



# Low-Power Red and Infrared Laser Effects on Cells Deficient in DNA Repair

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Published online July 6, 2019



## Abstract

**Introduction:** Low-level lasers are successfully used to prevent and treat diseases in soft oral and bone tissues, particularly diseases in oral cavity caused by chemotherapy and radiotherapy in oncology. However, controversy exists as to whether these lasers induce molecular side effects, mainly on DNA. The aim of this work was to assess the effects of low-power lasers on mutant *Escherichia coli* cells in DNA repair.

**Methods:** *Escherichia coli* wild type cultures as well as those lacking recombination DNA repair (*recA*) and la SOS responses (*lexA*) irradiated with lasers at different energy densities, powers, and emission modes for cell viability and morphology assessment were used in this study.

**Results:** Laser irradiation: (i) did not affect cell viability of non-mutant and *lexA*<sup>-</sup> cells but decreased viability in *recA*<sup>-</sup> cultures; (ii) altered morphology of wild type and *lexA*, depending on the energy density, power, emission mode, and wavelength.

**Conclusion:** Results show that low-level lasers have lethal effects on both recombination DNA repair and SOS response bacterial cells but do not induce morphological modifications in these cells.

**Keywords:** DNA repair; *Escherichia coli*; Laser therapy.

## Introduction

Monochromaticity, directionality, coherency, and light beams with high energy density differentiate lasers from regular radiation sources, making laser devices attractive for clinical applications. The light wavelength and optical properties of the biological tissue determine how deep laser radiation penetrates, and its high energy density and intensity allow high radiation doses to be delivered at high dose ratios.<sup>1</sup> Also, laser devices work as radiation sources, in continuous or pulsed emission mode, depend on the laser active medium or whether the emission is modulated by an electric current.<sup>1</sup> For low-level lasers, low energy densities or doses are applied to body tissues according to the therapeutic applications proposed by device guidebooks or laser practitioners. These lasers cause nonthermal and nondestructive effects but alter biological processes increasing the metabolism and

cellular division rate, an effect known as biostimulation or biomodulation. In most clinical applications, at a low level, this effect is considered as the photobiological basis for wound healing,<sup>2</sup> acceleration of tissue repair, nerves, cartilages, bone, soft tissues, as well as the relief from the inflammatory process<sup>3</sup> and pain.<sup>4</sup>

Absorption of radiation in the optical window of low-level lasers (600 up to 1100 nm) occurs by specific chromophores into cells.<sup>5</sup> Following photon energy absorption, a primary photo signal is generated and amplified in the cells as a consequence of transduction processes.<sup>6</sup> Free radicals, reactive oxygen, and nitrogen species were suggested as participating in these molecular mechanisms, reacting with biomolecules, and causing cellular function modifications.<sup>7</sup> However, as Kim<sup>8</sup> has suggested, photobiological side effects could occur if an increased free radicals pool is available to react with

biomolecules, like DNA, causing undesired effects. Side effects induced by low-level lasers are still a matter of controversy, but there are increasing experimental data from sublethal DNA lesions following irradiation of eukaryotic<sup>7,9,10</sup> and prokaryotic cells<sup>11,12</sup> with these lasers at therapeutic conditions. In other studies, it was demonstrated that previous exposure to low-level lasers induces resistance in *E. coli* cultures sensitive to hydrogen peroxide,<sup>13</sup> and cell viability is decreased in *E. coli* cultures after exposure to lasers.<sup>12,14</sup> In addition, these lasers are capable of inducing a filamentation phenotype, one of SOS responses, induced in some bacterial species exposed to harmful environments.<sup>15,16</sup>

Despite so, low-level laser therapies are used for treatment of diseases, particularly oral cavity diseases induced by chemotherapy and radiotherapy in oncology. The aim of this work was assessing the effects of low power lasers on mutant *Escherichia coli* cells in DNA repair.

