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BRIEF REPORT

Effect of nutritional stress and serum starvation on the optical absorbance of normal and malignant epithelial cell lines

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Abstract

This brief report aimed to investigate the optical absorbance spectra of normal, dysplastic, and malignant epithelial cell lines under normal and nutritional stress conditions. HaCAT (keratinocyte), DOK (oral dysplastic), and oral squamous cell carcinoma (OSCC) cell lines (CA1, Luc4, SCC9) were evaluated regarding their optical absorbance after culture with 0–10% fetal bovine serum. Absorbance measurements indicated that HaCAT under serum starvation exhibited higher absorbance at blue (430 nm) and near-infrared (906 nm) wavelengths. DOK showed absorption at 440 nm and 945 nm. OSCC cells showed absorption peaks at blue (400–428 nm) and near-infrared. These findings highlight the importance of tailoring PBM parameters to individual needs to achieve optimal absorption and effectiveness. Moreover, the higher absorption peaks in the blue region support further studies to elucidate the potential use of blue light in oral dysplastic lesions and OSCC.

Keywords Photobiomodulation · Oral squamous cell carcinoma · Oral dysplastic cells · Optical absorbance

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Introduction

Photobiomodulation (PBM) is capable of modulating the behavior and cellular response in different pathological conditions, acting through different signaling pathways and targeting transcription factors that ultimately activate effector cellular molecules [1]. Because of light absorption by cellular and/or tissue chromophores, distinct biological processes are triggered promoting cellular proliferation, differentiation, tissue repair, modulation of inflammation and analgesia [2]. This therapy typically employs light sources like lasers and LEDs, particularly in the red (600–700 nm) and near-infrared (770–1200 nm) spectras [3, 4]. The efficacy of PBM is influenced by factors such as light source, wavelength, pulse, exposure time, energy density, cell type, oxygen levels, and metabolic state [5]. In vitro studies suggest that PBM can enhance cell viability and proliferation under nutritional stress [6], probably because cells in their typical physiological state may not exhibit a significant response to PBM, as there is no underlying stress to alleviate. However, the mechanisms through which PBM influences stressed cells remain unclear.

In addition, the effects of PBM on malignant cells is still controversial [7]. It is hypothesized that the altered metabolic state in stressed and/or malignant cells may enhance PBM's effects due to changes in light absorption [8]. Thus, the effects of PBM may be variable according to a specific cell type and its patterns of light absorption, leading to different cellular behaviors after PBM [3]. In addition, it is important to consider the Warburg effect in cancer cells, in which the mitochondria metabolism is switched to carry out aerobic glycolysis and thus, the effects of PBM in normal and malignant cells will be different and will require different PBM parameters [3]. Furthermore, it is essential to study the behavior of cancer cells under nutritional stress to better simulate the real and complex tumor microenvironment, which has different cell types together with different nutrient gradients and metabolisms [9].

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer, accounting for about 90% of all oral malignancies. The 5-year survival rate for OSCC patients is between 50 and 60%, but this drastically declines to a median survival of 10 to 13 months in cases of recurrent and/or metastatic disease [10]. OSCC treatment is challenging and based on surgery, radiotherapy and chemotherapy. In addition, it is also associated with facial deformities and adverse side effects in the oral cavity, which significantly impact the patient's quality of life during and after cancer treatment [11]. In this context, PBM offers a promising adjunct therapy, reducing side effects and promoting tissue repair [9]. However, its dual effects, particularly in metabolically stressed conditions common in OSCC tumor microenvironment, require careful study to maximize benefits and avoid stimulating tumor growth [9].

This study investigates the optical absorbance spectrum of keratinocyte, oral dysplastic, and oral squamous cell carcinoma (OSCC) cell lines, cultured under both normal and nutritional stress conditions. The goal is to identify the most suitable wavelengths for PBM that should either be utilized or avoided in the treatment of oral malignant disorders and oral cancer.

