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# Antimicrobial blue light for decontamination of platelets during storage

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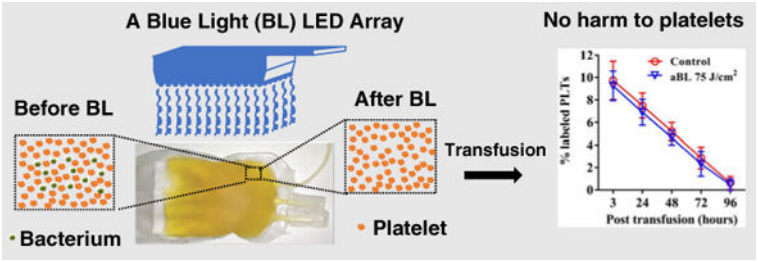
## Abstract

Platelet (PLT) storage is currently limited to 5 days in clinics in the United States, in part, due to an increasing risk for microbial contamination over time. In light of well-documented antimicrobial activity of blue light (405–470 nm), we investigated potentials to decontaminate microbes during PLT storage by antimicrobial blue light (aBL). We found that PLTs produced no detectable levels of porphyrins or their derivatives, the chromophores that specifically absorb blue light, in marked contrast to microbes that generated porphyrins abundantly. The difference formed a basis with which aBL selectively inactivated contaminated microbes prior to and during the storage, without incurring any harm to PLTs. In accordance with this, when contamination with representative microbes was simulated in PLT concentrates supplemented with 65% of PLT additive solution in a standard storage bag, all "contaminated" microbes tested were completely inactivated after exposure of the bag to 405 nm aBL at 75 J/cm$^2$ only once. While killing microbes efficiently, this dose of aBL irradiation exerted no adverse effects on the viability, activation or aggregation of PLTs ex vivo and could be used repeatedly during PLT storage. PLT survival in vivo was also unaltered by aBL irradiation after infusion of aBL-irradiated mouse PLTs into mice. The study provides proof-of-concept evidence for a potential of aBL to decontaminate PLTs during storage.

## Graphical Abstract

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AUTHOR CONTRIBUTIONS

M.L. designed and performed all the experiments, analyzed the data and wrote the manuscript. T.D. supervised and designed the experiments. S.H. contributed to antimicrobial experiments ex vivo. Q.Z. contributed to animal study. L.W. contributed to flow cytometry experiments. B.B. contributed to HPLC analyses. M.W. designed and supervised the study, analyzed the data and wrote the manuscript.

**Keywords**

activation; antimicrobial blue light; decontamination; function and survival; platelet storage

## 1 | INTRODUCTION

Platelet (PLT) transfusion is a critical first-line therapy for severe hemorrhages and the mainstay management of inherited and acquired thrombocytopenia of a variety of etiology [1]. Bacterial contamination of PLT concentrates during storage poses the greatest risk of bacterial sepsis and fatality in modern transfusion medicine due to unique storage conditions required for PLTs [2, 3]. While most of human blood products can be stored in refrigerators or freezers where microbial contamination can be well under control [4, 5], PLTs are uniquely stored at room temperature (20°C-24°C) with continuous agitation around 60 rpm/minute so as to sustain their survival and functionality [6–9]. However, room temperature favors the growth of various microorganisms, which is one of the main reasons to limit PLT storage for 5 days only in the United States, although PLTs are permitted to store for up to 7 days in the United Kingdom and Canada with pathogen inactivation technologies or in the United States in a 7-day container with bacterial detection devices approved by FDA [10].

Currently, light-based antimicrobial approaches to prevent from transfusion-associated bacterial sepsis include UVC irradiation and antimicrobial photodynamic inactivation. UVC irradiation can protect PLT concentrates from microbial contamination [2, 11, 12], which however adversely affects the function and viability of PLTs, as suggested by a decrease of ATP production, an increased expression of PLT surface marker P-selectin and disruption in S–S bonds of the integrin αIIbβ3 (GPIIb/IIIa) presumably because PLT proteins and mitochondrial nucleic acids can absorb UVC [11, 13–15]. Unlike UVC irradiation that does not alter the constituent of PLT concentrates directly, photodynamic inactivation of microorganisms requires exogenous photosensitizers, such as amotosalen, methylene blue and riboflavin. These photosensitizers can be excited by either UV light or visible light and lead to damages of the nucleic acids, thus preventing bacteria from growth [13, 16, 17]. Among these pathogen reduction technologies, only INTERCEPT Blood System (Cerus Corporation, Concord) was approved by FDA in the United States in 2014 and in Europe in 2002. The Mirasol Pathogen Reduction Technology system (Terumo BCT, Lakewood, Colorado) is now in phase III clinical trials in the United States, although it is approved in some European countries [16, 18, 19].

aBL represents a novel light-based approach and has attracted considerable attention lately due to its intrinsic antimicrobial effects without need of any exogenous compounds or photosensitizers [20–22]. The mechanism underlying the antimicrobial activity lies upon the ability of aBL to excite endogenous porphyrins that are abundantly generated by bacteria and fungi over mammalian cells [22]. aBL-mediated excitation of endogenous porphyrins resulted in the production of cytotoxic reactive oxygen species (ROS) that could be confined within the microbes following generation owing to their short-life and cell membrane impermeability [20,21]. aBL has been used clinically to treat jaundice in new-born infants for more than three decades with a long safety record [23] and recently for managing

inflammatory acne owing its superior safety relative to UV irradiation [24–26]. However, a potential for aBL for inactivation of contaminated bacteria and fungi in PLT concentrates has not been explored so far to the best of our knowledge. Here, we report that aBL sufficiently inactivates five representative bacteria and one fungus frequently in association with PLT concentrate contamination, while not adversely affecting the viability, activation and aggregation of PLTs ex vivo and their in vivo survival after transfusion into mice.

## 2 | RESULTS

### 2.1 | No detectable porphyrins in human PLTs

Endogenous porphyrins were investigated in human PLTs, MRSA USA300, *Pseudomonas aeruginosa* ATCC 19660 and *Candida albicans* CEC 749 by fluorescence spectrometer and high-performance liquid chromatography (HPLC) (Figure 1). The characteristic fluorescence emission spectra of PLT and microbe lysates were obtained, along with reference hematoporphyrin in Figure 1A. The absorbance peaks of USA 300, *P aeruginosa* ATCC 19660 and *C albicans* CEC 749 samples appeared at 622, 628 and 624 nm, respectively, characteristic for the typical fluorescence emission porphyrins at an excitation of 405 nm [20,21]. In parallel, hematoporphyrin absorption peaked similarly at 624 nm. To our surprise, there was no absorption peak of porphyrins in PLT lysates. Apart from absorbance, principal emissions peaked at 7.0, 11.1, 18.5, 20.2 and 21.0 minutes (red arrows) for USA 300, 15.8 and 22.5 minutes (green arrows) for *P aeruginosa* ATCC 19660 and 12.9, 18.5, 20.2 and 21.0 minutes (orange arrows) for *albicans* CEC 749, respectively, as revealed by HPLC (Figure 1B). Once again, we did not observe any emission peak of porphyrins when PLT lysates were analyzed by HPLC in the parallel study (Figure 1B). The results suggest that multiple chromophores that can absorb 405 nm light are presented in these microbes, but no such chromophores are presented in human PLTs, making it possible to selectively inactivate contaminated bacteria and fungus by aBL without any harm to PLTs.

