Monocytes	150 a 800/mm^3	330 ± 60	370 ± 60	270 ± 60	330 ± 60
Platelets	150,000 a 450,000/mm^3	188,000 ± 30,000	206,000 ± 10,000	167,000 ± 40,000	172,000 ± 40,000

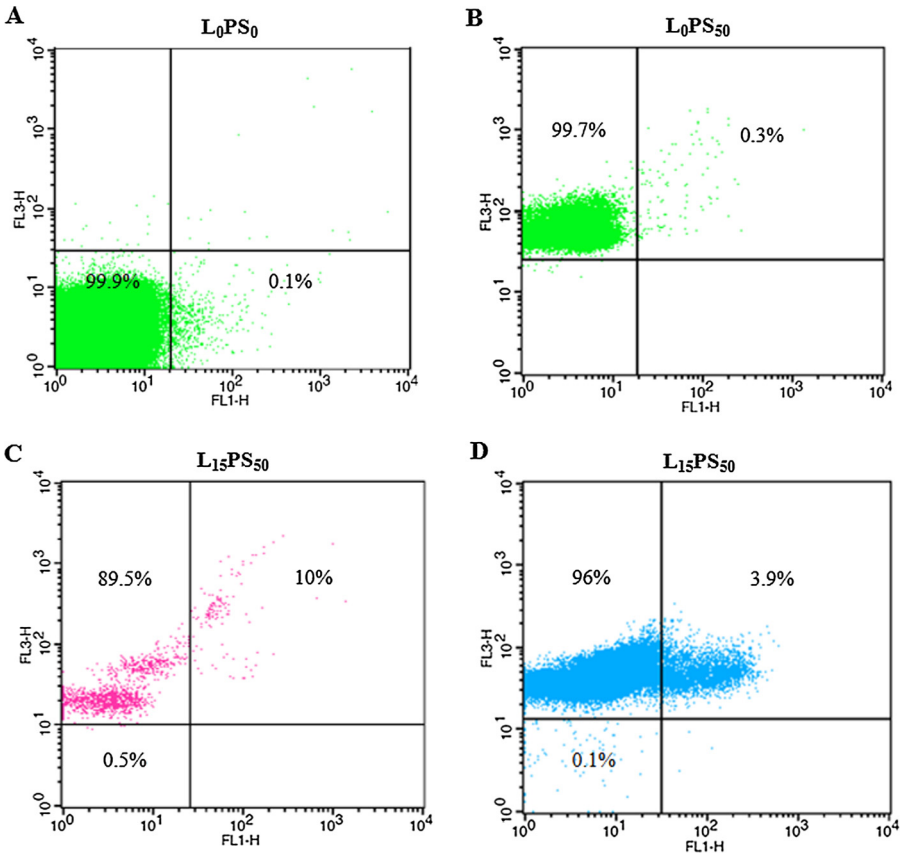

Fig. 3. Flow cytometry showing the dot plots with erythrocyte viability and apoptosis percentages in (A) control (L_0PS_0), (B) Photogem® 50 μg/mL (L_0PS_{50}), (C) normal size erythrocytes after PDI with Photogem® 50 μg/mL and 15 J/cm^2 ($L_{15}PS_{50}$), and (D) erythrocytes with reduced size after PDI with Photogem® 50 μg/mL and 15 J/cm^2 ($L_{15}PS_{50}$).

