

## Photobiomodulation assay of muscle cells C2C12 after irradiation with LED device

Ensaio de fotobiomodulação de células musculares C2C12 após irradiação com dispositivo LED

Ensayo de fotobiomodulación de células musculares C2C12 después de la irradiación con dispositivo LED

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### Abstract

**Introduction:** One of the ways that have been observed to reduce musculoskeletal fatigue is the use of protocols for the application of light sources (photobiomodulation) such as low-intensity laser and LED (Light Emitting Diode). Work involving photobiomodulation has shown promising results in strength performance or reduction of muscle fatigue. At the cellular level, photobiomodulation can modulate fibroblasts proliferation, the fixation and synthesis of collagen and procollagen, promote angiogenesis and improving energy metabolism in mitochondria. Compared with laser devices, LED has several advantages, such as being smaller, lighter, lower cost, and easier for operation. **Objective:** The present work objective is to verify if irradiation with LED device (650 nm and 860 nm) in muscle cells C2C12 modify the viability, morphology and cytoskeleton components. **Methodology:** C2C12 cells line (ATCC CRL - 1772) were cultured in 25 cm<sup>2</sup> bottles at 37°C under 5% CO<sub>2</sub> in DMEM. The cells were irradiated with the light-emitting diodes (LED) device, Sportllux Ultra that consists of 84 LEDs, each individual LED has 8 mW of power, emitting in 660±20 nm (42 LEDs) and 850±20 nm (42 LEDs), and covering an area (A) of 120 cm<sup>2</sup>. The power density of delivered light was 5,6 mW/cm<sup>2</sup>, and the exposure time was 10 minutes, totalizing the fluence of 3,4 J/cm<sup>2</sup>. Viability assay was performed where the cells were incubated with 100 µL of Crystal Violet (CV) solution and mitochondrial activity assay was evaluated by the colorimetric MTT assay. Nucleus (DAPI) and Cytoskeleton (Rhodamine Phalloidin) fluorescence assay was performed to study the cytoskeleton based on the change in the actin filaments. **Results:** Our results demonstrate that the synergism of LED irradiation (660nm and 850nm) induced the proliferation of C2C12 cells. The light-emitting diode (LED) device, Sportllux Ultra has a significant effect on C2C12 cell culture. Mitochondrial activity and cell viability showed a significative increase in their activities after irradiation. The microscopy fluorescence observations showed an alignment of cytoskeletal components of C2C12 cells after irradiation. **Conclusion:** The application of irradiation with the Sportlux Ultra LED device stimulated an increase of energy by mitochondrial activity assay, number of cells by cell viability assay and alignment of cytoskeleton components by fluorescence assay in C2C12 line cells. Our results suggest that organized cytoskeletal actin filaments normally contribute to cell survival and that induced major cell changes in the cytoskeleton that result in cell shape change. These results suggest that the Sport Lux Ultra LED device can help in the repair of tissue injuries and to collaborate to increase of performance in athletes in a faster way.

**Keywords:** Photobiomodulation; Cell culture; Muscle cells.

### Resumo

**Introdução:** Uma das formas observadas para a redução da fadiga musculoesquelética é a utilização de protocolos para aplicação de fontes de luz (fotobiomodulação) como laser de baixa intensidade e LED (Light Emitting Diode). Trabalhos envolvendo fotobiomodulação têm mostrado resultados promissores no desempenho de força ou redução da fadiga muscular. Em nível celular, a fotobiomodulação pode modular a proliferação de fibroblastos, a fixação e síntese de colágeno e procolágeno, promover a angiogênese e melhorar o metabolismo energético nas mitocôndrias. Comparado aos dispositivos a laser, o LED apresenta diversas vantagens, como ser menor, mais leve, de menor custo e de operação mais fácil. **Objetivo:** O objetivo do presente trabalho é verificar se a irradiação com dispositivo LED