Materials and methods

Cell culture

HaCaT cell line [12], dysplastic oral keratinocyte cell line DOK (ATCC), and the oral malignant cell lines SCC9 (ATCC), CA1 and Luc4 (kindly given by Prof. Ian Mackenzie, Barts and the London School of Medicine and Dentistry, UK) were cultivated according to previously established protocols [7, 13].

Experimental groups

The cell lines were seeded at a density of 7×10^3 cells/cm^2 and grown for 24 h with the regular medium supplemented with 10% FBS (control), 5% FBS (nutritional stress) and in the absence of FBS (0% FBS, serum starvation).

Analysis of optical absorbance

After 24 h, cells were detached, centrifuged, and counted. 5×10^4 cells from each treatment group were reconstituted in 1.5 mL PBS and placed in disposable plastic cuvettes. Absorbance was measured using a spectrophotometer (Ocean Optics, USB-2000 model, Florida, USA) with SpectraSuite software, following calibration with PBS. Data were analyzed using OriginPro (version 2017 SR2, Massachusetts, USA). Three independent experiments in triplicate were performed for each cell line to ensure the reproducibility and stability of the absorption analysis.

The mean absorbance was determined from triplicate experimental readings for each group, and the data were normalized for visualization, using the first-order derivative. The results were then smoothed employing the Savitzky-Golay method. The absorbance spectrum considered was 400 to 1000 nm. In the graphics, regions where the increase in absorbance was directly proportional to the concentration of FBS, that is, from 0 to 10% or inversely proportional, from 10 to 0% of FBS, were highlighted in light gray.

Results

Analysis of optical absorbance in normal and dysplastic epithelial cell lines

HaCAT cells absorb light over almost the entire spectrum (Fig. 1A), with one peak of absorption at blue, 430 nm and another peak of absorption in the near-infrared region, at 906 nm. The highest overall absorbance was observed in cells cultivated under serum starvation (0% FBS), followed by the cells cultivated with 5% FBS, when compared to cells cultivated with 10% FBS.

The DOK cells exhibited higher absorption at blue, 440 nm, with the second highest peak around 945 nm, also in the near-infrared region. Other peaks of absorption were noticed, but only from 635 to 670 nm (red region) and 840 to 955 nm (near infrared) the absorbances were proportional to the concentration, with the cells cultivated with 10% FBS showing higher absorbance than those cultivated with 5% FBS or in starvation condition. In some regions as 450 to 565 nm, 584 to 635 nm and 703 to 805 nm, cells cultivated under starvation showed higher absorbance than cells with