### 2.2 | Microbes are more susceptible to 405 nm aBL than 470 nm

In an attempt to define a more sensitive aBL, we compared antimicrobial activities of 405 and 470 nm aBL irradiation against *Bacillus cereus* ATCC 14579, *Staphylococcus epidermidis* ATCC 35985, *Streptococcus pyogenes*, USA 300, *P aeruginosa* ATCC 19660 and *C albicans* CEC 749 in PLT additive solution (PAS), which represent frequently contaminated Gram-positive bacteria, Gram-negative bacteria and fungus [27–29]. As shown in Figure 2, all six microbes were more susceptible to 405 nm aBL irradiation than 470 nm and more than 2-$\log_{10}$ reductions of CFU/mL (99% inactivation) were achieved for all the microbial strains by 405 nm aBL irradiation at a fluence of 50 J/cm$^2$. Among them, *S pyogenes* and *P aeruginosa* ATCC 19660 were most susceptible to 405 nm aBL and as high as 5-$\log_{10}$ CFU/mL bacteria could be completely eliminated by 405 nm aBL irradiation at 50 J/cm$^2$. Although 470 nm aBL also killed the bacteria sufficiently, it was significantly inferior to 405 nm aBL.

### 2.3 | aBL effectively inactivates bacteria and fungus in human PLT concentrates

Antimicrobial efficacies of 405 nm aBL irradiation against *B cereus* ATCC 14579, *S epidermidis* ATCC 35985, *S pyogenes*, USA 300, *P aeruginosa* ATCC 19660 and *C albicans*

CEC 749 were next validated in human PLT concentrates supplemented with 65% PAS and stored in a standard PLT storage condition. All six microorganisms were inactivated in stored PLT concentrates after exposure of the bags to aBL irradiation at 75 J/cm$^2$, and no microbial growth was seen after storage of the irradiated and "contaminated" PLT concentrates for 5 days. The bactericidal effects of aBL irradiation against all six microorganisms in PLT concentrates were closely correlated with those attained in PAS alone, with *S pyogenes* and *P aeruginosa* ATCC 19660 most susceptible to 405 nm aBL irradiation (Figure 3B). These two bacterial strains were eradicated completely by aBL irradiation at 50 J/cm$^2$ as evidenced with no any bacterial growth in 5 days of storage after aBL exposure.

### 2.4 | aBL does not affect PLT survival, activation or aggregation

The 2-day-old human PLT concentrates supplemented with 65% PAS at a ratio 35%:65% (vol/vol) of PLT concentrate to PAS in a storage bag at 3 mL/bag were irradiated by 405 nm aBL at a fluence of 25, 50 or 75 J/cm$^2$, and then stored for 2 hours, 2 days or 5 days. There were no significant differences on PLT counts up to 5 days of storage, as measured by an ABX Hematology Analyzer, between sham and aBL irradiated-PLTs irrespective of aBL fluences employed (Figure 4). The results indicate that 405 nm aBL irradiation at 75 J/cm$^2$ has no adverse effect on PLT survival, in good agreement with no porphyrins in PLTs.

We went on to evaluate PLT activations with surface marker CD62P expression in the presence or absence of agonists thrombin receptor activator-6 (TRAP-6) or thrombin and expression of activated GPIIb/IIIa with or without agonist ADP. All agonists activated PLTs and vigorously increased the expression of CD62P or activated GPIIb/IIIa on the surface of PLTs whether the PLTs were examined in 2 hours, 2 days or 3 days after aBL irradiation (Figure 5). However, there were no differences in the expression of CD62P or activated GPIIb/IIIa in the presence or absence of 405 nm aBL irradiation at 75 J/cm$^2$ (Figure 5A–C), even after three aBL treatments (Figure 5D). It was clear that aBL irradiation did not activate PLTs or interfere with agonist-mediated activation of PLTs. Likewise, when PLTs were stored for 2 hours (Figure 6A) or 2 days (Figure 6B) after 405 nm aBL irradiation at 75 J/cm$^2$, their aggregation function remained intact when stimulated with phorbol myristate acetate (PMA), as suggested by overlapping aggregation curves of PLTs in the presence or absence of aBL irradiation. Flow cytometric analysis of increasing double-colored PLT aggregates over time is commonly used to determine clotting functions of PLT ex vivo [30]. The overlapping aggregation curves of PLTs in the presence or absence of aBL irradiation prior storage are consistent with little effect of the irradiance on PLT activation shown above, corroborating that aBL exerts no any significant adverse effect on PLT functions.

### 2.5 | Similar recovery of mouse PLTs after infusion irrespective of 405 nm aBL irradiation

At last, freshly prepared mouse PLTs were treated with sham or 405 nm aBL at 75 J/cm$^2$ and stored for 2 hours or 5 days in a standard storage condition. At indicated times of storage, the PLTs were fluorescently stained and infused into mice to determine their recovery in vivo because there are significant differences in the recovery of PLTs between in vivo and ex vivo, a lesson learnt from PLTs stored in 4°C [7, 10]. Infused PLTs declined in number over time and reached a lowest level in 96 hours for PLTs stored for 2 hours after aBL irradiation

(Figure 7A,B) or 48 hours for 5-day-storaged PLTs (Figure 7C,D). The recovery of donor PLTs was however comparable in the presence or absence of aBL irradiation at 75 J/cm$^2$, irrespective of whether the PLTs were assayed in 2 hours (Figure 7B) or 5 days of storage (Figure 7D) after aBL exposure. Representative flow cytometric profiles were presented in Figure 7A,C showing decreased donor PLTs that were labeled with calcein, a vital green fluorescent dye, relative to a total number of PLTs marked by anti-CD41 staining in the blood samples taken at indicated times after infusion.

## 3 | DISCUSSION

For the past few years, bacterial sepsis as a consequence of PLT concentrate contamination has been the greatest risk of transfusion-transmitted infections in the United States [31]. A great deal of effort has been made to decontaminate PLT concentrates during storage, including UV light-based INTERCEPT, MIRASOL and THERAFLEX. INTERCEPT and MIRASOL both require an exogenous photoactive sub-stance such as amotosalen or riboflavin in combination with UV light to inactivate pathogens, whereas UVC can directly kill contaminated microorganisms in THERAFLEX [32, 33]. While photosensitizers required for INTERCEPT and MIRASOL may complicate in PLT transfusion, UVC-based reduction of pathogens shows some adverse effects on PLT activation and in vivo recovery because UVC can be absorbed by proteins and mitochondrial DNA of PLTs [2, 11, 14]. We present here a potential of aBL-based approach for pathogen inactivation in PLT concentrates during storage, which does not require any additives and would not impose any adverse effects on the survival and functions of PLTs. aBL irradiation at 405 nm inactivates bacteria or fungi by exciting endogenous porphyrins within bacteria and generation of ROS resulting in the lethal oxidative damage to the microbes [22]. In contrast, PLTs do not produce porphyrins or their derivatives and are not sensitive to aBL, and thus, aBL can be potentially used repeatedly during PLT storage to minimize bacterial sepsis of PLT transfusion. In marked contrast, UVC can be applied to a PLT storage bag only once owing to its adverse effects on PLT activation and survival [2, 14]. However, whether aBL can be effective in viral decontamination of PLTs remains to be determined, although antiviral activity of 405 nm aBL has been shown [34–36]. aBL was also shown to be sufficient in bacterial decontamination of ex vivo stored plasma [37], but it may not be suitable for decontaminating full-size blood components or red blood cells because red blood cells contain hemoglobin that can absorb blue light and may generate significant heat upon aBL.