## Methods

### Low-Power Lasers

Two therapeutic low-level lasers were used in this study: a red laser (InGaAlP), which emits in 660 nm, and an infrared laser (AsGaAl), which emits in 808 nm (DMC Equipamentos Ltda, São Paulo, Brazil).

### Cell Viability in *Escherichia coli* Cultures

In this study, exponentially *E. coli* AB1157 (not mutant), AB2463 (mutant in RecA protein), and AB2494 (mutant in LexA protein) were used. Following centrifugation (700x g, 15 minutes), washing and suspension in 0.9% NaCl, samples (50  $\mu$ L, n = 5 for each irradiation condition) of these cells (10<sup>8</sup> cells/mL) were irradiated with lasers (25, 50 and 100 J/cm<sup>2</sup>; 30, 50 and 100 mW; continuous, 10 and 100 Hz). Laser sources were placed immediately on the bacterial suspensions. Non-irradiated bacterial suspensions were used as controls.<sup>17</sup> Following incubation (18 hours, 37°C), bacterial colonies were counted and survival fractions were calculated as the measurement of cell viability.<sup>12</sup>

### Cellular Morphology Assessment

Bacteria area and perimeter were evaluated following laser irradiation. To do so, bacterial suspensions were irradiated with lasers, under the same conditions as those described in the item above, applied over the microscopic slides and stained by the Gram technique.<sup>17</sup> Microscopic images of bacteria (n = 300 for each laser irradiation condition, 40x magnification) were captured by Axion Vision software (Carl Zeiss, Germany) and quantified by Image-Pro Plus software (Media Cybernetics, Inc., USA).

### Statistical Analysis

Bacterial survival fractions, area, and perimeter are presented as means and standard deviation (means  $\pm$  SD). One-way analysis of variance was carried out to assess

differences between the groups, and Tukey post-test was carried out to compare and explore the differences between the groups.  $P < 0.05$  was chosen as the significant level. All statistical analyses were conducted by InStat GraphPad software (GraphPad Software Inc., California, USA).

## Results

### Cell Viability in *Escherichia coli* Cultures Irradiated With the Laser

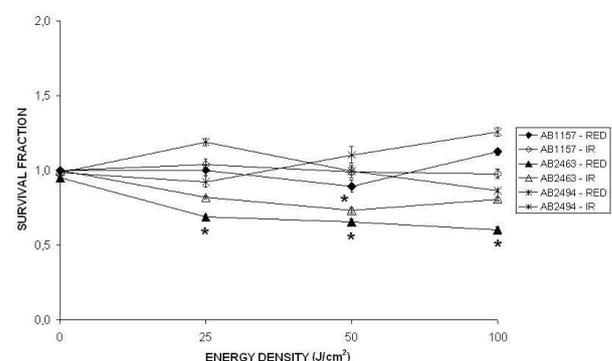
Survival fractions of *E. coli* AB1157, AB2463, and AB2494 irradiated with low-power lasers are shown in Figures 1, 2 and 3. At 100 mW and in continuous mode (Figure 1), no alteration in cell viability was obtained when *E. coli* AB1157 and AB2494 were irradiated with the lasers. However, cell viability was reduced in *E. coli* AB2463 following irradiation with an infrared laser at the middle energy density (50 J/cm<sup>2</sup>). Cell viability was also reduced following irradiation with a red laser at all energy densities. Based on these results, *E. coli* cultures were exposed to lasers at different powers and 100 J/cm<sup>2</sup> to evaluate whether laser power altered the laser effects.

Data in Figure 2 show that irradiation with both lasers at all powers evaluated did not modify cell viability in *E. coli* AB1157 and AB2494, but cell viability of *E. coli* AB2463 cells decreased following red laser irradiation at 100 mW.