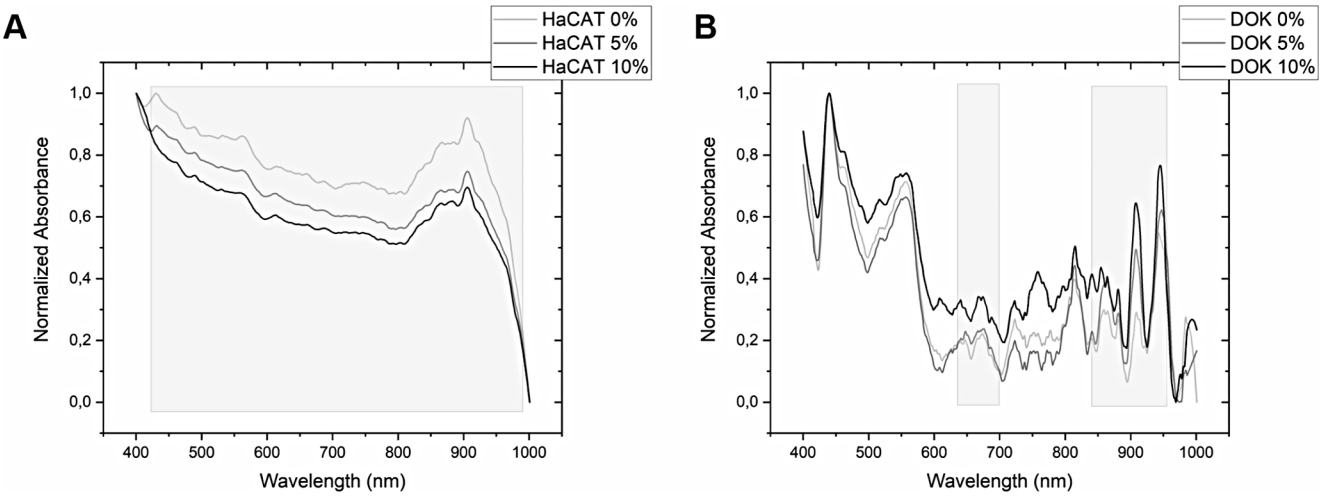

Fig. 1 Optical absorbance spectra (400 to 1000 nm) of HaCAT (**A**) and DOK (**B**) after 24 h of culture in the presence of 10% FBS, 5% FBS and in serum starvation (0% FBS). Graph represents mean of absorbance of three independent experiments. Regions where the increase in absorbance was directly proportional to the concentration of FBS, that is, from 0–10% or inversely proportional, from 10–0% of FBS, were highlighted in light gray

5% FBS, although the highest absorbance was with 10% FBS (Fig. 1B).

Analysis of optical absorbance in malignant epithelial cell lines

CA1 cells showed three high absorbance peaks in the visible range. In blue, at 400 and 428 nm, and in yellow, at 556 nm. However, only in the intervals from 428 to 457 nm there was a greater absorption with 10% FBS, followed by 5% FBS and 0% FBS, as well as between 497 and 584 nm and 681 to 695 nm. Another three peaks appear in the infrared region of the spectrum, at 904, 945 and 974 nm. However, only two regions, 871 to 917 nm and 963 to 982 nm showed absorbance related to the FBS concentration, being inversely proportional to concentrations with 0% FBS, followed by 5% FBS and then 10% FBS. Between 608 and 846 nm (red and near infrared) cells with 5% FBS exhibited higher overall absorbance than cells cultivated with 10% FBS or 0% FBS, with the lowest absorbance observed in the serum starvation condition in the red region (608 to 708 nm) (Fig. 2A).

LUC4 cells showed the highest absorption peak in the blue region of the spectrum, around 428 nm, followed by the peak at 976 nm (infrared). Two other peaks stand out, at 553 nm (yellow) and 744 nm (near infrared). Almost in the whole spectral range analyzed there was a higher absorbance for the concentration of 10% of FBS, followed by 5% FBS, with the lowest absorbance for 0% FBS. The exception is in the regions 445 to 500 nm, 752 to 760 nm, 902 to 922 nm and 965 to 991 nm, with the highest absorption using 5% FBS followed by absorbance of 10% FBS (Fig. 2B).

In contrast to the results observed for CA1 and Luc4, SCC9 showed the highest absorption peak in the red region, around 690 nm and the second highest peak at 818 nm. Cells cultivated in serum starvation exhibited higher optical absorbance in wavelengths up to 673 nm, at 719 nm and 783 nm when compared to those cells cultivated with 5% FBS or 10% FBS, which showed similar results. Interestingly, a switch in the optical absorbance was seen at 700 nm, in which cells cultivated in serum starvation showed lower absorption in relation to 10% FBS or 5%. Moreover, cells with 10% FBS demonstrated greater absorbance than 0% FBS and 5% FBS, in this sequence, at wavelengths 751 to 772 nm, 791 to 836 nm, 844 to 882 nm, 895 to 947 nm and above 958 nm (Fig. 2C).

The distinct absorbance peaks identified across the spectrum, such as the prominent blue peak, red and near-infrared peaks, suggest wavelength-specific interactions with cellular components that may influence processes such as oxidative stress modulation and mitochondrial activity, which are central to PBM-induced cellular responses.