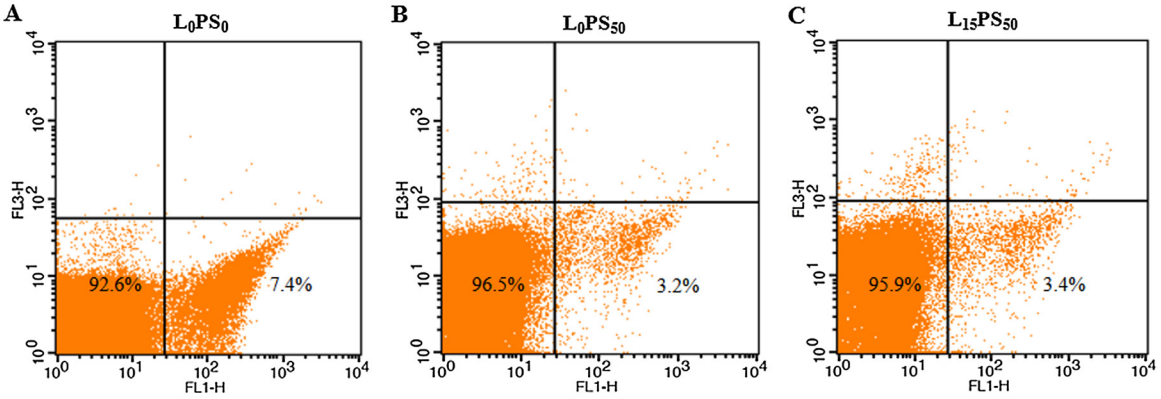

Fig. 4. Flow cytometry showing the dot plots with platelets viability and apoptosis percentages in (A) control (L_0PS_0), (B) Photogem® 50 μg/mL (L_0PS_{50}), and (C) PDI with Photogem® 50 μg/mL and 15 J/cm^2 ($L_{15}PS_{50}$).

These results of inactivation are in agreement with the study of Spesia et al. (2010), which also observed lower inactivation of bacteria in the presence of erythrocytes and plasma compared to saline solution [24]. The microbial reduction rate of *E. coli* and *S. aureus* were similar to this study. However, the hemolysis values reported in some studies were higher than those reported here. Marciel et al. (2017) showed inactivation of Gram-positive and Gram-negative bacteria in the presence of blood components using a tri-cationic porphyrin (Tri-Py^{+}-Me-PF). The efficiency of the selected PS to inactivate *E. coli* and *S. aureus* decreased with the increase in sample complexity, from PBS to whole blood. Nevertheless, the authors inactivated bacteria modifying the PS concentration and light doses [11].

Concerning of hemolysis observed in the PDI groups in the absence of plasma, PDI occurred in an environment where only erythrocytes and microorganism were present, so Photogem® interacted strongly with the cell membrane, attached to them. This interaction may justify the high percentage of hemolysis found in this situation under the conditions studied. These results are also explained by flow cytometry analysis, once was observed that PDI is acting on the erythrocyte membrane by deregulating the ionic pump and forming pores in the membrane, allowing the extravasation of the intracellular material and induced apoptosis by the phosphatidylserine externalization (Fig. 3C and D). In the absence of plasma, Photogem® interacts substantially with erythrocytes, mainly in the membrane of these cells.

It is known that some plasma components are responsible for protecting erythrocytes against hemolysis caused by mechanical trauma and hypotonicity. The albumin is the main plasma component responsible for this protection [35]. Thus, when the whole blood is separated into different blood components, the protective effect of plasma on erythrocytes is lost, and the erythrocytes become vulnerable to the effects of the photodynamic action. Lower hemolysis values were observed in the control groups for both whole blood and erythrocytes (Fig. 2). Hemolysis in low proportion is a normal and intrinsic process of the organism. About 0.8–1% of human red blood cells is hemolyzed daily which is offset by the production of new cells [36].

Regarding the effect of Photogem®-mediated photodynamic inactivation in the platelets present in the plasma, the results obtained from the flow cytometry analysis (Fig. 4) show that these cell fragments did not change their viability. These results make it possible to apply PDI for microbial reduction in this blood component.

Therefore, the use of this photonic method may be a possibility to eliminate microorganisms present in blood, preserving its characteristics. Nevertheless, PDI needs to present biological safety to be considered a technique with great potential for practical application. The biological safety of PDI relates to the choice of the PS and the dosimetry employed in the treatment, in order to minimize the damage to the non-target cells. This study evidences which PDI conditions result in damage to blood cells and which are ideal for preserving their functions, discussing the maintenance of the integrity of the blood constituents when subjected to PS and light. Thus, our results allow setting limitation and use conditions for PDI application in decontamination of whole blood.

5. Conclusion

The current study had established the characteristics when Photogem®-mediated photodynamic inactivation is applied in whole blood and its components. In this study, the decontamination process with Photogem® was possible depending on the blood component present during PDI. Safety is the primary consideration of any treatment and we reported the possibility of decontaminating whole blood with little hemolysis. In summary, this is certainly a first step in the direction of making photodynamic action an attractive technique for whole blood decontamination, aiming to be an alternative that exhibits the potential to the treatment of blood infection and sepsis. However, further studies are required to improve established conditions and to increase the efficiency of bacteria reduction in whole blood using PDI.

Declaration of Competing Interest

None.

Acknowledgments

The authors acknowledge the financial support provided by São Paulo Research Foundation (FAPESP) – grant number: 2013/07276-1 (CePOF – CePID Program), the National Council for Scientific and Technological Development (CNPq), and the Coordination for the Improvement of Higher Education Personnel (CAPES) for TQC's fellowship – grant number: 1500213.