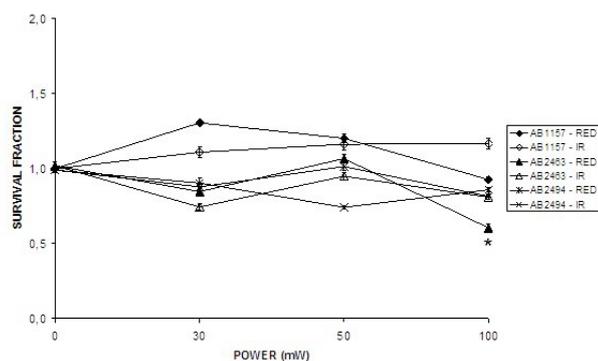
Also, bacterial cultures were irradiated with the lasers in the continuous and pulsed modes at higher energy density and power (100 J/cm<sup>2</sup>, 100 mW). As shown in Figure 3, cell viability was not altered in *E. coli* AB1157 and AB2494 following exposure to the lasers. However, the lasers altered *E. coli* AB2463 cell viability, except in the continuous infrared laser.

### Morphology of the Bacterial Cells Irradiated With the Lasers

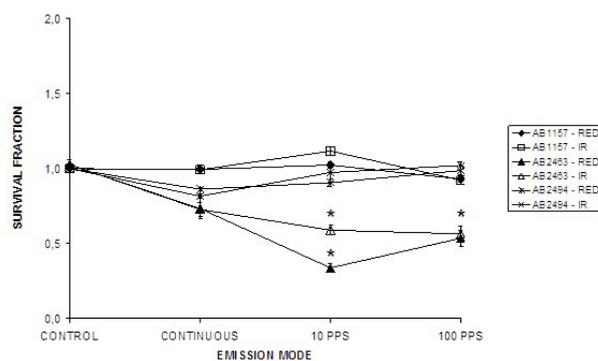
Area and perimeter of *E. coli* AB1157 cells were measured



**Figure 1.** Survival Fractions in *Escherichia coli* Irradiated With Lasers at Different Energy Densities. Exponentially bacterial cultures (2-5  $\times$  10<sup>8</sup> cells/mL) were irradiated with lasers (100 mW, continuous mode). Survival fractions were obtained between the number of irradiated viable cells and the number of non-irradiated viable cells. Data are from 3 experiments. \*  $P < 0.05$  for comparisons between the irradiated and control groups.



**Figure 2.** Survival Fractions in *Escherichia coli* Irradiated With Lasers at Different Powers. Exponentially bacterial cultures ( $2.5 \times 10^8$  cells/mL) were irradiated with lasers ( $100 \text{ J/cm}^2$ , continuous mode). Survival fractions were obtained between the number of irradiated viable cells and the number of non-irradiated viable cells. Data are from three experiments. \*  $P < 0.05$  for comparisons between the irradiated and control groups.



**Figure 3.** Survival Fractions in *Escherichia coli* Irradiated With the Lasers in Continuous and Pulsed Modes. Exponentially bacterial cultures ( $2.5 \times 10^8$  cells/mL) were irradiated with the lasers ( $100 \text{ J/cm}^2$ , 100 mW). Survival fractions were obtained between the number of irradiated viable cells and the number of non-irradiated viable cells. Data are from 3 experiments. \*  $P < 0.05$  for comparisons between the irradiated and control groups.

following irradiation with the lasers (Table 1). While in a continuous mode and at 100 mW,  $100 \text{ J/cm}^2$  of the red laser, the area of these cells decreased, the infrared laser increased both parameters in these cells. At the lowest powers (30 and 50 mW), the red laser did not make modifications, but the infrared laser increased the parameters of *E. coli* AB1157 cells. In a pulsed mode, at  $100 \text{ J/cm}^2$  and 100 mW, the red laser was not capable of causing alterations in area and perimeter, while these parameters increased after irradiation with an infrared laser, as it is shown in Table 1.

The area and perimeter values of *E. coli* AB2463 following irradiation with the lasers are shown in Table 2. The data show an increase in the area of the cells following irradiation with a red laser at the lowest energy densities ( $25$  and  $50 \text{ J/cm}^2$ ) in the continuous mode at 100 mW, but no perimeter modification. Under the same exposure conditions, the infrared laser did not induce any

modification in these morphological parameters in these cells. Morphological parameters were reduced when *E. coli* AB2463 were irradiated with the red and infrared lasers in the continuous mode at  $100 \text{ J/cm}^2$  and 30 mW, but the area of these cells irradiated with the infrared laser was not altered. Also, as shown in Table 2, the lasers did not induce any alteration in area and perimeter in the pulsed emission mode, except for the red laser at 100 Hz.

