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Optical techniques for the microbiological control of blood

Thaila Quatrini Corrêa[a,b]; Kate Cristina Blanco[b], Natalia Mayumi Inada[b], Cristina Kurachi[b] and Vanderlei Salvador Bagnato[a,b]

[a] Biotechnology Graduate Program, Federal University of São Carlos, SP, Brazil; [b] São Carlos Institute of Physics, University of São Paulo, PO Box 369, 13560-970, São Carlos, SP, Brazil

ABSTRACT

Blood can be the target of bacterial, viral and parasitic contamination, which can trigger serious diseases. In this study, photodynamic inactivation and ultraviolet radiation were evaluated in the *in vitro* decontamination of whole blood, erythrocytes, and platelet-rich plasma with *S. aureus*. For PDI, Photogem® and 630 nm light were evaluated, and risks of toxicity of the treatment were determined by hemolysis and cell viability assays. The reductions of *S. aureus* in whole blood, erythrocytes, and platelet-rich plasma at 15 J/cm^2 and 50 μg/mL porphyrin were 1.0 log, 1.3 log and 0.4 log CFU/mL, respectively. Hemolysis rate for erythrocytes in whole blood was 10.7%. However, erythrocytes hemolysis was 100% when in the absence of plasma. The cell viability assay showed 14% apoptosis rates in isolated erythrocytes, indicating damaging action of PDI, and no damage in platelet. For UVC radiation (254 nm), different light doses were analyzed, and the cell viability assay determined the toxicity of technique. The reductions of *S. aureus* in whole blood, erythrocytes and platelet-rich plasma at 23 J/cm^2 were 1.7 log, 1.1 log and 2.5 log CFU/mL, respectively. Relatively small differences were observed in plasma as a function of irradiation time, suggesting some degradation of plasma proteins with 23 J/cm^2. The cell viability assay showed normal rates for erythrocytes, however, in the platelets, a high apoptosis rate was observed (74%). Therefore, the optical techniques showed opposite damage effects in each blood component, and the use of one or another technique should be evaluated considering the better microbial inactivation and blood components preservation conditions.

Keywords: Photodynamic inactivation, Ultraviolet radiation, Blood, Erythrocytes, Platelet-rich plasma.

1. INTRODUCTION

The growing antibiotic resistance is one of the most important clinical challenges facing society today. This problem is everywhere, including solid-organ transplantations and blood transfusions. The presence of bacteria in the bloodstream can cause severe systemic inflammation and lead to sepsis, septic shock and death.[1] Incidence of sepsis has risen over the years and it is mainly due to the selection of resistant microorganisms to antibiotics and its inadequate use.[2]

All blood components can present contamination: red blood cells, white blood cells, platelet and plasma. Most bacterial species can rapidly proliferate in blood components reaching high levels within few hours.[3] Therefore, blood decontamination is being studied in order to increase the security of the blood supply.

In this study, photodynamic inactivation and ultraviolet radiation were evaluated in the *in vitro* decontamination of whole blood, erythrocytes, and platelet-rich plasma with *Staphylococcus aureus*.

Photodynamic inactivation (PDI) consists of the combined action of three elements: a non-toxic photosensitive molecule named photosensitizer (PS); a source of light at a specific spectral region to activate the PS; and the molecular oxygen.

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The combination of these three elements promotes the generation of reactive oxygen species (ROS) such as free radicals and singlet oxygen that cause cytotoxic damages.[4–6]

The PS chosen in this study was Photogem® (Limited Liability Company Photogem, Moscow, Russia). It is an approved PS for use in humans. The chemical structure of Photogem® is shown in Figure 1.

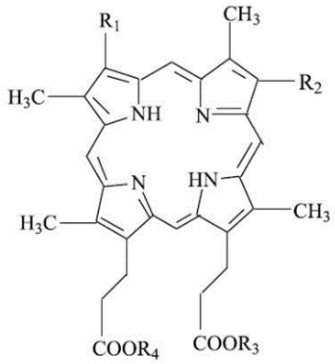

Figure 1: Chemical structure of Photogem®. The monomeric chemical structure of Photogem® has the formula represented by $[C_{34}H_{38}N_4O_6]_n$.

Ultraviolet C (UVC) radiation is considered the optimal germicidal range for microorganisms inactivation, as it is absorbed by the genetic material of bacteria, fungi, and viruses, rendering them incapable of replicating and causing diseases.[7] It has been used in the control of many microorganisms in a variety of application areas, such as water disinfection, sterilization of environments and air decontamination, sterilization of hospital rooms, surgical centers, in the production of medicines and food, and in the surfaces decontamination.[8–12]

UVC can also be used to decontaminate blood and its components. It was widely used in medical practice in the 1940s and 1950s to treat several serious diseases of infectious origin, such as tuberculosis, pneumonia and sepsis.[13,14]

The PDI and UVC were applied in this study to inactivate, reduce or eliminate microorganisms of the whole blood and its components. To evaluate the blood cells after the application of these optical techniques, hemolysis and cell viability assays were performed.