Discussion

PBM can influence cellular behavior and tissue response in pathological conditions, but its effectiveness varies with factors like wavelength, energy density, and cell type [2]. It is also important to highlight that different cell types respond differently to PBM, which can also be associated with their respective pattern of light absorption in a specific range of the spectrum.

Under conditions of nutritional stress induced by serum starvation, certain cell types, particularly normal cells, and dental-origin stem cells, exhibit a more favorable response to PBM [6, 14–16]. Conversely, cells in their typical

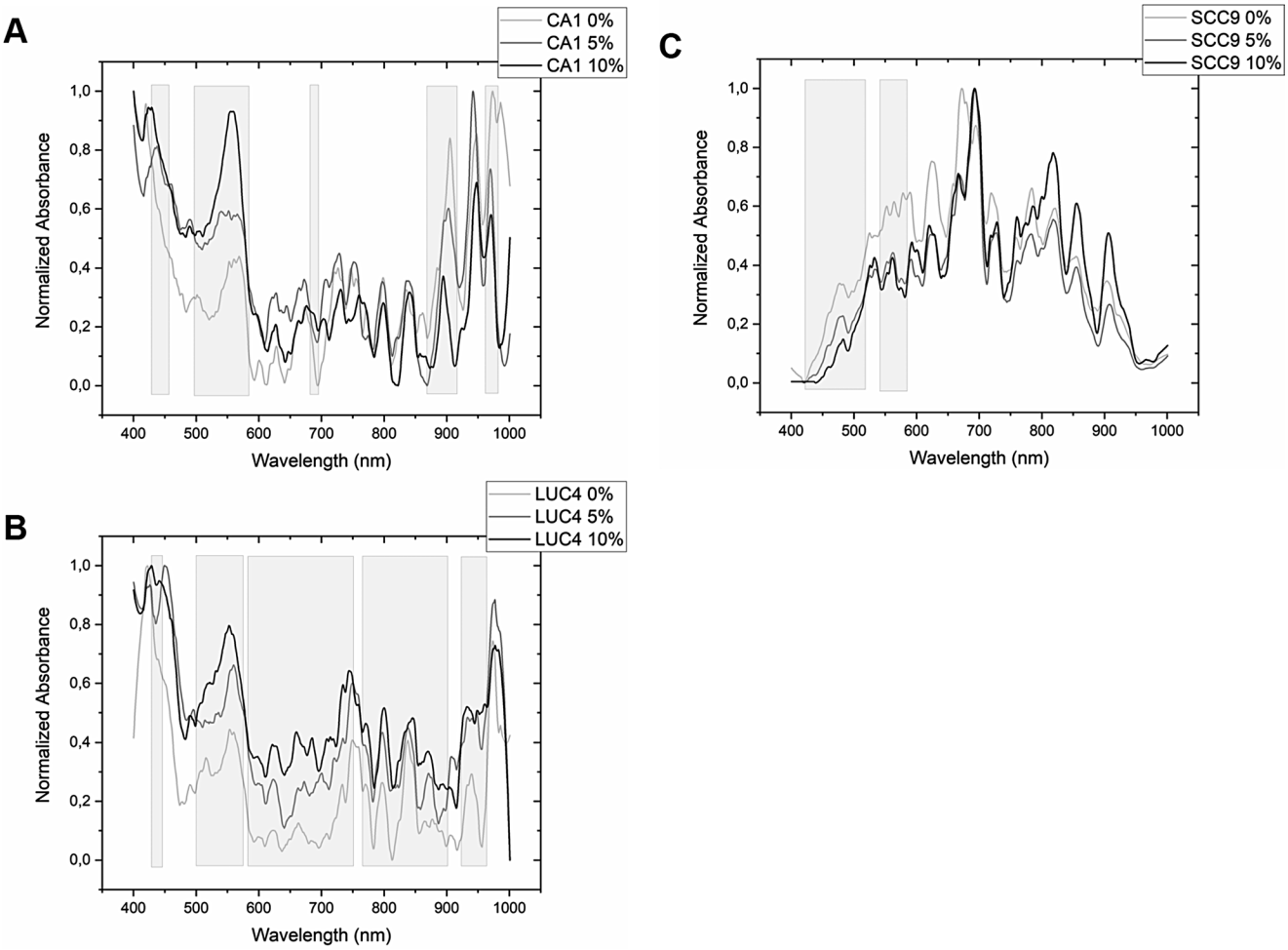

Fig. 2 Optical absorbance spectra (400 to 1000 nm) of CA1 (**A**), LUC4 (**B**) and SCC9 (**C**) after 24 h of culture in the presence of 10% FBS, 5% FBS and in serum starvation (0% FBS). Graph represents mean of absorbance of three independent experiments. Regions where the increase in absorbance was directly proportional to the concentration of FBS, that is, from 0–10% or inversely proportional, from 10–0% of FBS, were highlighted in light gray

physiological state may not exhibit a significant response to PBM, as there is no underlying stress to alleviate [8]. However, this response does not appear to be uniform across all cell types. Considering nutritional stress, HaCAT showed the most linear and stable behavior across the analyzed spectral range, with the absorbance being highest for 0% FBS and the lowest absorbance for 10% FBS, with peaks at 430 nm and 906 nm. Fushimi et al. [17] investigated the impact of red (638 nm), blue (456 nm), and green (518 nm) LEDs. They observed that green light (518 nm) significantly accelerated wound healing, promoting faster cell proliferation and modulating growth factors and cytokines, resulting in more efficient wound recovery in vitro and in vivo. Blue light (456 nm) and red light (638 nm) had a moderate effect, but less effectively than green light.