References

[1] Uppu DSSM, C. Ghosh, J. Haldar, Surviving sepsis in the era of antibiotic resistance: are there any alternative approaches to antibiotic therapy? Microb. Pathog. 80 (2015) 7–13.
[2] M.E. Brecher, N. Means, C.S. Jere, D. Heath, S. Rothenberg, L.C. Stutzman, Evaluation of an automated culture system for detecting bacterial contamination of platelets: an analysis with 15 contaminating organisms, Transfusion 41 (4) (2001) 477–482.
[3] S. Zaza, J.I. Tokars, R. Yomtovian, N.V. Hirschler, M.R. Jacobs, H.M. Lazarus, et al., Bacterial contamination of platelets at a university hospital: increased identification due to intensified surveillance, Infect. Control Hosp. Epidemiol. 15 (2) (2014) 82–87.
[4] M.E. Brecher, S.N. Hay, Bacterial contamination of blood components, Clin. Microbiol. Rev. 18 (1) (2005) 195–204.
[5] D. Védy, D. Robert, G. Canellini, S. Waldvogel, J. Tissot, Bacterial contamination of platelet concentrates: pathogen detection and inactivation methods, Hematol. Rep. 1 (1) (2009) 5.
[6] S. Sapatnekar, E.M. Wood, J.P. Miller, M.R. Jacobs, M.J. Arduino, S.K. McAllister, et al., Methicillin-resistant Staphylococcus aureus sepsis associated with the transfusion of contaminated platelets: a case report, Transfusion 41 (11) (2001) 1426–1430.
[7] M. Wainwright, Pathogen inactivation in blood products, Curr. Med. Chem. 9 (1) (2002) 127–143.
[8] M.J. Casteel, K. Jayaraj, A. Gold, L.M. Ball, M.D. Sobsey, Photoinactivation of hepatitis A virus by synthetic porphyrins, Photochem. Photobiol. 80 (2) (2004) 294–300.
[9] T. Burnouf, M. Radosevich, Reducing the risk of infection from plasma products: specific preventative strategies, Blood Rev. 14 (2) (2000) 94–110.
[10] M.S. Baptista, M. Wainwright, Photodynamic antimicrobial chemotherapy (PACT) for the treatment of malaria, leishmaniasis and trypanosomiasis, Braz. J. Med. Biol. Res. 44 (1) (2011) 1–10.
[11] L. Marciel, L. Teles, B. Moreira, M. Pacheco, L.M.O. Lourenço, M.G.P.M.S. Neves, et al., An effective and potentially safe blood disinfection protocol using tetrapyrrolic photosensitizers, Future Med. Chem. 9 (4) (2017) 365–379.
[12] L. Huang, T. Dai, M.R. Hamblin, Antimicrobial photodynamic inactivation and photodynamic therapy for infections, Methods Mol. Biol. 635 (8) (2010) 155–173.
[13] T.G. St. Denis, T. Dai, L. Izikson, C. Astrakas, R.R. Anderson, M.R. Hamblin, et al., All you need is light: antimicrobial photoinactivation as an evolving and emerging discovery strategy against infectious disease, Virulence 2 (6) (2011) 509–520.
[14] M.R. Hamblin, Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes, Curr. Opin. Microbiol. 33 (2016) 67–73.
[15] E. Ben-Hur, H. Margolis-Nunno, P. Gottlieb, S. Lustigman, B. Horowitz, Photodynamic decontamination of blood for transfusion, Photodyn. Ther. Cancer II 2325 (1995) 166–173.
[16] P. Gottlieb, H. Margolis-Nunno, R. Robinson, L.G. Shen, E. Chimezie, B. Horowitz, et al., Inactivation of Trypanosoma cruzi trypomastigote forms in blood components with a psoralen and ultraviolet A light, Photochem. Photobiol. 63 (5) (1996) 562–565.
[17] S. Lustigman, E. Ben-Hur, Photosensitized inactivation of Plasmodium falciparum in human red cells by phthalocyanines, Transfusion 36 (6) (1996) 543–546.
[18] E. Ben Hur, W.S. Chan, Z. Yim, M.M. Zuk, V. Dayal, N. Roth, et al., Photochemical decontamination of red blood cell concentrates with the silicon phthalocyanine PC 4 and red light, Dev. Biol. Stand. 102 (1999) 149–155.
[19] M. Wainwright, Methylene blue derivatives - suitable photoantimicrobials for blood product disinfection? Int. J. Antimicrob. Agents 16 (4) (2000) 381–394.
[20] H. Mohr, B. Bachmann, A. Klein-Struckmeier, B. Lambrecht, Virus inactivation of blood products by phenothiazine dyes and light, Photochem. Photobiol. 65 (3) (1997) 441–445.
[21] H.G. Klein, Pathogen inactivation technology: cleansing the blood supply, J. Intern. Med. 257 (3) (2005) 224–237.
[22] S.J. Wagner, J.R. Storry, D.A. Mallory, R.R. Stromberg, L.E. Benade, L.I. Friedman, Red cell alterations associated with virucidal methylene blue phototreatment, Transfusion 33 (1) (1993) 30–36.
[23] S.J. Wagner, A. Skripchenko, D. Robinette, D.A. Mallory, L. Cincotta, Preservation of red cell properties after virucidal phototreatment with dimethylmethylene blue, Transfusion 38 (8) (1998) 729–737.
[24] M.B. Spesia, M. Rovera, E.N. Durantini, Photodynamic inactivation of Escherichia

