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LETTER

Effect of near-infrared and blue laser light on vero E6 cells SARS-CoV-2 infection model

Luisa Zupin[1] | **Libera Clemente**[2] | **Francesco Fontana**[2] | **Sergio Crovella**[3]

[1]Institute for Maternal and Child Health, IRCCS "Burlo Garofolo", Trieste, Italy

[2]Division of Laboratory Medicine, University Hospital Giuliano Isontina (ASU GI), Trieste, Italy

[3]Department of Biological and Environmental Sciences, College of Arts and Sciences, University of Qatar, Doha, Qatar

Correspondence
Luisa Zupin, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, 34137, Trieste, Italy.
Email: luisa.zupin@burlo.trieste.it

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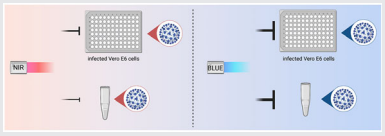
Abstract



Photobiomodulation therapy (PBMT) employing laser light has been emerging as a safe strategy to challenge viruses. In this study the effect of blue and near-infrared (NIR) laser light was assessed in an in vitro model of SARS-CoV-2 infection. PBMT at blue wavelength inhibited viral amplification when the virus was directly irradiated and then transferred to cell culture and when cells already infected were treated. The NIR wavelength resulted less efficacious showing a minor effect on the reduction of the viral load. The cells receiving the irradiated virus or directly irradiated rescued their viability to level comparable to not treated cells. Virion integrity and antigenicity were preserved after blue and NIR irradiation, suggesting that the PBMT antiviral effect was not correlated to viral lipidic envelope disruption. Our results suggested that PBMT can be considered a valid strategy to counteract SARS-CoV-2 infection, at least in vitro.

KEYWORDS

antiviral, blue and near-infrared laser light, photobiomodulation therapy, SARS-CoV-2, vero E6 cells

1 | INTRODUCTION

Different studies reported that photobiomodulation therapy (PBMT), employing laser light at various wavelengths can counteract viral infections.

Laser light in the red range has been described as an effective tool to fight herpes simplex virus type 1 (HSV-1) in infected patients. Indeed, multiple irradiation of patients on blister in prodromal stage and on the wound shortened the HSV-1 outbreaks and reduced the relapses [1]. An interesting study, where the PBMT was performed during the recurrence free-interval, showed that, also in this setting, PBMT increments the recurrence-free interval to 37 weeks, compared to 3 weeks in the placebo group [2]. The near-infrared wavelength application on affected patients was also beneficial in reducing the herpes lesions as well as the inflammatory edema [3]. PBMT at 780 nm was also successfully applied to a child, that at the second session reported no longer pain sensation and itching, showing that PBMT can be an interesting alternative in pediatrics [4].

Beyond herpes labialis, PBMT in the infrared band is also applied on the verrucae caused by human papillomavirus (HPV) infection showing an improvement of the

recalcitrant ones when treated with 595 nm pulsed dye laser light [5].

Very recently, laser light was used to treat patients with coronavirus disease 2019 (COVID-19).

A case series employing red and NIR wavelengths on mild–severe COVID-19 affected patients showed significant recovery of the subjects after treatment. PBMT was delivered through LED pads applied directly on skin in a whole organ dynamic approach of 84 min session on sinus and lungs, the treatments were performed every 2–3 days for a maximum of three sessions in patients with mild–moderate disease. The symptomatic acute condition relief observed included breathing improvement, elimination of sinus congestion, reduction of dry coughing, fever, and severe eye inflammation and occurred in less than 2–3 days [6].

Recovery of pulmonary involvement in COVID-19 was also reported in one case study where the patient was treated on the thorax at level of the lungs with the laser maintained 20 cm above the area for 4 days [7], while in another PBMT was combined with static magnetic field and delivered for 45 days on six areas in the lower thorax/upper abdominal cavity plus two sites at neck level [8].

The therapeutic effects of PBMT are generally referred to a systemic impact of laser light leading to the reduction of inflammation and fibrosis, and promotion of healing. Nevertheless, in vitro studies revealed that PBMT can impact on virus itself uncovering a direct antiviral property of laser light.

Several studies showed that PBMT at the blue wavelength possesses an inhibitory effect on Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) replication [9–13].

Blue light was also described as effective in counteracting HCoV-229 E, HCoV-OC43 [14], SARS-CoV-1, MERS-CoV [11], HSV-1 [15, 16], and Zika virus [17].