Similarly, Kim et al. [18] found that in HaCAT cells, green light (525 nm) greatly increased ROS production, leading to strong activation of focal adhesion kinase (FAK) and promoting cell viability and proliferation. Blue light (470 nm) caused a smaller ROS increase and moderate FAK activation, enhancing viability to a lesser degree. Red light (630 nm) had the least impact on ROS, FAK activation, and cell viability. Also Basso FG et al., [19] showed effects of PBM (780 nm) in HaCAT with positive biostimulator effect, promoting proliferation, viability, and migration, thereby aiding in wound healing and tissue regeneration. Taken together, these studies indicate positive effects of PBM at various wavelengths, which can be associated with the absorption of light over almost the entire optical spectrum by HaCAT cells. Although all studies used 10% serum for cell supplementation, no impact on the evaluated outcomes was noticed. However, the conditions with highest light absorption were under nutritional stress and serum starvation, which is more similar to the clinical context when PBM is used, where the ATP levels and the cellular metabolic states are disturbed.

For the DOK, we observed higher absorption at blue light (440 nm), with a second peak around 945 nm in the near-infrared region. The literature is scarce regarding the DOK and PBM. Notably, Sperandio et al. [20] demonstrated that PBM wavelengths of 660 nm (red) and 780 nm (near-infrared) can increase the aggressiveness of dysplastic cells by activating the Akt/mTOR pathway, with the 780 nm wavelength showing a more pronounced effect compared to 660 nm. Our findings corroborate with this study, as we observed higher absorption near 780 nm than 660 nm in the DOK. In regards to the blue light, future studies should address whether this wavelength will have inhibitory effects in dysplastic oral lesions.

