Light-induced toxicity and reactive oxygen species production in HeLa cervical cancer cells

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INTRODUCTION

Photodynamic therapy (PDT) represents a minimally invasive treatment strategy that relies on light-induced production of reactive oxygen species (ROS) to selectively destroy diseased cells. While PDT typically requires a photosensitizer, it has been demonstrated that light alone, in the form of phototherapy (PT), can also stimulate ROS generation in cancer cells, leading to their elimination [1-3]. This approach offers a major advantage by avoiding the need for exogenous drugs, thereby simplifying treatment, reducing potential side effects, and accelerating therapeutic response. Due to its anatomical accessibility, the uterine cervix allows precise and localised light delivery, making PT an especially promising modality for cervical cancer management.

MATHERIALS AND METHODS

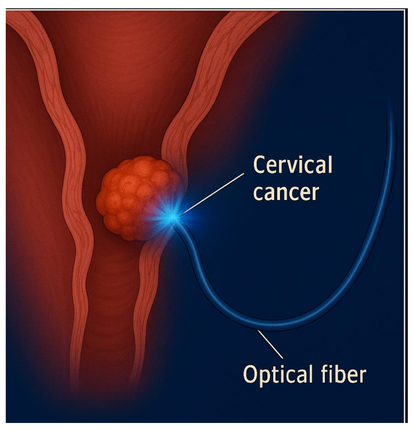
HeLa cells were seeded in 96-well plates and incubated overnight until reaching confluency, after which they were subjected to illumination. Figure 1b illustrates the experimental setup for cell illumination. Blue laser system (Creality (Shenzhen, China) in a continuous-wave regime was used for cell illumination. Cells were illuminated for 20 min with green light (661, 332, 260 J/cm², Verdi 5, Coherent, Saxonburg, USA) and 5 min with blue light (125, 67, 29 J/cm²). After 48 hours, cell viability was determined using an SRB assay [4] with a Wallac VICTOR2 microplate reader (PerkinElmer, Turku, Finland).

Intracellular ROS levels were measured with the DCFDA cellular ROS assay following the standard protocol previously described in the literature [5].

Cell imaging before and after illumination was performed using the Zoe™ Fluorescent Cell Imager (Bio-Rad Laboratories, Hercules, CA, USA).

AIM

Cervical cancer represents the fourth most common cancer in women globally, with more than 660,000 newly diagnosed cases and approximately 350,000 deaths reported annually [6]. Despite the availability of HPV vaccination and regular screening programs, many women remain undiagnosed until the disease reaches advanced stages. Standard therapies, including surgery, radiotherapy, and chemotherapy, are often effective in early-stage disease, but their success is limited in advanced or recurrent cases. Consequently, cervical cancer continues to have a high mortality rate worldwide, highlighting the urgent need for novel, effective, and accessible therapeutic approaches.



This study systematically compares the effects of two laser wavelengths, blue (405 nm) and green (532 nm), on HeLa cells. The aim is to provide new insights into wavelength-dependent phototoxicity and its link to ROS production. Additionally, it lays the foundation for potential translation using fibre-mediated light delivery. Such approaches could allow precise, minimally invasive phototherapy for early-stage cervical lesions. Targeted cytotoxic effects in tumor cells could be achieved while sparing surrounding healthy tissue. Moreover, these strategies support the potential for repeated treatments or combination therapies in a clinically relevant context. Overall, this work highlights the promise of fibre-guided phototherapy as a selective and adaptable approach for cervical cancer management.

RESULTS AND DISCUSSION

HeLa cells were illuminated with blue and green laser light for the purpose of cervical cancer PT. All applied fluences reduced cell viability below 50% compared to the control, with lower fluences of blue light showing a more substantial effect (Figure 1a). The fluence of 125 J/cm² decreased cell viability to 14%, while the most effective green light treatment (661 J/cm²) resulted in 24% viability.

We have also monitored cellular morphology up to 48 h post-irradiation. Control HeLa cells (non-illuminated) displayed normal morphology and proliferation, whereas blue and green irradiated cells showed shrinkage, detachment, and cell death (Figure 1c). Illuminated cells displayed typical signs of cellular stress, indicating a clear disruption of normal morphology.