Less recent investigations highlighted that also other wavelengths possess antiviral effects. PBMT at 830 nm inhibits HSV-1 replication in the viral post-absorption phase [18] and at 970 nm repress Zika virus replication [17].

In this study a class IV laser medical device, commonly employed in the clinical practice in both adult and pediatric patients suffering oral mucositis wounds [19, 20], skin scars [21], and asthma [22], was employed in a cell model of SARS-CoV-2 infection by using both the blue and the near-infrared wavelengths.

2 | MATERIALS AND METHODS

2.1 | Cell line

Vero E6 epithelial normal kidney cell line (*Cercopithecus aethiops*, ATCC CRL-1586) was cultured in MEM + 10% fetal bovine serum, 2 mM glutamine, and 100 U/ml penicillin/streptomycin (Euroclone, Pero, Italy). Cells were seeded at a density of 1.5×10^4 cells for well in 96 multi-well plates and at a density of 2×10^5 cells for well in 24 multi-well plate.

2.2 | Photobiomodulation therapy

The PBMT was performed with a medical laser device (class IV diode laser, K-Laser Blue series, K-laser d.o.o., Sežana, Slovenia) equipped with a zoom tip. Different protocols were initially tested to define the maximum dose tolerated by the cells employing 445 and 970 nm of wavelengths, irradiance 0.25 W/cm^2 (0.5 W output power), fluence 5–30 J/cm^2.

During the experiments, the cells seeded in the 96 multi-well plate were used in medium without phenol red to avoid light absorption (DMEM, BE12-917F, Lonza, Basel, Switzerland) supplemented with 2% FBS, 2 mM glutamine, and 100 U/ml penicillin/streptomycin (Euroclone, Pero, Italy).

The irradiations were carried out in the cabinet hood in dark conditions with the lid of the plate left open to avoid the interference from environmental light and plastic support. The power emission was priorly checked with a power meter and the beams were settled to cover uniformly a 96 multi-well plate.

The cells were stained in crystal violet after 24 h of irradiation. After medium removal, crystal violet (10% in phosphate buffer saline—PBS) solution was added to the cells for 30 min, then after washing the cells were air-dried and finally lysed in 1% dodecyl sodium sulfate in PBS. The absorbance was read at 600 nm after dilution 1:4 in water.

The following protocol, already tested and known not to be cytotoxic, was selected: irradiance 0.25 W/cm^2 (0.5 W output power), fluence 30 J/cm^2, continuous wave. This protocol was tested on Vero E6 cells at 24, 48 and 72 h, showing no detrimental effect on survival (Figure SS1).

2.3 | PBMT antiviral activity

SARS-CoV-2 was employed in the experiments at a multiplicity of infection (MOI) of 0.05, at the BLS3 facility (San Polo Monfalcone hospital, GO, Italy).

Two experimental settings were used:

Setting 1: SARS-CoV-2 was irradiated alone and then transferred to the cells for 1 hour, then the cells were washed in PBS and new medium was added.

Setting 2: cells were infected with SARS-CoV-2 for 1 h, washed in PBS, then new medium was added and the PBMT was carried out.

At the end of the procedures, the medium was removed and replaced with a fresh one.

The development of cytopathic effect was monitored by employing the EVOS XL Core Cell Imaging System (Thermo Fisher Scientific, Waltham, MA, USA).

After 3 and 7 days post infection/irradiation, 15 µl of cell medium supernatants were thermolyzed after the mixing with 45 µl of water (98°C for 3 s, 4°C for 5 s). The viral RNA was quantified using Real-Time Quantitative Reverse Transcription PCR on the 7500 Fast Real-Time PCR platform (Thermo Fisher Scientific, Waltham, MA, USA) with the Luna® Universal Probe One-Step RT-qPCR Kit (New England Biolabs, Ipswich, MA, USA).

The RT-qPCR was performed by using the CDC primers and probe (Eurofins, Luxembourg) for *N* gene (nucleocapsid, set N1, 500 nM forward primer GAC CCC AAA ATC AGC GAA AT, 500 nM reverse primer TCT GGT TAC TGC CAG TTG AAT CTG, 125 nM probe FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1 [23]) and the nCoV-CDC-Control Plasmid (Eurofins) was used to generate the standard curve.