Regarding the OSCC cell lines, the LUC4 showed a spectral absorption response to nutritional stress inversely proportional to the HaCAT, In CA1 and SCC9, a variable absorption spectrum was noticed depending on the FBS concentration and wavelength, indicating that the response to PBM may be variable according to the nutritional condition and cancer cell line. In SCC9, the highest absorbance peak occurred at 690 nm (red region) and the second highest peak was at 818 nm (near infrared). CA1 and LUC4 also showed absorption peaks at red and infrared regions lower than SCC9. Research on OSCC cells, especially SCC9, has indicated that PBM might promote tumor growth in a dose-dependent manner, enhancing cell viability and proliferation, activating Akt/mTOR pathways [7, 20]. Given that OSCC cells are capable of absorbing photons in the red and infrared regions, which can potentially modulate tumor cell behavior, studies with PBM in tumor cells have focused more on these regions of the absorbance spectrum as they are used in the treatment of oral mucositis, the main adverse effect of the oncological treatment, mainly in patients with head and neck cancer submitted to radiotherapy. However, there is no consensus regarding whether PBM could stimulate the remaining tumor cells in oral mucosa, possibly leading to tumor progression or recurrence, so studies are still essential to thoroughly investigate the safety and efficacy of PBM with different wavelength and dosage in cancer patients, particularly concerning its potential effects on malignant cells.

Interestingly, both OSCC cell lines CA1 and Luc4 showed higher absorption in wavelengths up to 440 nm (blue region) and between 904 and 976 nm (near infrared). The blue light has recently been investigated as a possible tool for cancer treatment. Several studies in vitro and in vivo have already shown inhibitory effects of blue light irradiation alone on specific cancer cells, through the induction of autophagy, apoptosis, necrosis, production of reactive oxygen species and DNA damage [21]. Furthermore, the effect of blue light has already been shown to decrease the activity of cancer-associated fibroblasts and cancer-associated macrophages, important therapeutic targets in the tumor microenvironment [22]. In OSCC cells, especially, studies with blue light irradiation showed a suppressive effect on cellular proliferation and migration, mainly via oxidative stress, DNA damage and a pronounced mitochondrial dysfunction [21, 23]. In fact, the positive anti-tumoral effects of blue light in OSCC might be related with the highest absorption of light at this spectrum region, which was observed in the present study, corroborating to future studies aiming to evaluate the therapeutic use of blue light in oncology.

In summary, future research should focus on evaluating the potential effect of PBM on OSCC, taking into account different nutritional states, especially studies in vivo, in order to better mimic the tumor microenvironment, being able to define correct parameters for the use of blued light for oncological treatment and to confirm the safety of its use, especially in red and infrared wavelengths, in oncological patients. In addition, a large-scale study incorporating a diverse series of cell lines, including those representing potentially premalignant disorders and additional malignant cell types, would provide a broader context and deeper understanding of cellular responses under metabolic stress and different PBM parameters.

Conclusion

This study revealed that the absorption profile of normal, dysplastic and OSCC cells is variable according to the cell type and metabolic state, with normal cells (HaCaT) showing higher absorption under serum starvation. Dysplastic (DOK) and OSCC cells had distinct absorption peaks, with the highest peak in blue region, encouraging future studies to evaluate the anti-tumoral effects of blue light in oral dysplasia and initial stages of OSCC. In addition, the second-highest peak in near-infrared regions indicates that PBM may modulate OSCC cell behavior and caution must be taken when PBM is applied to treat the adverse effects of cancer treatment. Taken together, findings highlight the importance of tailoring PBM parameters to individual needs and underscore the need for further research to guarantee both the safety and effectiveness of its application, especially, to elucidate the potential use of blue light in oral dysplastic lesions and in OSCC.

Author contributions J. S. Nobile: Writing – original draft, visualization, validation, methodology, investigation, formal analysis, data curation, conceptualization. D. Heguedusch: Writing – original draft, methodology, investigation, formal analysis, conceptualization. G. L. Carvalho: Writing – original draft, methodology, investigation, formal analysis, conceptualization. D. de F. T. da Silva: Writing – original draft, methodology, investigation. R. B. Cecatto: Writing – original draft, methodology, investigation. R. L. Marcos: Validation, methodology, investigation, formal analysis F. D. Nunes: Writing- review &

editing, investigation, methodology, formal analysis. M.F.S.D. Rodrigues: Writing –review & editing, supervision, resources, project administration, methodology, conceptualization.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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