(650 nm e 860 nm) em células musculares C2C12 modifica a viabilidade, morfologia e componentes do citoesqueleto. **Metodologia:** A linhagem celular C2C12 (ATCC CRL - 1772) foi cultivada em frascos de 25 cm<sup>2</sup> a 37°C sob 5% CO<sub>2</sub> em DMEM. As células foram irradiadas com o dispositivo de diodos emissores de luz (LED), Sportlux Ultra que consiste em 84 LEDs, cada LED individual possui 8 mW de potência, emitindo em 660±20 nm (42 LEDs) e 850±20 nm (42 LEDs), e cobrindo uma área (A) de 120 cm<sup>2</sup>. A densidade de potência da luz emitida foi de 5,6 mW/cm<sup>2</sup>, e o tempo de exposição foi de 10 minutos, totalizando a fluência de 3,4 J/cm<sup>2</sup>. O ensaio de viabilidade foi realizado onde as células foram incubadas com 100 µL de solução de Cristal Violeta (CV) e o ensaio de atividade mitocondrial foi avaliado pelo ensaio colorimétrico MTT. O ensaio de fluorescência de núcleo (DAPI) e citoesqueleto (rodamina faloidina) foi realizado para estudar o citoesqueleto com base na alteração nos filamentos de actina. **Resultados:** Nossos resultados demonstram que o sinergismo da irradiação LED (660nm e 850nm) induziu a proliferação de células C2C12. O dispositivo de diodo emissor de luz (LED), Sportlux Ultra tem um efeito significativo na cultura de células C2C12. A atividade mitocondrial e a viabilidade celular mostraram um aumento significativo em suas atividades após a irradiação. As observações de microscopia de fluorescência mostraram um alinhamento dos componentes do citoesqueleto das células C2C12 após a irradiação. **Conclusão:** A aplicação da irradiação com o aparelho Sportlux Ultra LED estimulou aumento de energia por ensaio de atividade mitocondrial, número de células por ensaio de viabilidade celular e alinhamento de componentes do citoesqueleto por ensaio de fluorescência em células da linhagem C2C12. Nossos resultados sugerem que os filamentos organizados de actina do citoesqueleto normalmente contribuem para a sobrevivência da célula e que induzem grandes mudanças celulares no citoesqueleto que resultam na mudança da forma celular. Esses resultados sugerem que o aparelho Sport Lux Ultra LED pode auxiliar no reparo de lesões teciduais e colaborar para aumentar o desempenho em atletas de forma mais rápida.

**Palavras-chave:** Fotobiomodulação; Cultura de células; Células musculares.

## Resumen

**Introducción:** Una de las formas observadas para reducir la fatiga musculoesquelética es el uso de protocolos para la aplicación de fuentes de luz (fotobiomodulación) como láser de baja intensidad y LED (Light Emitting Diode). Los trabajos de fotobiomodulación han mostrado resultados prometedores en el rendimiento de la fuerza o la reducción de la fatiga muscular. A nivel celular, la fotobiomodulación puede modular la proliferación de fibroblastos, la unión y síntesis de colágeno y procolágeno, promover la angiogénesis y mejorar el metabolismo energético en las mitocondrias. En comparación con los dispositivos láser, el LED tiene varias ventajas, como ser más pequeño, más liviano, menos costoso y más fácil de operar. **Objetivo:** El objetivo del presente trabajo es verificar si la irradación con dispositivo LED (650 nm y 860 nm) en células musculares C2C12 modifica la viabilidad, morfología y componentes del citoesqueleto. **Metodología:** La línea celular C2C12 (ATCC CRL - 1772) se cultivó en matraces de 25 cm<sup>2</sup> a 37°C bajo 5% de CO<sub>2</sub> en DMEM. Las celdas fueron irradiadas con el dispositivo de diodo emisor de luz (LED), Sportlux Ultra, que consta de 84 LED, cada LED individual tiene 8 mW de potencia, emitiendo a 660±20 nm (42 LED) y 850±20 nm (42 LEDs), y cubriendo un área (A) de 120 cm<sup>2</sup>. La densidad de potencia de la luz emitida fue de 5,6 mW/cm<sup>2</sup> y el tiempo de exposición de 10 minutos, totalizando una fluencia de 3,4 J/cm<sup>2</sup>. El ensayo de viabilidad se realizó donde las células se incubaron con 100 µL de solución Crystal Violet (CV) y el ensayo de actividad mitocondrial se evaluó mediante el ensayo colorimétrico MTT. Se realizó el ensayo de núcleo de fluorescencia (DAPI) y citoesqueleto (faloidina de rodamina) para estudiar el citoesqueleto en función del cambio en los filamentos de actina. **Resultados:** Nuestros resultados demuestran que el sinergismo de la irradación LED (660nm y 850nm) indujo la proliferación de células C2C12. El dispositivo de diodo emisor de luz (LED), Sportlux Ultra tiene un efecto significativo en el cultivo de células C2C12. La actividad mitocondrial y la viabilidad celular mostraron un aumento significativo en sus actividades después de la irradación. Las observaciones de microscopía de fluorescencia mostraron una alineación de los componentes del citoesqueleto de las células C2C12 después de la irradación. **Conclusión:** La aplicación de irradación con el dispositivo Sportlux Ultra LED estimuló un aumento de energía por ensayo de actividad mitocondrial, número de células por ensayo de viabilidad celular y alineación de componentes del citoesqueleto por ensayo de fluorescencia en células de la línea C2C12. Nuestros resultados sugieren que los filamentos de actina del citoesqueleto organizados normalmente contribuyen a la supervivencia celular y que indujeron cambios celulares importantes en el citoesqueleto que dan como resultado un cambio en la forma de la célula. Estos resultados sugieren que el dispositivo Sport Lux Ultra LED puede ayudar en la reparación de lesiones en los tejidos y colaborar para aumentar el rendimiento en los atletas de una manera más rápida.