coli and Streptococcus mitis by cationic zinc(II) phthalocyanines in media with blood derivatives, Eur. J. Med. Chem. 45 (6) (2010) 2198–2205.
[25] L.N. Dovigo, A.C. Pavarina, E.G. de Oliveira Mima, E.T. Giampaolo, C.E. Vergani, V.S. Bagnato, Fungicidal effect of photodynamic therapy against fluconazole-resistant Candida albicans and Candida glabrata, Mycoses 54 (2) (2011) 123–130.
[26] M.M. Gois, C. Kurachi, E.J.B. Santana, E.G.O. Mima, D.M.P. Spolidório, J.E.P. Pelino, et al., Susceptibility of Staphylococcus aureus to porphyrin-mediated photodynamic antimicrobial chemotherapy: an in vitro study, Lasers Med. Sci. 25 (3) (2010) 391–395.
[27] J.M. Soares, T.Q. Corrêa, N.M. Inada, V.S. Bagnato, K.C. Blanco, In vitro study of photodynamic therapy for treatment of bacteremia in whole blood, J. Pharm. Pharmacol. 6 (9) (2018) 863–869.
[28] L.M. Souza, N.M. Inada, S. Pratavieira, J.J. Corbi, C. Kurachi, V.S. Bagnato, Efficacy of Photogem® (Hematoporphyrin Derivative) as a photoactivatable larvicide against Aedes aegypti (Diptera: Culicidae) larvae, J. Life Sci. 11 (2) (2017) 74–81.
[29] H.A. Ricci Donato, S. Pratavieira, C. Grecco, A. Brugnera-Júnior, V.S. Bagnato, C. Kurachi, Clinical comparison of two photosensitizers for oral cavity decontamination, Photomed. Laser Surg. 35 (2) (2016) 105–110.
[30] S. Pratavieira, P.L.A. Santos, P.F.C. Menezes, C. Kurachi, C.H. Sibata, M.T. Jarvi, et al., Phototransformation of hematoporphyrin in aqueous solution: anomalous behavior at low oxygen concentration, Laser Phys. 19 (6) (2011) 1263–1271.
[31] I.J. MacDonald, T.J. Dougherty, Basic principles of photodynamic therapy, J. Porphyr. Phthalocyanines 5 (2) (2001) 105–129.
[32] P. Yagupsky, F.S. Nolte, Quantitative aspects of septicemia, Clin. Microbiol. Rev. 3 (3) (1990) 269–279.
[33] O. Opota, A. Croxatto, G. Prod'hom, G. Greub, Blood culture-based diagnosis of bacteraemia: state of the art, Clin. Microbiol. Infect. 21 (4) (2015) 313–322.
[34] O. Opota, K. Jaton, G. Greub, Microbial diagnosis of bloodstream infection: towards molecular diagnosis directly from blood, Clin. Microbiol. Infect. 21 (4) (2015) 323–331.
[35] T. Butler, C.A. Bradley, J.E. Owensby, Plasma components protect erythrocytes against experimental haemolysis caused by mechanical trauma and by hypotonicity, Int. J. Exp. Pathol. 73 (1) (1992) 27–33.
[36] S.O. Sowemimo-Coker, Red blood cell hemolysis during processing, Transfus. Med. Rev. 16 (1) (2002) 46–60.