The viability of the cells after 7 days was assessed by crystal violet staining as described above. Briefly, the medium was removed, the cells stained with 10% crystal violet in PBS for 30 min, then after washing the cells were air-dried and lysed in 1% SDS in PBS. After dilution 1:4 in water, the 600 nm absorbance was read.

2.4 | PBMT antiviral mechanism of action

RNAse protection assay was performed to assess the integrity of the virions after the irradiation.

Briefly after PBMT, the 15 µl of the samples were treated with RNAse A (1 µg of Ribonuclease A, R4875, Merck KGaA) at 37°C for 30 s, then the samples were submitted to thermolysis and quantified by RT-qPCR as previously described.

To further assess virus integrity and specifically the antigenicity of nucleocapsid protein, the antigen rapid test COVID-19 Antigen Rapid Test Cassette (cod. COV-2C25B BTNX, Inc., Markham, ON, Canada) was performed. Briefly, 50 µl of samples (irradiated and not irradiated SARS-CoV-2) were lysed with 50 µl of extraction buffer and loaded into the cassette, and the results were read after 10 min.

2.5 | Statistical analysis

R software was employed to perform the statistical analysis by using Kruskal–Wallis (KW) test corrected for multiple comparisons with Dunn's test. The cells infected with no illuminated virus were used as positive control and the cells not infected as negative control for the comparison with the 2 PBMT experimental settings [24]. The experiments were performed in triplicate.

3 | RESULTS AND DISCUSSION

PBMT at both near infrared and blue wavelength was able to inhibit SARS-CoV-2 amplification, although the blue one was more efficient. When the blue laser light was delivered to the virus itself or on already (1 h) infected cells, the viral amplification was blocked (10^5 viral copies/ml), while the not irradiated virus replicated reaching the 10^7 viral copies/ml at 3 days and 10^9 viral copies/ml at day 7 (Figures 1 and 2, KW test, setting 1, day 7, blue vs. not irradiated SARS-CoV-2 p-value = 0.008; setting 2, day 3, blue vs. not irradiated SARS-CoV-2 p-value = 0.01; day 7, blue vs. not irradiated SARS-CoV-2 p-value = 0.001).

Our results agree with our previous findings [12, 13], which reported that blue laser light at not cytotoxic doses is able to block viral replication with long-lasting (7 days) effect. In our previous paper [12] employing an LED device, inhibition of virus replication was observed at 24 and 48 h post irradiation, while at 72 h, although a negative trend was found, it did not reach the statistical significance. Instead, in the present work, a complete block of viral replication was detected; indeed the viral titer was the same between day 3 and day 7, both when the virus was irradiated itself or the photons were delivered on already infected cells. In our second article [13], utilizing the same LED instrument of the first work, a suppression of SARS-CoV-2 expansion was demonstrated, exploiting a protocol that resulted slightly cytotoxic with 80% of viable cells after irradiation, while in the current work, no harmful effect was detected on cell survival by blue PBMT, while revealing a strong antiviral impact.

Instead, the NIR wavelength was less effective with a minor reduction (∼1 Log) in the level of SARS-CoV-2 RNA in both experimental settings (Figures 1 and 2, KW test, setting 2, day 3, NIR vs. not irradiated SARS-CoV-2 p-value = 0.01; day 7, NIR vs. not irradiated SARS-CoV-2 p-value = 0.03), although also in this case PBMT emerged as a potential antiviral agent.

As expected, the inhibition of viral amplification by laser light also rescued the cells from the cytopathic effect, preserving their viability at level comparable to the not infected cells at 7 days post infection, while in the cells infected with the not irradiated virus the survival dropped at 75% respect to not infected cells (Figure 3).