Light illumination is known to induce the generation of ROS [7,8]. The excessive accumulation of ROS disrupts redox homeostasis, triggers oxidative stress, and activates downstream signalling pathways leading to mitochondrial dysfunction and apoptotic or necrotic cell death. Because of this central role in phototoxicity, ROS production is considered one of the primary mechanisms underlying the effectiveness of light-based anticancer therapies. Therefore, we examined the intracellular ROS production in HeLa cells following illumination. The highest ROS production was observed upon blue laser irradiation (Figure 1d), correlating with the pronounced photocytotoxicity, whereas green light exposure induced ROS levels comparable to those of the positive control, further indicating substantial ROS generation following irradiation. The stronger photocytotoxic response observed with blue light may be attributed to its higher efficiency in ROS generation compared to green light.

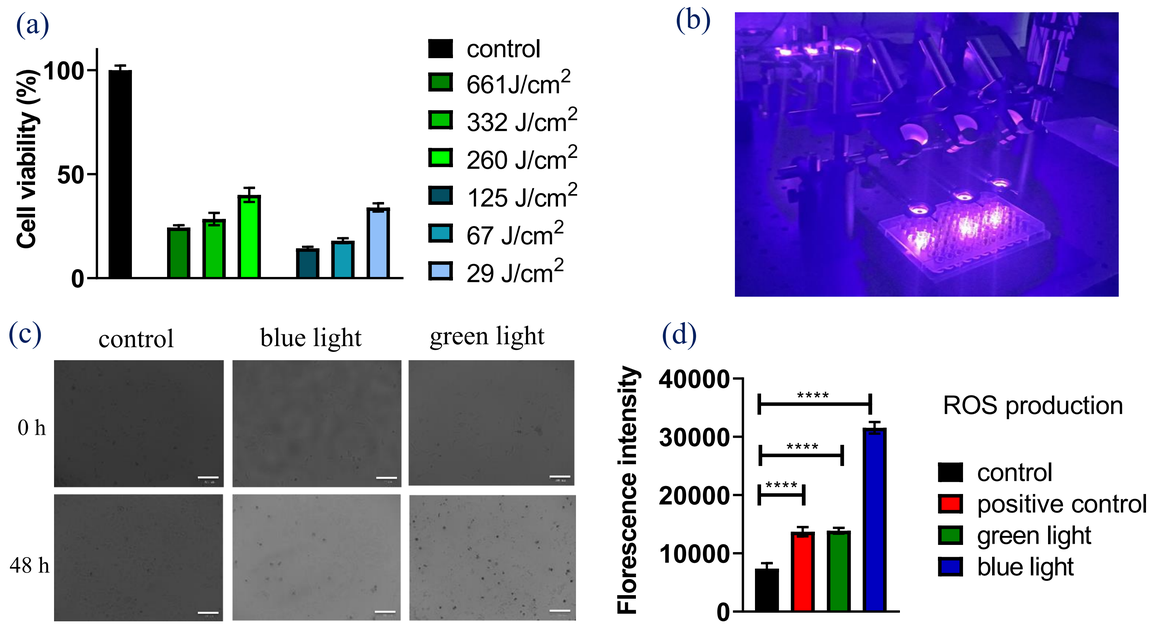


Figure 1. HeLa cell phototocixity 48 h after green and blue light exposure (a), Experimental set-up for cell illumination (b). Photomicrographs of HeLa cells before, and 48 hours after illumination. (c). Intracellular ROS production after light exposure (d). Scale bar: 100 μm.

CONCLUSION

In vitro results on HeLa cervical cancer cells indicated photocytotoxic effects under both blue and green light irradiation, with blue light exhibiting higher efficiency at lower doses, confirming that direct light application to tumour cells can achieve therapeutic effects. Although the penetration depth of blue (405 nm) and green (532 nm) light in biological tissues is limited due to strong absorption and scattering, this limitation can be effectively mitigated through the use of optical fibres positioned near the tumour. By delivering light directly to the lesion site, intracervically, optical fibres bypass the superficial tissue layers responsible for most attenuation, ensuring sufficient light intensity reaches the target. Additionally, specially designed fibre tips, such as cylindrical or spherical diffusers, can provide more uniform light distribution within the tumour volume. These findings support the potential of fibre-mediated photodynamic or photothermal therapy for cervical cancer, particularly for early-stage of anatomically accessible tumours.

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