A number of studies have demonstrated the presence of endogenous porphyrins in clinical pathogens such as Gram-negative strains *Acinetobacter baumannii* [20, 21], *Helicobacter pylori* [38], *P aeruginosa* [21], *Porphyromonas gingivalis* [39] and *Prevotella intermedia*[39], Gram-positive strains MRSA [21, 40] and *Propionibacterium acnes* [41] and fungus *C albicans* [42]. In accordance with these investigations, we also found endogenous porphyrins in the strains of USA 300, *P aeruginosa* ATCC 19660 and *C albicans* CEC 749 by fluorescence spectrometer and HPLC. Porphyrins are believed necessary for the majority of pathogens to utilize heme as an iron source that is essential for survival and pathogenesis of these bacteria and fungi [43, 44], which explains the wide antimicrobial activities of blue laser spectrum (405–470 nm) [22, 45–47]. The abundance of porphyrins generated in microbial cells is the reason behind the more susceptibility of all six representative microbes

to 405 nm aBL irradiation than 470 nm, in agreement with the previous study showing that foodborne pathogens *Lactobacillus plantarum*, *S aureus* and *Vibrio parahaemolyticus* were all more susceptible to 405 nm than 460 nm or 520 nm light irradiation [48]. Apart from the six microbes tested, *Klebsiella pneumoniae* and *Escherichia coli* were also commonly detected in contaminated PLT concentrates [49] and particularly sensitive to 405 nm aBL reported in the previous studies [45, 46, 50, 51].

PLT activation is one of the key adverse events associated with PLT storage, which can compromise their clotting and retraction function initiated by agonists in vivo. Our investigation demonstrates that aBL does not activate PLTs in the absence of an agonist or reduce their activation in the presence of a specific agonist. The expressions of two PLT activation biomarkers, CD62P and GPIIb/IIIa, were not affected by aBL irradiation at 75 J/cm$^2$ [52, 53]. On the contrary, INTERCEPT or MIRASOL treatments have been shown to increase CD62P expression on PLTs [2, 16]. Likewise, UVC treatment elevated the expression of GPIIb/IIIa on PLTs owing to a reduction of the disulfide bonds of the receptors [14]. No aBL-mediated PLT activation was also corroborated by unaltered PLT aggregation that is directly associated with clotting function and retraction of stored PLTs [30]. These ex vivo observations were in good agreement with almost identical recovery between sham and aBL-treated PLTs in vivo after infusion of aBL-treated PLTs into mice, which is in sharp contrast to a reduced recovery of INTERCEPT-treated PLTs by 16% [54] or MIRASOL-treated PLTs by 25% [55].

Bacterial sepsis due to PLT concentrate contamination remains a public health concern despite current interventions, which is one of the major reasons to limit PLT storage for 5 days only. To minimize the risk, FDA permits extension of PLT storage to 7 days only if additional strategy is implemented to reduce a risk of bacterial sepsis. A simulation study finds that each additional day that PLT shelf life is extended may reduce PLT wastage by as much as 16% [56]. Recently we showed that near infrared low-level light could extend the shelf-life of stored PLTs beyond 7 days with improved quality and survial and the light also protected PLTs from apoptosis induced by anti-PLT antibody [57,58]. Thus, incorporation of both 405 nm and near infrared 810 nm light-emitting diode (LED) lights into the current PLT storage system may extend PLT shelf-life significantly. Our results indicate that the LED-based approach may offer an alternative to inactivate bacteria and fungi in PLT concentrates. However, further studies are needed to establish the clinical potential.

## 4 | MATERIALS AND METHODS

### 4.1 | Light source

An experimental device equipped with a LED with peak emission at 405 nm (Thorlabs, Newton, New Jersey) was used in this study (Figure 3A). The LED was held firmly by a clamp on a support stand, and the distance between the LED and the target surface was fixed to obtain 55 mW/cm$^2$ as measured by a PM100D power/energy meter (Thorlabs, Newton, New Jersey) for all the experiments. The fluence was verified each time.

### 4.2 | Microorganisms and cultures

Six representative microbial species associated with PLT concentrate contamination were selected in this study, among which four were Gram-positive bacteria, *B cereus* ATCC 14579, *S epidermidis* ATCC 35985, *S pyogenes* and methicillin-resistant *S aureus* USA 300 (MRSA USA300), one Gram-negative bacterium, *P aeruginosa* ATCC 19660 and one fungus, *C albicans* CEC 749 [16, 27–29]. These microbial strains were purchased from ATCC. The bacteria and fungus were grown in brain heart infusion (BHI) medium and yeast extract peptone dextrose (YPD) medium, respectively.

### 4.3 | Fluorescence spectroscopy and high-performance liquid chromatography

Endogenous porphyrins in human PLTs, MRSA USA300, *P. aeruginosa* ATCC 19660 and *C. albicans* CEC 749 were determined by fluorescence spectroscopy and HPLC as previously described [20, 21, 42]. The PLT concentrates were obtained from Blood Donor Center at Massachusetts General Hospital with approval from Partners Human Research Ethics Committee as apheresis products of healthy de-identified volunteers and released to us after 2 days of sequestration to ensure no bacterial contamination as a standard clinical practice. The 2-day-old human PLT concentrates ($10^{10}$ PLTs) or overnight bacterial and fungal cultures of $10^{10}$ colony-forming units (CFU) were centrifuged, washed twice with PBS and pelleted. The pellets were resuspended in extraction solvent composed of ethanol: dimethyl sulfoxide: acetic acid at a ratio of 80:20:1 (vol/vol/vol) and then stored at −80°C for 48 hours. The extracts were centrifuged, and the supernatants were collected and filtered through a Sep-Pak $C_{18}$-Cartridge. Fluorescence of the PLT and microbe extracts was measured on a Fluoromax 3 (Horiba, Edison, New Jersey) with an excitation at 405 nm and an emission width ranging from 560 to 700 nm. The extracts were also analyzed for the presence of porphyrins by an Agilent's 6430 Triple Quad LC/MS system (Agilent, Lexington, Massachusetts) in conjunction with a fluorescence detector at an excitation of 405 nm and the emission spectra of 590, 610 and 630 nm. All assays were performed in triplicate.