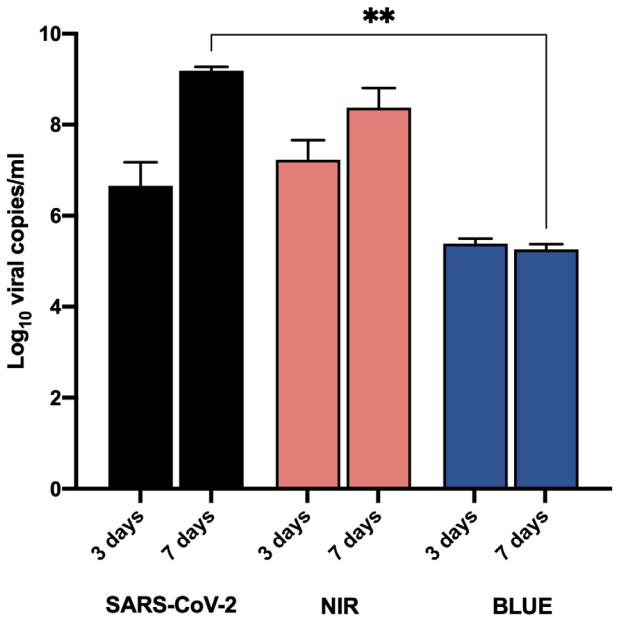


FIGURE 1 PBMT antiviral effect when laser (NIR and BLUE protocols of irradiation) was delivery on virus itself (setting 1). The viral load was displayed as Log_{10} viral copies/ml at 3- and 7-days post-infection/treatment. Cells infected with not irradiated virus was also shown (designed as SARS-CoV-2). $^{**}p$-value < 0.01

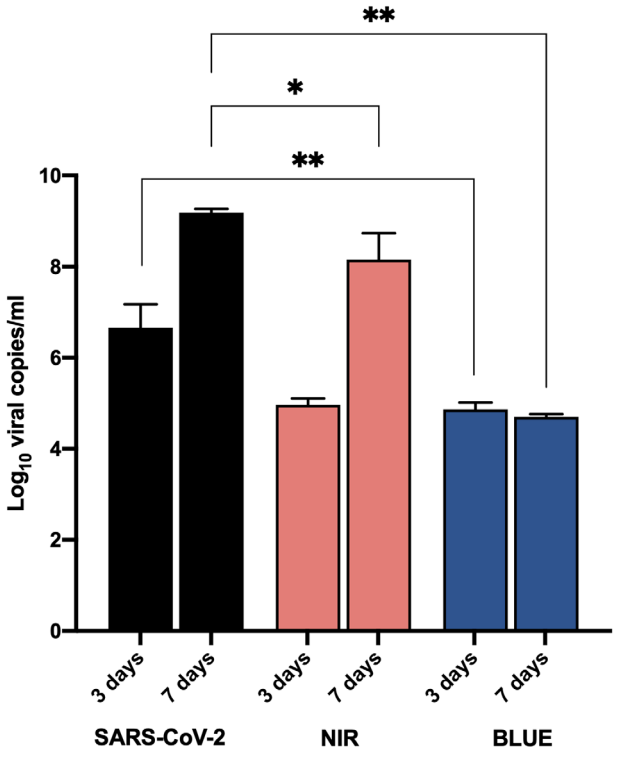


FIGURE 2 PBMT antiviral effect when laser light (NIR and BLUE protocols of irradiation) was delivery on already infected cells (setting 2). The viral load was displayed as Log_{10} viral copies/ml at 3- and 7-days post-infection/treatment. Cells infected with not irradiated virus was also shown (designed as SARS-CoV-2). $^{*}p$-value < 0.05, $^{**}p$-value < 0.01

Aiming at understanding the virucidal effect of PBMT, RNAse protection assay was performed; the virion integrity was preserved after the irradiation to level comparable to no irradiated virus, possibly suggesting that PBMT did not disrupt the lipidic envelope of SARS-CoV-2 (Figure 4) and the antigenicity of nucleocapsid protein was also retained (Figure S2).

These observations were not in agreement with our previous findings [13] where a reduction of intact virions was determined, nevertheless, the different devices, blue LED lamps in the previous study and class IV medical laser in the current one can account for the divergent outcomes; moreover, the protocols employed in the previous study were slightly cytotoxic [13], while the current ones, both employing NIR or blue wavelengths, did not have any impact on cells' survival.

Our results are in agreement with previous findings showing the antiviral effect of blue light against β-coronavirus [9–11].