**Palabras clave:** Fotobiomodulación; Cultivo de células; Células musculares.

## 1. Introduction

Photobiomodulation by light in the red to the spectrum's infrared region (630-1000 nm) modulates numerous cellular functions. The clinical and experimental applications of photobiomodulation have expanded over the past 30 years. Low-power lasers and light-emitting diodes (LEDs) are well-accepted therapeutic tools for treating infected, ischemic, and hypoxic wounds

and other soft tissue injuries. The positive effects of photobiomodulation include accelerated healing, better recovery from ischemic lesions in the heart, and attenuated degeneration of the injured optic nerve (Desmet et al., 2006). At the cellular level, photobiomodulation can modulate fibroblasts proliferation, the fixation and synthesis of collagen and procollagen, promote angiogenesis and stimulate macrophages and lymphocytes, improving energy metabolism in mitochondria (Yu et al. 1994). Besides, photobiomodulation has demonstrated the ability to increase the production of growth factors, such as keratinocyte growth factor (KGF), transforming growth factor (TGF), and platelet-derived growth factor (PDGF) (Desmet et al., 2006; Hamblin, 2017).

Every type of tissue or cell responds differently even in the same experimental conditions. Low intensity irradiation can inhibit as well as stimulate cellular activity (Al-Ghamdi et al., 2012). Furthermore, irradiation with the same wavelength delivered at low doses may produce better results compared to higher doses. This biphasic dose response may also be a cause of inconsistent reports on photobiomodulation. Therefore, the potential effect of photobiomodulation should be tested by a complete and reliable method that will investigate the effect of the light in a multistage manner (Ates et al., 2017). Although the biological mechanisms underlying the biostimulatory effects of LLLT are not fully understood, it has been reported by several investigators that LLLT modulates cellular metabolic processes, leading to an enhanced regenerative potential for biological tissues (Mester et al., 1985).

In recent years, the light emitting diodes (LEDs)-mediated photobiomodulation is going to provide diabetic foot ulcers patients with a more feasible and lower-cost therapeutic option (Zhao et al. 2022). Compared with laser devices, LED has several advantages, such as being smaller, lighter, lower cost, and easier for operation (Frangez et al., 2017; Al-Watban & Andres, 2017). Although a few studies have demonstrated that LED irradiation is able to promote the healing of diabetic ulcers in clinics (Silveira et al., 2016; Barolet, 2008), the dose-effect relationship and the mechanism of LED-mediated photobiomodulation are not clear. Several parameters are important for photobiomodulation, which include wavelength, light irradiance, energy, time, and frequency of the treatment. Generally, red or near-infrared (NIR) light (600–1000 nm) was considered as the optical window (Li et al. 2020). Laser-mediated photobiomodulation (630nm and 810 nm) has already achieved positive results in the treatment of diabetic foot ulcers.

Recently, photobiomodulation in the form of light-emitting diode (LED) therapy has been applied over human skeletal muscles before or after bouts of exercises in order to (i) accelerate muscle recovery, (ii) protect against muscle damage induced by exercise, and (iii) improve performance, such as increasing muscle strength and fatigue resistance (Ferraresi et al., 2011; Ferraresi et al., 2012; Borsa et al., 2013). Moreover, there has been no in vitro study reporting the effects of phototherapy on muscle cells culture morphology.

For several decades solar UVA (ultraviolet-A, 320–400 nm) irradiation have been considered environmental carcinogens that contribute to the development of skin cancer (Hopkins et al., 2016). In this context, a novel generation of violet LED light (LED) for tooth bleaching has raised concerns on possible side effects due to the use of light as a bleaching protocol considering the lack of evidence supporting both its efficacy and safety (Kuri et al., 2020). Violet LED operates under an approximate 405 nm wavelength, and it is speculated that its radiation presents the same absorbance peak of pigments on the enamel surface, causing a photolytic effect (Rastelli et al., 2018).