### 4.4 | Comparison of antimicrobial activity of aBL at 405 nm vs 470 nm

To determine optimal laser parameters to kill the representative bacteria and fungus above, 3 mL of microbial suspensions at $10^5$ CFU/mL in PAS (Fenwal, Lake Zurich, Illinois) was added to 35-mm Petri dishes and irradiated with aBL at 405 or 470 nm at an irradiance of 55 mW/cm$^2$. Aliquots of 50 μL of the suspension were withdrawn at 0, 3, 6, 9, 12 and 15 minutes during aBL exposure, corresponding to 0, 10, 20, 30, 40 and 50 J/cm$^2$, respectively. The resident microbes were quantified on BHI agar plates for bacteria or YPD agar plates for *C. albicans* CEC 749 as previously described [59]. All assays were performed in quintuplicate.

### 4.5 | Microbial inactivation in PLT concentrates by aBL at 405 nm

The 2-day-old human PLT concentrates supplemented with PAS at a ratio 35%:65% (vol/vol) of PLT concentrate to PAS were split into PLT storage bags at 3 mL/bag using syringe with a 22 G needle, along with an estimate of 100 CFU/bag of indicated microbes to simulate PLT concentrate contamination. The storage bag was made in house with the

polyvinylchloride (PVC)/tri-(2-ethylhexyl)- trimellitate (PVC-TEHTM) film that is currently used to fabricate standard PLT storage bags (a kind gift from American RENOLIT Corp. LA, California). The PVC-TEHTM film offers high oxygen and carbon dioxide permeability for PLT storage. The bags each were positioned under the LED and illuminated with 405 nm aBL for 0, 7.5, 15 or 22.5 minutes, corresponding to 0, 25, 50 and 75 J/cm$^2$, respectively. A soft white light bulb irradiation was used as controls throughout the study. The irradiated PLT storage bags were stored for 5 days at 22°C with a gentle agitation of 60 to 70 rpm in a standard PLT storage incubator (Boekel Scientific, Pennsylvania). After storage, all contents in the PLT storage bag were removed, centrifuged and examined for resident microbes as above [59]. All assays were performed with eight independent experiments, each assayed in triplicate.

### 4.6 | Effects of 405 nm aBL on human PLT survival, activation and aggregation

The 2-day-old human PLT concentrates supplemented with PAS were split into PLT storage bag as above and exposed to 0, 25, 50 or 75 J/cm$^2$ 405 nm aBL, respectively. PLTs were then counted to determine viability in 2 hours after aBL or 2 or 3 days after storage by a HemaTrue Veterinary Hematology Analyzer (Heska Corporation, Loveland, Colorado) as depicted in Figure 3A. All assays were performed with six independent experiments, each assayed in triplicate.

PLT activation was assessed by expression of activated integrin GPIIb/IIIa and CD62P, two common PLT activation biomarkers, at varying times after aBL irradiation [52, 60]. In some samples, the PLTs in a bag were exposed to 75 J/cm$^2$ aBL or sham light on day 2. Around 200 μL of samples was taken in 2 hours after aBL or 2 or 3 days after storage to determine the PLT activation (Figure 3A). To verify whether repeated irradiations could be conducted safely during PLT storage to maximize the decontamination effect, the PLTs in a bag were also treated with aBL once a day for three consecutive days before PLT activation was evaluated on day 5 as depicted in Figure 3A. To evaluate PLT activation in the presence or absence of a specific stimulator, the PLTs were stained with FITC-PAC-1 antibody specific for activated GPIIb/IIIa (1:100; BD Bioscience, San Jose, California) and APC-antibody directed at CD62P (1:100; BD Bioscience, San Jose, California). FITC-mouse IgM and APC-IgG1 served as isotype controls. Alternatively, PLTs were activated with 100 μM TRAP-6, 2 U/mL thrombin, 50 μM ADP or vehicle control for 30 minutes at 25°C, after which the PLTs were fixed by 1 mL of 0.5% paraformaldehyde in PBS for 20 minutes and stained with specific anti-bodies against GPIIb/IIIa and CD62P as above, followed by flow cytometric analysis. The percentages of PLTs positive to either activated GPIIb/IIIa, CD62P or both were determined by FlowJo software. All assays were performed with six independent experiments, each assayed in triplicate.

To examine PLT aggregation, an important function for clotting, irradiant PLTs were collected at indicated times, diluted with HEPES/Tyrode's solution to a final concentration of $50 \times 10^6$ cell/mL, and then divided into two parts equally: one was labeled with PE-anti-CD31 antibody and another with APC-anti-CD9 antibody as previously detailed [30, 61]. APC-mouse IgG1 and PE-mouse IgG1 served as isotype controls. After incubation for 30 minutes, the two populations of labeled PLTs were washed with HEP- ES/Tyrode's solution

and mixed at a 1:1 ratio, followed by activation with 100 ng/mL of PMA at 37°C while shaking at 500 rpm. At different times during the activation, samples were fixed by 0.5% paraformaldehyde in PBS for 20 minutes and analyzed by flow cytometry. The percentages of double-colored events were determined by FlowJo software as aggregated PLTs according to the reported method [30]. All assays were performed with six independent experiments, each assayed in triplicate.

### 4.7 | Recovery of mouse PLTs in vivo after aBL irradiation

The animal protocol was approved by the subcommittee on Research Animal Care of the Massachusetts General Hospital in compliance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animal. C57BL/6 mice at 12 weeks of age were purchased from Charles River Laboratories. Blood samples were collected from mouse retro-orbital venous plexus into a tube containing 30 U/mL heparin in PBS and pooled. Concentrated PLTs were obtained by centrifugation of the blood samples at 200 g for 10 minutes to remove blood cells, followed by centrifugation at 1000 g to prepare $1 \times 10^8$ PLTs/mL. The concentrated PLTs were slowly injected into PLT storage bags using syringe with a 22 G needle. The bags containing concentrated PLTs were exposed to 75 J/cm$^2$ aBL irradiation or sham light on day 0 and stored for 2 hours or 5 days under a standard storage condition. The PLTs were then stained with a vital fluorescent dye calcein at 5 μg/mL for 20 minutes, washed twice, resuspended in a modified Tyrode-HEPES buffer and intravenously infused into retro-orbital vein of mice at $5 \times 10^6$ PLTs per gram body weight. At indicated times after transfusion, blood samples were collected and labeled with APC-anti-CD41 or PE-anti-CD41 antibody. Percentages of calcein-positive PLTs relative to a total number of PLTs (CD41+) were quantified by flow cytometry to determine a recovery of infused PLTs.

### 4.8 | Statistical analysis

Data are represented as mean ± SEM. Statistical analysis was performed with a paired Student *t* test between two groups or one-way ANOVA for multiple group comparisons. A *P*-value of $< .05$ is considered significant. All statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, San Diego, California).