Similar to our results obtained with blue wavelength, Stasko et al. [11] showed that light at 425 nm (50 mW/cm^2) was able to inhibit SARS-CoV-2 on already infected cells and on the virus itself. The irradiation of Vero E6 cells after 1 h of infection, with fluence of 7.5 J/cm^2, 15 J/cm^2 and 30 J/cm^2 reduced the viral load of >1, >2, and >3 Logs, respectively, at 24 h, at 48 h the viral load incremented with the first 2 protocols, while the 30 J/cm^2 the reduction of >3 Logs was maintained. Higher fluences (45 and 60 J/cm^2) were also effective in virus inhibition. PBMT applied to cell-free virions was also effective in the reduction of infective virus determined with tissue culture infectious dose 50 ($TCID_{50}$) assay, with reduction of >1 Log at 7.5 J/cm^2, > 2 Logs at 15 J/cm^2, > 3 Logs at 30 J/cm^2, and >4 Logs at 60 J/cm^2. These results were comparable to our findings, nevertheless, by using very low irradiance the time of irradiation in the work by Stasko et al. was quite long when compared to our findings (7.5 J/cm^2–150 min, 15 J/cm^2–300 min, 30 J/cm^2–600 min, 45 J/cm^2–900 min, 60 J/cm^2–1200 min).

Oh et al. [9] observed a viral load reduction after irradiation of already infected (1 h) cells by employing a 450 nm LED device (5.56 mW/cm^2) with 1.6, 5 and 10 J/cm^2 (5, 10, 20 s of irradiation), and the effect was prompted by a double treatment. The treatment of the cells with N- Acetylcysteine (NAC) a free-radical scavenger slightly diminished the impact, possibly suggesting a possible partial role of ROS in the antiviral activity of blue light. Interestingly, the blue light promoted the expression of ER stress proteins that are responsible for the blocking of mRNA translation, as well as host cell defensive signaling pathway, inhibiting apoptosis and inducing autophagy suggesting an indirect effect of PBMT on infection through the influence of the host target cells.

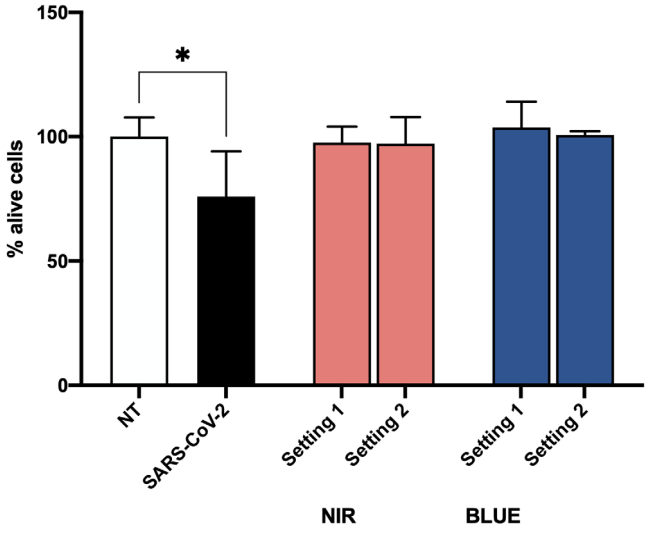


FIGURE 3 Viability of the cells at day 7 after irradiation/infection with setting 1 and 2 by employing NIR and blue PBMT. The results are reported as percentage respect to the not infected cells (NT). Survival of cells infected with not irradiated virus was also shown (designed as SARS-CoV-2). *p-value < 0.05

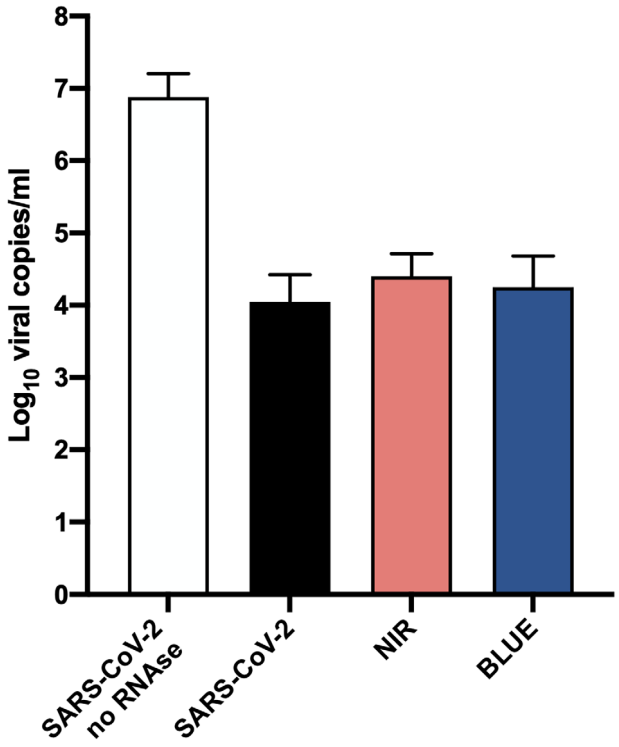


FIGURE 4 RNAse protection assay assessing virion integrity. The viral load was displayed as Log_{10} viral copies. Not irradiated virus was also displayed (designed as SARS-CoV-2) as well as the viral load of the virus not submitted to RNAse digestion