At 100 mW in a continuous wave, the lasers did not alter the morphological parameters of *E. coli* AB2494 in the middle energy density used ( $50 \text{ J/cm}^2$ ), but the values of both parameters were reduced at 25 and  $100 \text{ J/cm}^2$  (see Table 3). The morphological parameters analyzed were reduced in *E. coli* AB2494 following irradiation with the lasers at the lowest powers in the continuous mode and at  $100 \text{ J/cm}^2$ . As shown in Table 3, these parameters were also reduced in the cells irradiated with the lasers at 100 Hz.

**Table 1.** Morphological Parameters of *Escherichia coli* AB1157 Following Irradiation With the Lasers

	Area ( $\mu\text{m}^2$ )		Perimeter ( $\mu\text{m}$ )	
	Red	Infrared	Red	Infrared
Energy density ( $\text{J/cm}^2$ ) <sup>a</sup>				
Control	$1.9 \pm 0.3$	$1.9 \pm 0.3$	$6.0 \pm 1.3$	$6.0 \pm 1.3$
25	$2.0 \pm 0.3$	$1.6 \pm 0.6$	$6.4 \pm 0.7$	$6.1 \pm 1.4$
50	$1.8 \pm 0.2$	$1.8 \pm 0.6$	$6.3 \pm 0.6$	$6.5 \pm 1.4$
100	$1.6 \pm 0.2^d$	$2.2 \pm 0.8^d$	$5.8 \pm 0.8$	$6.6 \pm 1.4^d$
Power (mW) <sup>b</sup>				
30	$1.8 \pm 0.7$	$2.3 \pm 0.7^d$	$6.4 \pm 1.5$	$6.8 \pm 1.2^d$
50	$1.8 \pm 0.6$	$2.1 \pm 0.6^d$	$6.3 \pm 1.4$	$6.5 \pm 1.5^d$
100	$1.6 \pm 0.2^d$	$2.2 \pm 0.8^d$	$5.8 \pm 0.8$	$6.6 \pm 1.4^d$
Frequency (Hz) <sup>c</sup>				
Continuous	$1.6 \pm 0.2^*$	$2.2 \pm 0.8^d$	$5.8 \pm 0.8$	$6.6 \pm 1.4^d$
10	$1.7 \pm 0.6$	$2.0 \pm 0.5^d$	$6.3 \pm 1.4$	$6.5 \pm 1.7^d$
100	$1.7 \pm 0.6$	$2.2 \pm 0.6^d$	$6.0 \pm 1.3$	$6.5 \pm 1.6^d$

<sup>a</sup>The continuous wave emission mode at 100 mW.

<sup>b</sup>The continuous mode at  $100 \text{ J/cm}^2$ .

<sup>c</sup> $100 \text{ J/cm}^2$  and 100 mW.

<sup>d</sup> $P < 0.05$  for comparisons between the irradiated and control groups.

