**To:** Reviewers of the SAIP2016

**From:** Anine Crous

**Date:** 24/11/2017

**Title:** Photobiomodulation of Isolated Lung Cancer Stem cells

**Authors:** Anine Crous and Heidi Abrahamse

**CORRECTIONS TO REVIEWER’S COMMENTS: MANUSCRIPT (id: 415)**

Referee: To be corrected

Comments

**Review 1:**

The work is extremely relevant however the novelty has not been demonstrated clearly. The authors should however be commended for a clear and concise manuscript. The manuscript can be submitted provided the following corrections are incorporated and/or defended.

**Corrections:**

In the abstract:

* The following words “background”, “methodology” and “discussion” should be removed.

Corrected.

Page 2:

* Paragraph 1, sentence 2: after energy output… add ….of the incident light.

Corrected: “metabolic reactions depending on the wavelengths and energy output of the incident light”

* The range defined (1 to 15 J/cm^2) for LF-LILI overlaps with the HF-LILI (>10 J/cm^2). Is this a mistake? Or is this intended? Furthermore how was the fluence altered? I assume that the irradiation time was varied. This must be mentioned.

Corrected: “using LILI with low fluences (LF-LILI) ranging from 1 – 15 J/cm2 and wavelengths of 600 nm – 700 nm it stimulated biological processes [14-18], but have an inhibitory effect when using increased wavelengths of 800–830 nm and fluences larger than 10 J/cm2 “

* Paragraph 2, the defined range for LF-LILI changes to from 5 to 20 J/cm^2. This implies that the above HF-LILI is now included in the range of LF-LILI. A clear contradiction.

Corrected: “evaluated the effects of LF-LILI (5 – 20 J/cm2) and high fluence LILI (HF-LILI) (40 J/cm2), where different fluences were calculated through laser time exposure and output (mW)”

* Under 2.Methodology second paragraph. Mention if the number 1x10^5 is the number of CSCs or is it a surface concentration? In that case it must have an accompanying unit.

Corrected: “seeded at a number of 1 x 105 cells in culture plates”

* Instead of stating that the laser powers were +-85mW, state “ all lasers were kept at a constant power of 85 mW.”

Corrected: “All lasers were kept at a constant power output of 85 mW”

Page 3:

* The table is a summary of results not shown in the manuscript. Add the appropriate data and the rest (uninteresting) in the supplementary information.

Corrected: Results that were not statistically significant were added to the table as black arrows and results discussed accordingly in the discussion and conclusion section.

Page 4:

* Paragraph 3: give greater detail summarizing the information found in refs 22 and 23.

Corrected:” Photobiomodulation relies on specific parameters such as wavelength, fluence, power density, pulse structure, and treatment time when applied to biological tissue. This allows for targeting of specific light-absorbing molecules in specific tissues, operating on the principle of photochemistry, as opposed to photo thermogenesis. The light energy absorbed causes singlet state excitation of oxygen molecules, leading to triplet state excitation causing an energy transfer to ground state molecular oxygen (a triplet) to form the reactive species, singlet oxygen. Alternatively superoxide may be formed as a result of electron reduction. LILI operates at an exact wavelength of light, which influences the depth of tissue penetration. Similar to normal cells, cancer cells also contain with intracellular chromophores. Different cellular chromophores are stimulated at different wavelengths. Therefore, the prediction can be made that in targeting cancerous cells, the outcome expected can be controlled by the wavelength as well as by the energy output that will lead to either stimulation or inhibition. The exact mechanism behind the stimulation of the light-absorbing molecules producing these two different effects is still being investigated.”

**Review 2:**

The text includes some vague statements like “stimulate biological processes” which really should be clarified.

Corrected: “it stimulated biological processes by increasing proliferation and viability”

The absorption spectrum of the media as well as the CSC’s should be provided in order to validate the proposed mechanism.

Corrected: According to previous literature, the media used to conduct this study has no effect on the wavelengths used.

CSCs: “Similar to normal cells, cancer cells also contain with intracellular chromophores. Different cellular chromophores are stimulated at different wavelengths. Therefore, the prediction can be made that in targeting cancerous cells, the outcome expected can be controlled by the wavelength as well as by the energy output that will lead to either stimulation or inhibition. The exact mechanism behind the stimulation of the light-absorbing molecules producing these two different effects is still being investigated” Hence the proposed range of wavelengths were used in the study.

The results should be compared to those of the non-cancerous cells. The idea of killing CSC’s using HF-LILI is only practical if non-cancerous cells are unaffected at these fluencies.

Corrected: This study only focused on the effects LF-LILI and HF-LILI has on CSCs and results were interpreted as such. Previous studies did show that HF-LILI has a bio-inhibitory effect on normal cells. Thus concluding if similar effects are seen when comparing previous results of normal cells to CSCs.

The temperature of the cells before and after treatment should be noted to verify that this is not simply a thermal effect (this in combination with the absorption spectra is essential).

Corrected: No thermal effects were/are achieved when using LILI at a power output below 100 mW.

If these corrections are possible, then the paper is acceptable for publication.