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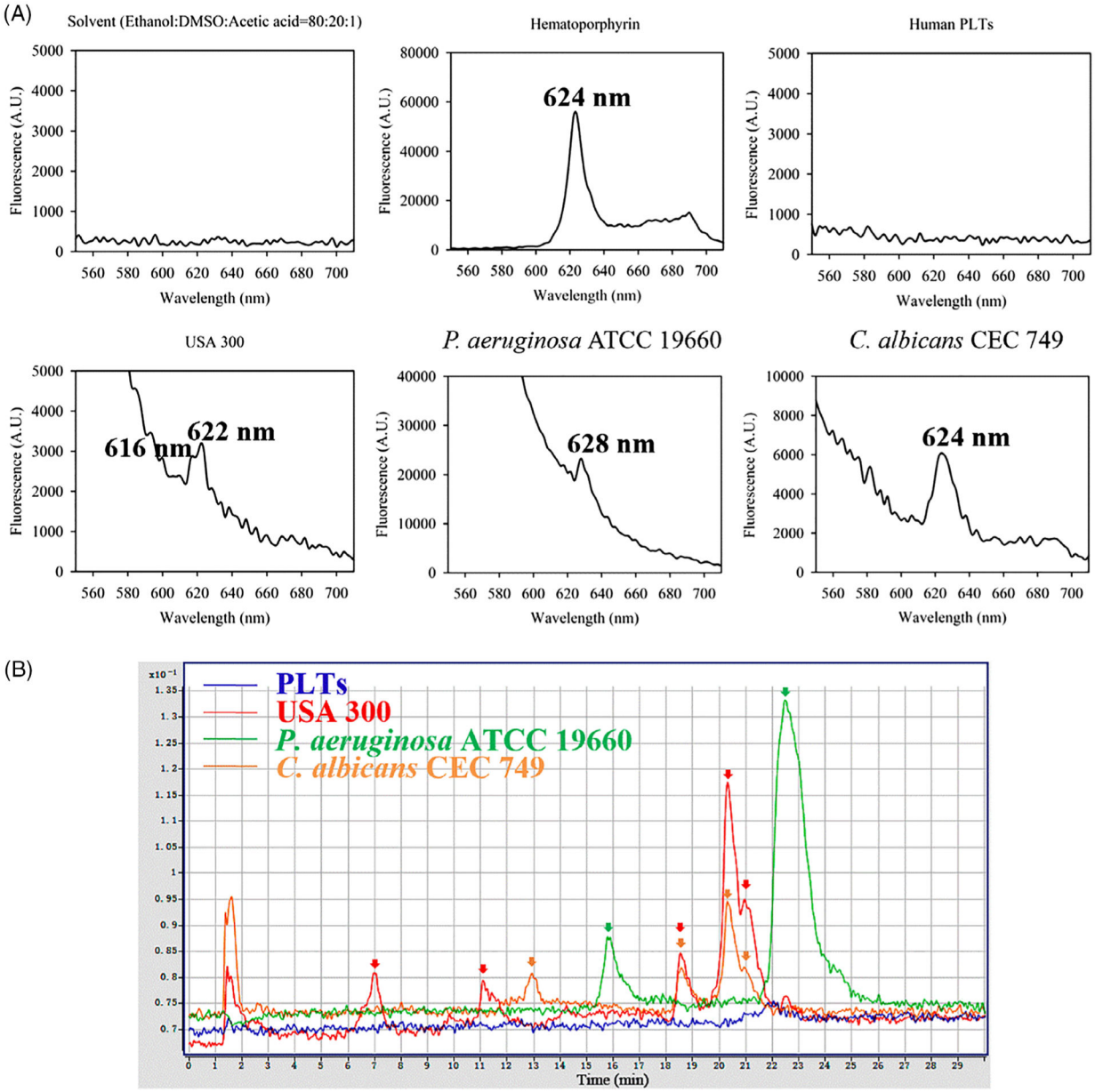


**FIGURE 1.**

Determination of intracellular porphyrins in platelet (PLT) cells and representative microbes by fluorescence spectrometer (A) and high-performance liquid chromatography (HPLC) (B). A, Fluorescence emission spectra of solvent, positive control (hematoporphyrin), human PLT lysate and lysates of USA 300, *Pseudomonas aeruginosa* ATCC 19660 and *Candida albicans* CEC 749 at an excitation wavelength 405 nm and an emission width ranging from 560 to 700 nm. B, HPLC chromatograms of human PLT lysate (blue line) and lysates of USA 300 (red line), *P aeruginosa* ATCC 19660 (green line) and *C albicans* CEC 749 (orange line). Excitation wavelength was 405 nm, and emission wavelengths were 590, 610 and 630 nm