**Table 2.** Morphological Parameters of *Escherichia coli* AB2463 Following Irradiation With the Lasers

	Area (µm <sup>2</sup> )		Perimeter (µm)	
	Red	Infrared	Red	Infrared
<b>Energy density (J/cm<sup>2</sup>)<sup>a</sup></b>				
Control	2.1±1.0	2.1±1.1	6.8±2.3	6.8±2.3
25	2.6±1.1 <sup>a</sup>	2.4±1.3	7.4±2.4	6.9±2.9
50	2.6±1.1 <sup>a</sup>	2.0±0.9	7.2±2.2	6.4±2.3
100	2.1±0.9	1.9±0.7	6.6±2.3	6.4±1.9
<b>Power (mW)<sup>b</sup></b>				
30	1.9±0.9 <sup>a</sup>	1.9±0.9	6.3±2.2 <sup>a</sup>	6.2±2.2 <sup>a</sup>
50	2.0±1.0	2.3±1.1	6.5±2.7	6.7±2.3
100	2.1±0.9	1.9±0.7	6.6±2.3	6.4±1.9
<b>Frequency (Hz)<sup>c</sup></b>				
Continuous	2.1±0.9	1.9±0.7	6.6±2.3	6.4±1.9
10	2.0±0.9	2.3±0.9	6.0±1.9	6.4±1.9
100	1.8±0.8 <sup>a</sup>	2.2±1.0	6.0±2.1	6.6±2.1

<sup>a</sup>The continuous wave emission mode at 100 mW.

<sup>b</sup>The continuous mode at 100 J/cm<sup>2</sup>.

<sup>c</sup>100 J/cm<sup>2</sup> and 100 mW.

<sup>d</sup>P<0.05 for comparisons between the irradiated and control groups.

**Table 3.** Morphological Parameters of *Escherichia coli* AB2494 Following Irradiation With the Lasers

	Area (µm <sup>2</sup> )		Perimeter (µm)	
	Red	Infrared	Red	Infrared
<b>Energy density (J/cm<sup>2</sup>)<sup>a</sup></b>				
Control	1.8±0.6	1.8±0.6	5.8±1.3	5.8±1.3
25	1.6±0.5 <sup>d</sup>	1.4±0.5 <sup>d</sup>	5.4±1.3 <sup>d</sup>	5.2±1.5 <sup>d</sup>
50	1.9±0.6	1.7±0.6	5.8±1.3	5.5±1.2
100	1.1±0.3 <sup>d</sup>	1.6±0.5 <sup>d</sup>	5.5±1.2 <sup>d</sup>	5.3±1.2 <sup>d</sup>
<b>Power (mW)<sup>b</sup></b>				
30	1.1±0.3 <sup>d</sup>	1.2±0.3 <sup>d</sup>	4.4±0.8 <sup>d</sup>	4.6±0.8 <sup>d</sup>
50	1.2±0.4 <sup>d</sup>	1.1±0.3 <sup>d</sup>	4.6±0.9 <sup>d</sup>	4.4±0.8 <sup>d</sup>
100	1.1±0.3 <sup>d</sup>	1.6±0.5 <sup>d</sup>	5.5±1.2 <sup>d</sup>	5.3±1.2 <sup>d</sup>
<b>Frequency (Hz)<sup>c</sup></b>				
Continuous	1.1±0.3 <sup>d</sup>	1.6±0.5 <sup>d</sup>	5.5±1.2 <sup>d</sup>	5.3±1.2 <sup>d</sup>
10	1.7±0.5	1.5±0.6	5.7±1.3	5.4±1.6
100	1.4±0.4 <sup>d</sup>	1.2±0.4 <sup>d</sup>	5.3±1.2 <sup>d</sup>	4.8±1.2 <sup>d</sup>

<sup>a</sup>The continuous wave emission mode at 100 mW.

<sup>b</sup>The continuous mode at 100 J/cm<sup>2</sup>.

<sup>c</sup>100 J/cm<sup>2</sup> and 100 mW.

<sup>d</sup>P<0.05 for comparisons between the irradiated and control groups.

**Discussion**

Cells have different DNA repair mechanisms to maintain chromosomal stability and resist various aggressive agents in the environment. *E. coli* strains presenting deficiency in a specific DNA repair mechanism allow for evaluating the participation of this specific mechanism as part of the survival strategy or adaptation to changes in environmental conditions or exposure to harmful agents.