2. MATERIAL AND METHODS

2.1 Blood samples

Blood samples were provided by healthy volunteers and performed on blood collection tubes (BD Vacutainer) containing anticoagulant EDTA K2 7.2 mg. The tubes were carefully homogenized immediately after collection by inversion of 5 to 8 times to avoid hemolysis and blood clotting. To obtain the erythrocytes and the platelet-rich plasma, the whole blood samples were centrifuged at 1,000 × g for 5 minutes at 25 °C.

2.2 Microorganism

Staphylococcus aureus (ATCC 25923) was grown in Brain Heart Infusion (BHI) medium at 37 °C for 16 hours under shaking. After, the sample was homogenized, centrifuged at 1,000 × g for 15 minutes at 25 °C and resuspended in phosphate buffered saline (PBS) solution. The suspension density was measured in a spectrophotometer (Cary 50 Bio UV-Vis, Varian, Australia) at 600 nm to obtain the inoculum at 10^7 CFU/mL, approximately.

2.3 Photodynamic inactivation

Photogem® was prepared by dissolving 5 mg in 1 mL of saline solution (0.9%, w/v, NaCl). PS solutions were prepared in saline to obtain the final concentrations of 25 and 50 μg/mL. The incubation time of blood samples with the PS, in the dark, was 30 minutes at 37 °C. The irradiation was performed with light emitting diodes (LEDs) at 630 nm, with irradiance at 30 mW/cm^2 and light doses of 10 and 15 J/cm^2. The *S. aureus* survival in whole blood, erythrocytes, and platelet-rich plasma samples was evaluated by counting the colony forming units (CFU/mL) on Petri plates with BHI.

2.4 Ultraviolet radiation

For UVC radiation assay, it was used a device with a UVC lamp at wavelength 254 nm, with an irradiance of 13 mW/cm^2 when used at 1 cm distance from the samples. The irradiation times used for inactivation of *S. aureus* in whole blood, erythrocytes, and platelet-rich plasma were: 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 minutes. The *S. aureus* survival in whole blood, erythrocytes, and platelet-rich plasma samples was evaluated by counting the colony forming units (CFU/mL) on Petri plates with BHI.

2.5 Hemolysis assay

Whole blood was centrifuged (1,000 × g for 5 minutes) to remove platelet-rich plasma and leukocyte layer, washed three times with PBS and centrifuged again. Erythrocytes were resuspended in saline solution (0.9%, w/v, NaCl) to be used in PDI assay. After PDI, erythrocytes were maintained at 4 ºC in the dark for 24 hours.

The samples were centrifuged (1,000 × g for 5 minutes) again 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).[15] Absorbance measurements were also performed on the supernatants of erythrocytes in whole blood.

2.6 Cell viability assay

Erythrocytes and platelets were obtained from whole blood by centrifugation (1,000 × g for 5 minutes) to be used in PDI assay. Photogem® concentration was 50 μg/mL and light dose was 15 J/cm^2. After PDI, 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. After 15 minutes 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 and PAINT-A-GATE BD software (Becton Dickinson, San Jose, CA, USA).

3. RESULTS

3.1 Photodynamic inactivation

The results of *S. aureus* reduction in whole blood, erythrocytes, and platelet-rich plasma with 50 μg/mL of Photogem® and light dose of 15 J/cm^2 were 1.0 log, 1.3 log and 0.4 log CFU/mL, respectively.

The effect of PDI to inactivate *S. aureus* in whole blood was diminished compared with the results in aqueous media.[16] It was observed that bacterial cells in a complex media, such as whole blood and plasma, are much less susceptible to the Photogem® due to the blocking action of the blood components, whose presence decreases the activity of PS. Moreover, the interaction between Photogem® and blood components also contributed for the diminished inactivation of *S. aureus*.[17]

Hemolysis rate for erythrocytes in whole blood was 10.7% with 50 μg/mL of Photogem® and light dose of 15 J/cm^2. However, hemolysis rate for erythrocytes in the absence of plasma was 100% at the same conditions of PS concentration and light dose.

The cell viability assay showed that after PDI with 50 μg/mL of Photogem® and light dose of 15 J/cm2 a significant displacement of the erythrocytes population into two distinct groups was observed: one with normal size and granularity, and another with both lower size and granularity. The level of apoptosis detected in these populations was 14%, indicating damaging action of PDI for these cells.

For platelets, the cell viability assay showed no damage after PDI with 50 μg/mL of Photogem® and light dose of 15 J/cm^2. The platelets viability was 95.5%, indicating no damage to these cell fragments.

3.2 Ultraviolet radiation

The results of *S. aureus* reduction in whole blood, erythrocytes and platelet-rich plasma with 30 minutes of irradiation (light dose of 23 J/cm^2) were 1.7 log, 1.1 log and 2.5 log CFU/mL, respectively. These results can be seen in Figure 2.