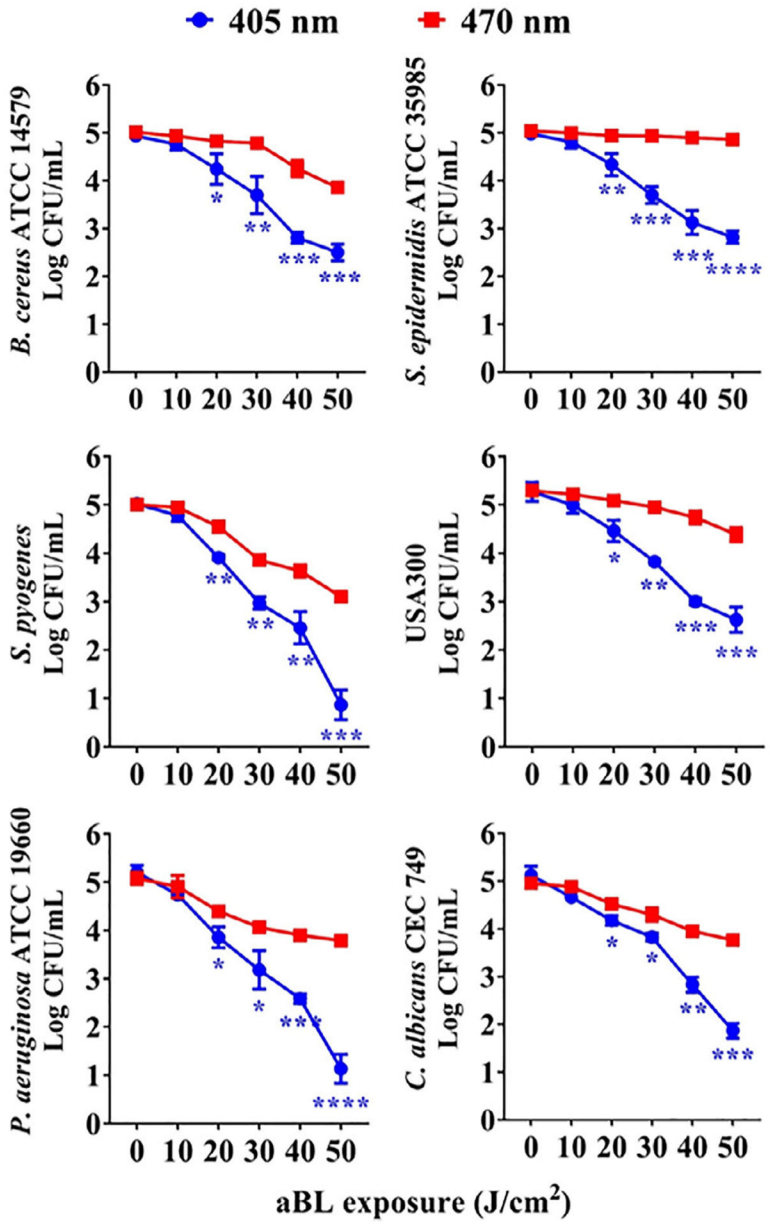


**FIGURE 2.**

Comparison of antimicrobial activity of antimicrobial blue light (aBL) irradiation at 405 () and 470 nm (). *Bacillus cereus* ATCC 14579, *Staphylococcus epidermidis* ATCC 35985, *Streptococcus pyogenes*, USA 300, *Pseudomonas aeruginosa* ATCC 19660 and *Candida albicans* CEC 749 suspended in PLT additive solution (PAS) were illuminated with aBL at indicated fluences, after which colony-forming unit (CFU) was determined and expressed as mean ± SEM log CFU/mL, n = 5. **P* < .05, ***P* < .01, ****P* < .001 and *****P* < .0001 between 405 and 470 nm aBL

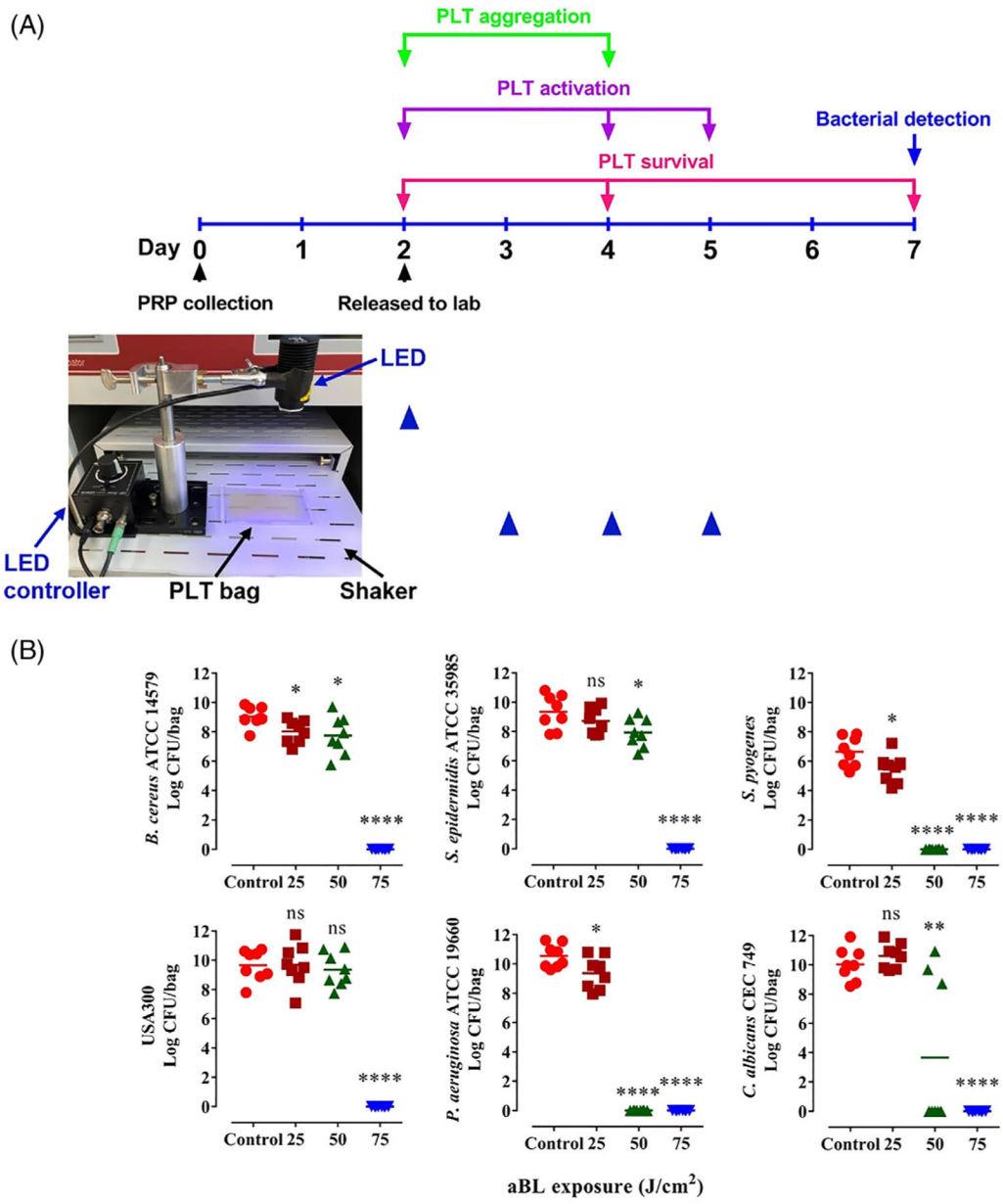


**FIGURE 3.**

Depict of time lines of antimicrobial blue light (aBL) irradiation (A) and evaluation of its effects on platelets (PLTs) (B). A, The 2-day-old human PLT concentrates were irradiated with sham or 405 nm aBL once during storage or once a day for three consecutive days (from day 3 to day 5 after PLT collection) (blue triangle). The light-emitting diode (LED) was held firmly by a clamp on a support stand, and the distance between the LED and the PLT storage bag was fixed to obtain 55 mW/cm$^2$ for the experiments (inset photo). The PLTs were irradiated at indicated times controlled by a LED controller while gently shaking on the shaker at 60 to 70 rpm. Residual bacteria in the PLT concentrates were determined on day 5 after aBL irradiation (day 7 after PLT collection). PLTs were counted to determine viability in 2 hours (day 2 after PLT collection), 2 days (day 4 after PLT collection) and 5

days (day 7 after PLT collection) after sham or aBL irradiation (red). PLT activations were analyzed in 2 hours (day 2 after PLT collection), 2 days (day 4 after PLT collection) and 3 days (day 5 after PLT collection) after sham or aBL irradiation (purple). PLT aggregations were assayed in 2 hours (day 2 after PLT collection) and 2 days (day 4 after PLT collection) (green). B, *Bacillus cereus* ATCC 14579, *Staphylococcus epidermidis* ATCC 35985, *Streptococcus pyogenes*, USA 300, *Pseudomonas aeruginosa* ATCC 19660 or *Candida albicans* CEC 749 was cultured overnight and added to PLT storage bags at 100 CFU/bag containing 3 mL PLT concentrates supplemented with 65% PLT additive solution (PAS). The bags each were exposed to sham light (●) or 405 nm aBL at 25 (■), 50 (▲) or 75 (▼) J/cm$^2$ and then stored in a standard condition for 5 days. The number of residual bacteria in each bag was determined on day 5 after irradiation as Figure 2, n = 8. *$P$ < .05, **$P$ < .01 and ****$P$ < .0001 in the presence or absence of aBL. ns, no significance