Terrosi et al. [10] reported the virucidal effect of blue light emitting at 410–430 nm (120 mW/cm^2, with a complete inactivation after 15 s of irradiation). The researchers showed that PBMT did not degrade the RNA or spike protein in accord with our findings regarding the maintenance of protein antigenicity, after photons delivery. Moreover, they showed the antiviral effect of PBMT when the virus was directly irradiated from nasopharyngeal swab in physiologic solution, possibly suggesting endogenous photosensitizers derived from human respiratory droplets or organic materials as mucins may enhance the antiviral effect, as well as the universal transport medium (UTM) medium of the swab. This aspect will be particularly important considering a possible application for the patients' treatment. Nevertheless, considering that viruses budded from the host cellular membrane it cannot be ruled out the contributor role of photosensitizers on plasma membrane [25–27].

When considering the antiviral effect of NIR PBMT, few studies have been addressed this topic. NIR PBMT has been used to treat different human ailments in pediatric patients as oral mucositis wound [19, 20], skin scars [21], and asthma [22]. The effect of NIR PBMT on virus was previously investigated by Donnarumma on HSV-1 (830 nm) replication in the viral post-absorption phase [18] and by our research group against Zika virus, both delivering PBMT on already infected cells and on the virus itself [17]. In this case the biomodulation effects, widely known for this type of wavelength can be combined with an antiviral activity in a synergistic approach.

PBMT has been arisen as an alternative nonpharmacological powerful option in the fight viruses. Being aware that our observations present the limitation of having been obtained in an in vitro cellular model, our study contributed to further elucidate laser light effects on SARS-CoV-2 in vitro, showing that blue wavelength but also the NIR one possess antiviral activity when the virus itself was irradiated but also when it is intracellularly. Our promising findings are contributing to pave the way for in vivo studies, necessary before translating PBMT to a clinical trial, and also to unravel the possible mechanisms of action of PBMT on the virus as well as on the infected cells. Preclinical measures/treatements are now well worth since they will endorse the employment of the laser device in the long lasting challenge against infectious agents, supporting its use as a potential antiviral strategy.

AUTHOR CONTRIBUTIONS

Luisa Zupin and Libera Clemente were involved in performing the experiments and writing—original draft. Francesco Fontana was involved in supervision of the experiments conducted in the BLS3 facility, writing—review and editing. Sergio Crovella was involved in conceptualization of the study, writing—review and editing, project management.

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CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Luisa Zupin https://orcid.org/0000-0001-5886-9129