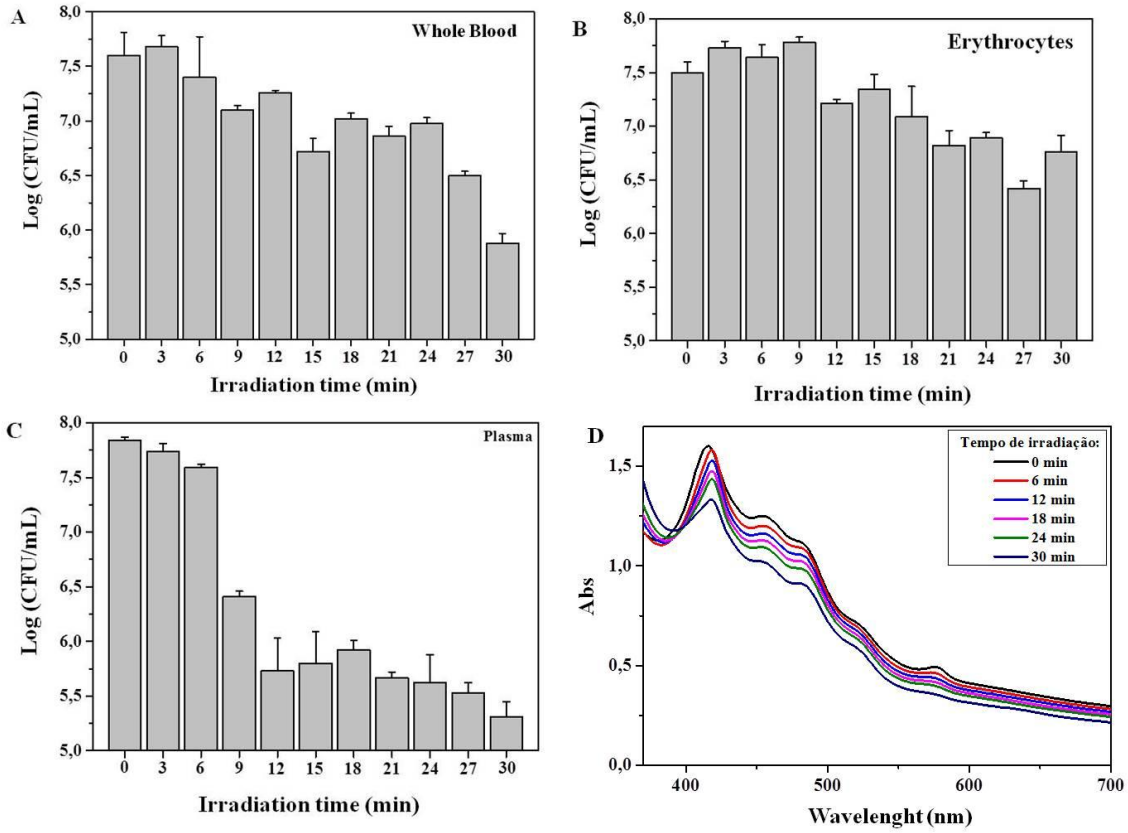

Figure 2: Log of *S. aureus* in whole blood (A), erythrocytes (B) and platelet-rich plasma (C) after ultraviolet light (254 nm) depending on the irradiation time (0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 minutes). (D) Absorption spectra of platelet-rich plasma after irradiation with ultraviolet light at irradiation times: 0, 6, 12, 18, 24 and 30 minutes.

The best results of *S. aureus* inactivation can be seen in the plasma, and the worst results can be seen in the erythrocytes. The presence of erythrocytes impaired the *S. aureus* inactivation, preventing all light from reaching the microorganisms.

This result can be explained by the dispersion that UVC radiation may have undergone in this medium, since the variety of molecules present in the blood, herewith the presence of erythrocytes may have hindered the penetration of light into the microorganisms.

Figure 2D shows the result of plasma analysis after UVC radiation. Measurements were performed by UV-Vis absorption spectroscopy every 6 minutes for 30 minutes. According to the spectra, it is possible to observe small differences in plasma as a function of irradiation time, suggesting some degradation of plasma proteins with 23 J/cm^2. These results show that exposure to UVC radiation may be damaging some plasma protein; however, all peaks are observed at all irradiation times, which suggests no degradation of plasma proteins, but one probable loss in its initial concentration.

The cell viability assay showed normal rates for erythrocytes after UVC irradiation at 30 minutes (light dose of 23 J/cm^2). However, for the platelets, the cell viability assay showed 74% of apoptosis, indicating damaging action of UVC radiation for these cells.

4. CONCLUSION

In this study, we have presented the characteristics observed when PDI and UVC were applied in whole blood and its components. The results allow setting limitation and use conditions for PDI and UVC in blood.

The two optical techniques analyzed, PDI and UVC, showed opposite damage effects in each blood component. PDI was toxic to erythrocytes presenting a percentage of apoptosis, while in platelets no cell death rates were observed. Therefore, it can be an alternative technique for this blood component decontamination. UVC radiation, on the other hand, showed to be toxic to platelets, in which high rates of apoptosis were observed, whereas in erythrocytes the viability and functionality were maintained.

These results are interesting since, depending on the target to be decontaminated, the use of one or another technique should be evaluated together considering the better microbial inactivation and blood components preservation conditions.

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