Cell viability in *E. coli* AB1157 cultures, not mutant for DNA repair mechanisms, is not altered following irradiation with the lasers at energy densities, powers, and frequencies for therapeutical protocols (Figures 1, 2 and 3). These results could suggest that the therapeutic lasers have no lethal effect on or risk to chromosomal

stability in cells presenting all DNA repair mechanisms. However, there are error-prone DNA repair mechanisms which repair DNA lesions but can compromise the genetic code and DNA stability causing mutations. In bacterial cells, these mechanisms are represented by inducible mechanisms participating in SOS responses, which include filamentation phenotype, prophage lytic cycle induction and mutagenic DNA repair. Thus, an agent capable of inducing SOS responses could be able to induce mutations in DNA. *E. coli* AB2463 cells present deficiency in *recA* protein, a recombinase, and this does not present an inducible SOS response, neither does this present recombination and postreplication repair mechanism.<sup>18</sup> Cell viability in *E. coli* AB2463 culture

was reduced following irradiation with an infrared laser, mainly after irradiation with a red laser at 100 mW in the continuous and pulsed modes (Figures 1, 2 and 3). This suggests that SOS responses and recombination repair are mechanisms used in cell survival or adaptation to the effects of the infrared and red monochromatic radiation at low intensities. However, which gene involved in the control of SOS responses is important for cell survival or adaptation to these radiations? For this, cell viability in *E. coli* AB2494 cultures was evaluated because these cells, like *E. coli* AB2463, are deficient in SOS responses and proficient in recombination DNA repair, but they present a mutation in *lexA* gene, which codes SOS response repressor.<sup>19</sup> Interestingly, our results showed that the lasers did not affect cell viability in cultures of these cells (Figures 1, 2 and 3). This demonstrates that the global molecular mechanism of the survival or adaptation of cells to the low-level lasers is *lexA*-independent but dependent on recombination repair.

Induction of filamentation phenotype is reported in *E. coli* cells irradiated with the low-level lasers, as in those with mutations in genes related to DNA repair pathways for oxidative damages.<sup>15,16,20</sup> However, the filamentation assay performed in these studies did not allow for evaluating what happened with cells which do not present this phenotype. To attempt this need, area and perimeter were measured in mutant *E. coli* cells in SOS functions (AB2463 and AB2494) and compared to *E. coli* wild type cells (AB1157). Table 1 shows that the infrared laser, compared to the red laser, seems to have more effect on wild type cells, increasing both area and perimeter. Area and perimeter of wild type cells are reduced by the red laser and increased by the infrared laser radiation depending on the energy density, power, and emission mode. These findings confirm that the laser effects depend on physical laser parameters.<sup>12</sup> In comparison with wild type cells, morphological parameters analyzed in *E. coli* AB2463 cells were less modified by laser radiation (Table 2). The red laser increased area at the lowest energy densities and powers in the pulsed emission mode while the infrared laser caused a slight perimeter reduction. Similar to wild type cells, the lasers induced striking changes in the morphology of *E. coli* AB2494 cells (Table 3). Results from these two mutant *E. coli* cells confirm that the laser effects depend on the repair of damages to the DNA molecule.<sup>12</sup>

On the other hand, a low-level red laser modifies slow potassium currents,<sup>21</sup> and the ion flux through K<sup>+</sup> and Ca<sup>2+</sup> channels is high following irradiation with a red laser.<sup>22</sup> Moreover, an infrared laser increases transmembrane potential in mitochondria and decreases Ca<sup>2+</sup> levels into cells.<sup>23</sup> Alterations of area and perimeter could be related to modifications in the membrane ion channels of the *E. coli* cells.

However, these laser-induced effects on cellular morphology could be dependent on RecA protein but not on LexA protein albeit experiments are necessary to

evaluate the relations between the ionic currents through cellular membrane and SOS response.

### Conclusion

This study suggests that the low-power red and infrared lasers have a lethal effect on mutant cells in both recombination DNA repair and SOS response but do not induce morphological modifications in these cells. Our results reinforce the point that DNA repair mechanisms participate in the effects of the low-power lasers, which should be taken into account when laser therapy is performed.

### Ethical Considerations

Not applicable.

### Conflict of Interests

The authors declare no conflict of interest.

### Acknowledgements

The Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) sponsored this research.

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