Lam et al. (1986) demonstrated that in vitro irradiation of fibroblasts with 633 nm wavelength light increased fibers synthesis four-fold from baseline. Irradiation with this red light increased fibroblastic growth factor synthesis from photoactivated macrophages (Young et al., 1989). Light at 830 nm (near infrared) wavelength is absorbed in the cellular membrane rather than in cellular organelles which remain the target when using light in the visible spectrum. Irradiation at 830 nm has accelerated chemotaxis and phagocytic activity of leucocytes and macrophages on cellular stimulation by this

wavelength (Osani et al., 1990; Dima et al., 1997). It is hypothesized that the synergy of 633 nm and 830 nm wavelength light will combine these effects to enhance fibroblast proliferation and thus increase fibers synthesis, as well as stimulating inflammatory cell lines such as mast cells and macrophages (Russel et al., 2005).

Myoblast cell lines have been applied as valuable in-vitro models in past two decades. In terms of C2C12 cell line, it is derived from mouse and has been recognised as an effective examination tool in several aspects including ageing, diabetes mellitus, obesity, hyperlipidaemia, hepatic steatosis, growth impairment and muscle growth (Mangnall et al., 1993; Wong, et al., 2020). The rat-derived muscle cell line L6 and mouse-derived muscle cell line C2C12 are frequently used to study intracellular signaling in skeletal muscle cells. The advantages of these cell lines include reducing the use of animals, easy transfection of exogenous DNA or siRNA, a shorter experimental period, homogeneous and easy to culture as clone cells, and avoidance of the influence of systemic factors from other organs (Manabe et al., 2012)

A new device of light-emitting diodes (LEDs) Sportlux Ultra has become commercially available, with power density of delivered light 5,6 mW/cm<sup>2</sup> and fluence of 3,4 J/cm<sup>2</sup>, emitting in 660±20 nm and 850±20 nm at the same time simultaneously. The present study was designed to assess the effectiveness of a LED therapy system commercially available in muscle cells culture C2C12 where the viability, morphology and cytoskeleton components were avalueted.

## 2. Methodology

That experimental study, *in vitro*, used a deductive hypothetical method, with a quantitative approach through statistical analysis of the collected data (Pereira et al., 2018).

### 2.1 Cell Culture

C2C12 cells line (Immortalized Mouse Myoblast Cell Line ATCC – CRL - 1772) differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. C2C12 cell line were cultured in 25 cm<sup>2</sup> bottles at 37°C under 5% CO<sub>2</sub> in DMEM (Dulbecco's modified Eagles medium – Thermo Fisher Scientific, Gibco, Waltham, Massachusetts, EUA) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Gibco, Waltham, MA, EUA) and 1% penicillin and streptomycin (Thermo Fisher Scientific, Invitrogen, Waltham, MA, EUA).

### 2.2 Experimental Groups

The cultures of C2C12 cells were divided into two groups, control group (not irradiated) that were cultured in DMEM supplemented with 10% FBS and treatment group (irradiated group) that were cultured in DMEM supplemented with 10% FBS and were submitted to LED device Sportlux Ultra irradiation (Cosmedical – Every Eletroeletrônica Imp. Exp. LTDA, Mauá, São Paulo, Brazil).

### 2.3 Irradiation process

The culture medium was removed 24 h after plating (1x10<sup>4</sup> cells/mL per well) in 24-well plates and the wells were washed with PBS. New culture medium was put in each well. The light-emitting diodes (LED) device, Sportlux Ultra (Cosmedical – Every Eletroeletrônica Imp. Exp. LTDA, Mauá, São Paulo, Brazil), used for irradiation consists of 84 LEDs, each individual LED has 8 mW of power, emitting in 660±20 nm (42 LEDs) and 850±20 nm (42 LEDs), and covering an area (A) of 120 cm<sup>2</sup>. After turn on the LED device, all light emitters (660nm and 850nm) will light up together and the exposure time is start. The power density of delivered light was 5,6 mW/cm<sup>2</sup>, and the exposure time was 10 minutes, totalizing the fluence of 3,4 J/cm<sup>2</sup>. The power density was calculated according to the following formula:  $(84 \times 8) / A = I$ , and the fluence was

calculated according to the following one:  $I \text{ (W/cm}^2\text{)} \times t \text{ (s)} = F \text{ (J/cm}^2\text{)}$ . After irradiation, cells were incubated for 24 h at 37°C under 5% CO<sub>2</sub>. Thus, two groups were investigated: Control group (not irradiated) and the treatment group, submitted to LED irradiation.