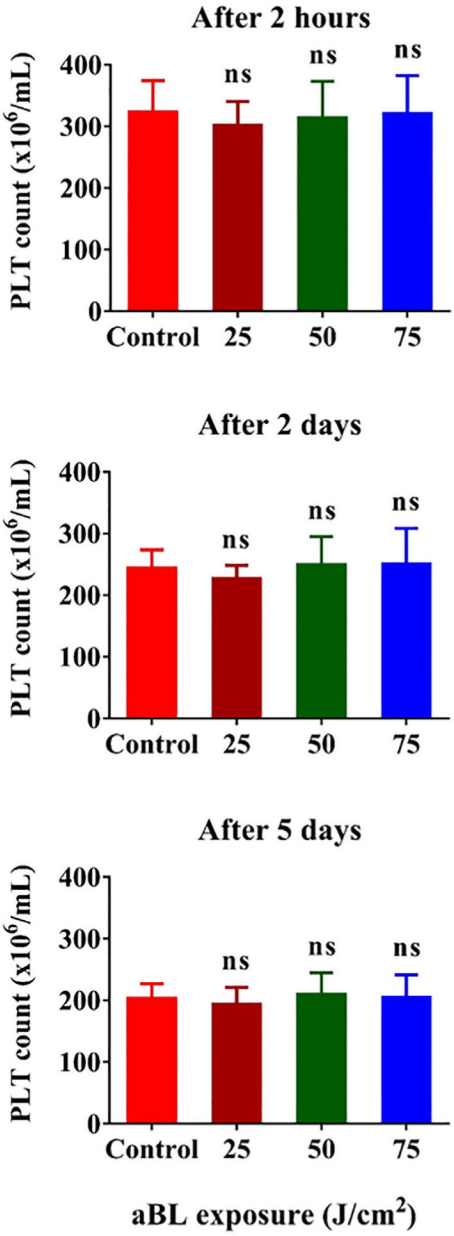


**FIGURE 4.**

Effects of 405 nm antimicrobial blue light (aBL) irradiation on platelet (PLT) survival in vitro. The PLT concentrate was irradiated with aBL at 25, 50 or 75 J/cm$^2$, respectively. PLT counts were determined in 2 hours or 2 or 5 days after aBL irradiation (red) as depicted in Figure 3A. Data represent mean ± SEM, n = 6. ns, no significance between sham- and aBL-treated samples

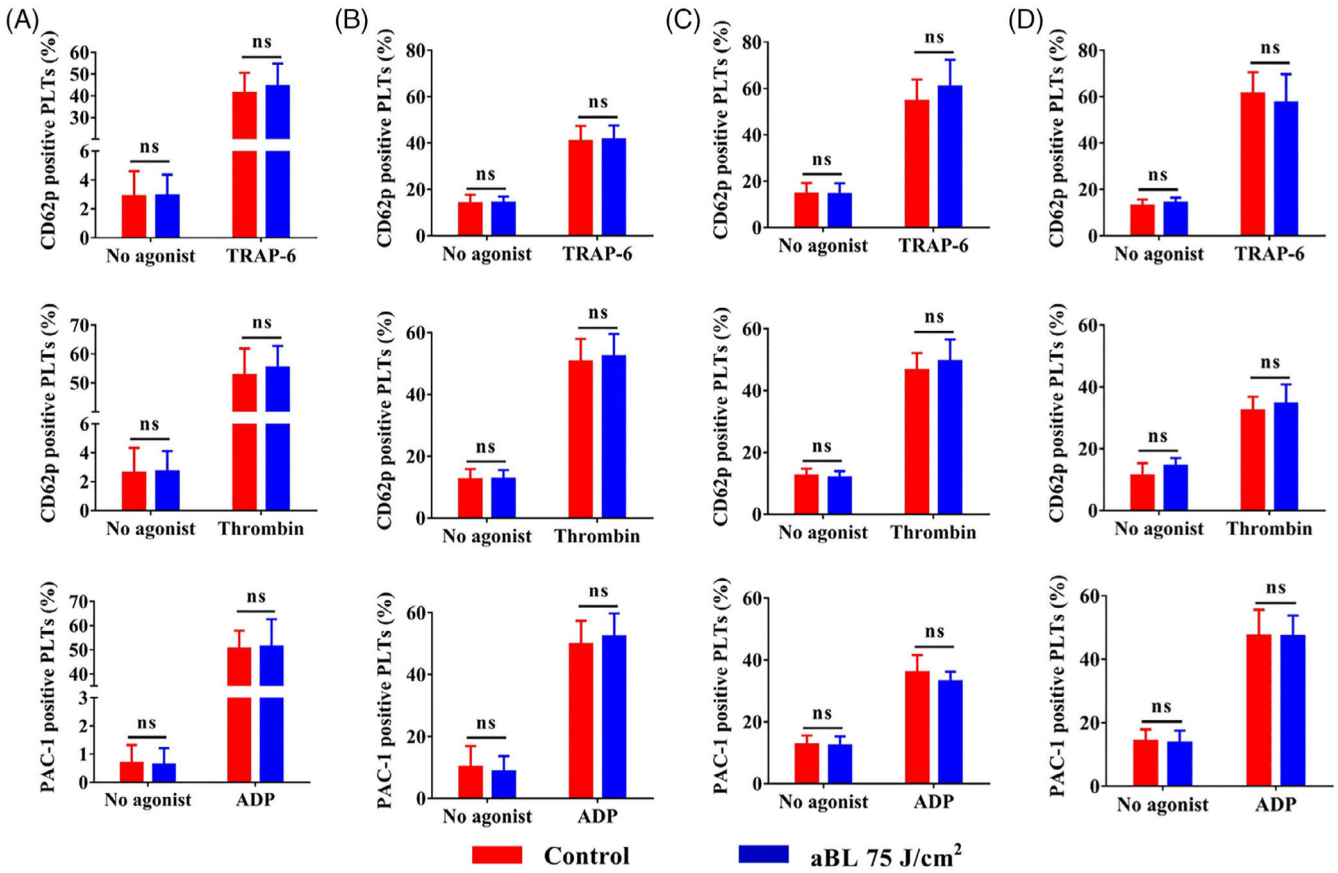


**FIGURE 5.**

Effects of 405 nm antimicrobial blue light (aBL) on platelet (PLT) activation in the absence or presence of agonists. PLT concentrates were irradiated with sham or 405 nm aBL at 75 J/cm$^2$ once and then evaluated for PLT activations in 2 hours (A), 2 days (B) or 3 days(C) after irradiation. Alternatively, the PLT concentrate was irradiated once a day for three consecutive days and determined for PLT activation in2 hours after the final irradiation (D). Percentages of PLTs positive for CD62P or GPIIb/IIIa expression in the absence or presence of an indicated agonist were evaluated by flow cytometry. The agonists tested were TRAP-6 at 10 μM, thrombin at 2 U/mL or ADP at 50 μM. Data represent mean ± SEM of six independent experiments with each assayed in triplicate. ns, no significance between sham- and aBL-treated samples