REFERENCES

[1] P. J. Muñoz Sanchez, J. L. Capote Femenías, A. Díaz Tejeda, J. Tunér, *Photomed. Laser Surg.* **2012**, *30*, 37.
[2] A. Schindl, R. Neumann, *J. Invest. Dermatol.* **1999**, *113*, 221.
[3] R. R. de Carvalho, F. de Paula Eduardo, K. M. Ramalho, J. L. F. Antunes, L. M. Bezinelli, M. H. C. G. de Magalhães, T. Pegoretti, P. M. de Freitas, C. de Paula Eduardo, *Lasers Med. Sci.* **2010**, *25*, 397.
[4] P. Stona, E. da Silva Viana, L. dos Santos Pires, J. B. Blessmann Weber, P. Floriani Kramer, *Int. J. Clin. Pediatr. Dent.* **2014**, *7*, 140.
[5] Y. S. Shin, E. B. Cho, E. J. Park, K. H. Kim, K. J. Kim, *J. Dermatol. Treat.* **2017**, *28*, 411.
[6] R. K. Williams, J. Raimondo, D. Cahn, A. Williams, D. Schell, *J. Biophotonics* **2022**, *15*, e202100194.
[7] S. A. Sigman, S. Mokmeli, M. Monici, M. A. Vetrici, *Am. J. Case Rep.* **2020**, *21*, e926779.
[8] S. S. Tomazoni, D. S. Johnson, E. C. P. Leal-Junior, *Life* **2021**, *11*, 1124.
[9] P. Oh, K. W. Kang, S. R. Ryu, S. Lim, M. Sohn, S. Lee, H. Jeong, *Photochem. Photobiol.* **2022**, *98*, 461.
[10] C. Terrosi, G. Anichini, J. D. Docquier, G. Gori Savellini, C. Gandolfo, F. S. Pavone, M. G. Cusi, *Pathogens* **2021**, *10*, 1590.
[11] N. Stasko, J. F. Kocher, A. Annas, I. Henson, T. S. Seitz, J. M. Miller, L. Arwood, R. C. Roberts, T. M. Womble, E. G. Keller, S. Emerson, M. Bergmann, A. N. Y. Sheesley, R. J. Strong, B. L. Hurst, D. Emerson, E. B. Tarbet, S. S. Bradrick, A. S. Cockrell, *Sci. Rep.* **2021**, *11*, 20595.
[12] L. Zupin, R. Gratton, F. Fontana, L. Clemente, L. Pascolo, M. Ruscio, S. Crovella, *J. Biophotonics* **2021**, *14*, 14.
[13] L. Zupin, R. Gratton, M. Milani, L. Clemente, F. Fontana, M. Ruscio, S. Crovella, *J. Biophotonics* **2022**, *15*, e202100375.
[14] C. S. Enwemeka, V. V. Bumah, J. L. Mokili, *J. Photochem. Photobiol. B* **2021**, *222*, 112282.
[15] L. Zupin, I. Caracciolo, P. M. Tricarico, G. Ottaviani, P. D'Agaro, S. Crovella, *Microbiol. Immunol.* **2018**, *62*, 477.
[16] L. Zupin, S. Crovella, *Life* **2022**, *12*, 55.
[17] L. Zupin, I. Caracciolo, P. M. Tricarico, G. Ottaviani, P. D'Agaro, S. Crovella, *Lasers Med. Sci.* **2018**, *33*, 2011.
[18] G. Donnarumma, V. De Gregorio, A. Fusco, E. Farina, A. Baroni, V. Esposito, M. Contaldo, M. Petruzzi, G. Pannone, R. Serpico, *Int. J. Immunopathol. Pharmacol.* **2010**, *23*, 1167.
[19] M. Chermetz, M. Gobbo, L. Ronfani, G. Ottaviani, G. A. Zanazzo, F. Verzegnassi, N. S. Treister, R. Di Lenarda, M. Biasotto, S. Zacchigna, *Int. J. Paediatr. Dent.* **2014**, *24*, 441.
[20] M. Gobbo, F. Verzegnassi, L. Ronfani, D. Zanon, F. Melchionda, S. Bagattoni, A. Majorana, E. Bardellini, R. Mura, A. Piras, M. G. Petris, M. L. Mariuzzi, A. Barone, E. Merigo, N. Decembrino, M. C. Vitale, M. Berger, P. Defabianis, M. Biasotto, G. Ottaviani, G. A. Zanazzo, *Pediatr. Blood Cancer* **2018**, *65*, e27098.
[21] J. Alsharnoubi, K. E.-S. Shoukry, M. W. Fawzy, O. Mohamed, *Lasers Med. Sci.* **2018**, *33*, 1991.
[22] S. V. Moskvin, A. A. Khadartsev, *BioMedicine* **2020**, *10*, 1.
[23] X. Lu, L. Wang, S. K. Sakthivel, B. Whitaker, J. Murray, S. Kamili, B. Lynch, L. Malapati, S. A. Burke, J. Harcourt, A. Tamin, N. J. Thornburg, J. M. Villanueva, S. Lindstrom, *Emerg. Infect. Dis.* **2020**, *26*, 1654.
[24] R Core Team, **2021**, http://www.R-project.org.
[25] R. Rathnasinghe, S. Jangra, L. Miorin, M. Schotsaert, C. Yahnke, A. García-Sastre, *Sci. Rep.* **2021**, *11*, 19470.
[26] P. Vatter, K. Hoenes, M. Hessling, *Photochem. Photobiol.* **2021**, *97*, 122.
[27] R. Lavi, R. Ankri, M. Sinyakov, M. Eichler, H. Friedmann, A. Shainberg, H. Breitbart, R. Lubart, *Photomed. Laser Surg.* **2012**, *30*, 14.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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