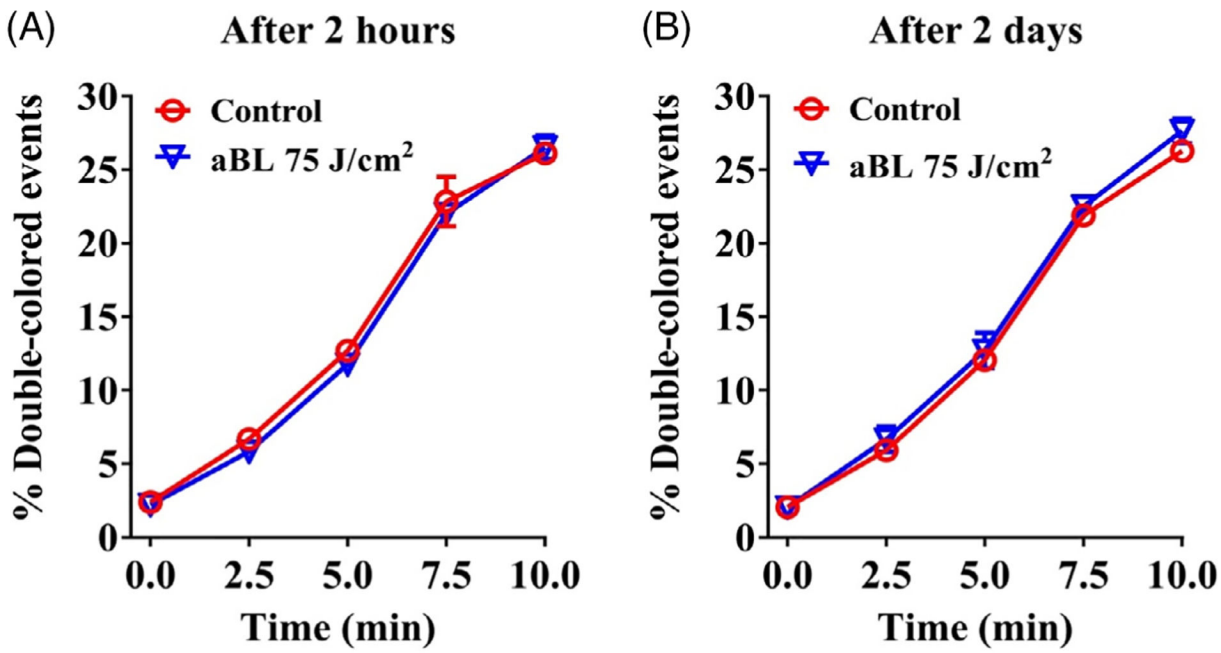


**FIGURE 6.**

Effects of 405 nm antimicrobial blue light (aBL) irradiation on platelet (PLT) aggregation. PLTs were treated with sham or405 nm aBL at 75 J/cm$^2$, and then stored for2 hours (A) or 2 days (B). PLTs were then divided into two groups, labeled with either PE-CD31 or APC-CD9, and mixed at a 1:1 ratio before stimulation. Aggregation was measured by flow cytometry over time after stimulation with PMA at 100 ng/mL. Double-colored events were quantified and presented as mean percentages ± SEM of six independent experiments with each assayed in triplicate

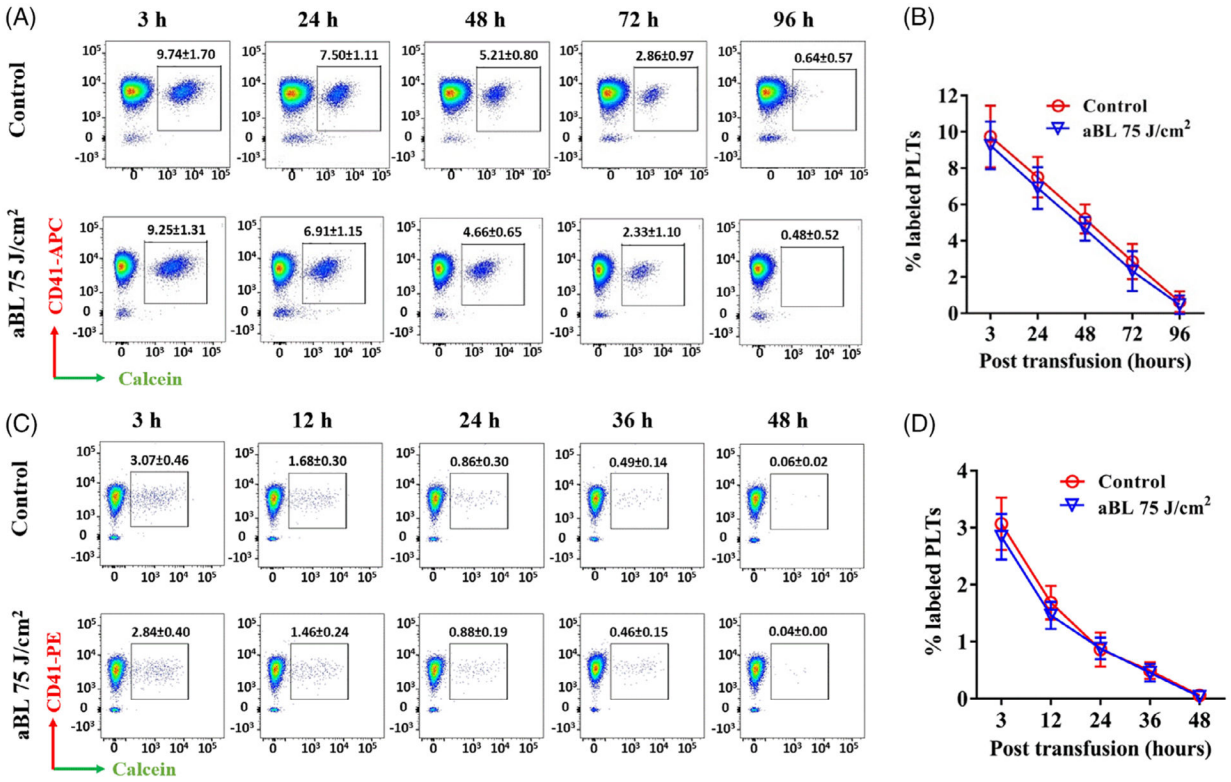


**FIGURE 7.**

Antimicrobial blue light (aBL) did not affect recovery of mouse platelets (PLTs) following transfusion into mice. Concentrated PLTs were freshly prepared from pooled mouse blood samples, treated with sham or 405 nm aBL at 75 J/cm$^2$ and stored for 2 hours (A and B) or5 days (C and D), after which the PLTs were labeled with calcein and intravenously infused into recipient mice. Blood was drawn at indicated times after infusion in alternative mice, labeled with anti-CD41 and analyzed by flow cytometry. A and C are representative dot plots of 2-hour- (A) and 5-day-storaged (C) PLTs from recipients labeled with calcein and CD41 at different time points after transfusion. B and D are changes of percentage double-labeled events in sham- and aBL irradiated-PLTs at indicated times after 2 hours (B) or 5 days (D) of storage. Results are shown as percent double-colored PLTs ± SEM with five mice in each time point