**Figure 1** – A - LED device, Sportlux Ultra (Cosmedical – Every Eletroeletrônica Imp. Exp. LTDA, Mauá, São Paulo, Brazil), used for irradiation; B - 24-well plate containing the C2C12 cell culture being irradiated.



Source: Authors.

#### 2.4 Mitochondrial activity assay

Mitochondrial activity was evaluated by the colorimetric MTT [(bromide 3–4,5-dimethylthiazol-2-yl) -2,5-difeniltetrazolol] (Sigma) assay. After treatments, 100 µL of MTT solution (5 mg/mL) were added to the wells and incubated for 2 h at 37°C in 5% CO<sub>2</sub>. After this period, cells were incubated with 200µL of DMSO (dimethyl sulfoxide) for 30 min under stirring and the absorbance was measured at 570 nm (SpectraCount - Packard spectrophotometer). The whole process was carried out in the dark. Data collected were statistically analyzed.

#### 2.5 Viability assay

Using a protocol adapted for the viability assay, adhered cells were incubated with 100 µL of Crystal Violet (CV) solution for 4 min at room temperature. At the end of incubation, the plate was washed with water to remove excess dye and 200µL of elution solution (SDS – sodium dodecyl sulfate) were added for incubation for 1 h before reading using a SpectraCount – Packard 570 nm spectrophotometer. The whole process was carried out in the dark. Data collected were statistically analyzed.

#### 2.6 Nucleus and Cytoskeleton fluorescence assay

The fluorescent dyes were acquired from Molecular Probes. Intervals of 24 h after irradiation cells were doubly



labeled with fluorescent dyes under the following conditions: for analysis of the nucleus, cells were fixed in 4% paraformaldehyde in a PHEM buffer (60 nM Pipes, 20 nM Hepes, 10 nM EGTA, 5 nM MgCl<sub>2</sub>) for 10 min at room temperature. After fixation, cells were stained with DAPI (40,6-diamidino-2-phenylindole dihydrochloride) at 300 nM for 10 min. After staining, cells were labeled with Rhodamine Phalloidin (1:100 for 1 h) to study the cytoskeleton based on the change in the actin filaments. After these procedures, the coverslips were rinsed in the dark with PHEM and mounted on slides with n-propyl gallate and were analyzed in a fluorescence microscope Leica DMLB equipped with a Leica MPS-30 photographic system and Confocal Microscope ZEISS LMS700.

### 2.7 Statistical analysis

Statistical calculations were performed using the GraphPad Prism® version 6.0 statistical data analysis software. Data were compared by unpaired t-test (also known as an independent t-test) defining the significance level at 5% ( $p \leq 0.05$ ). All experiments were performed in triplicate. An unpaired t-test (also known as an independent t-test) is a statistical procedure that compares the means of two independent or unrelated groups to determine if there is a significant difference between the two. The t-test can be used, for example, to determine if the means of two sets of data are significantly different from each other.

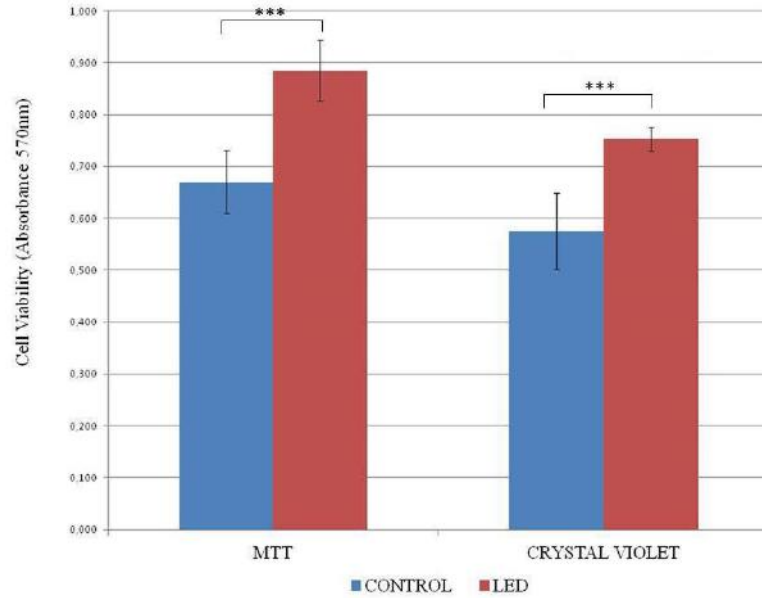
## 3. Results

The MTT assay quantifies the mitochondrial activity by analyzing formazan crystals formed by reducing the salt 3-[(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT). Our results shown a significant difference when the LED device, Sportlux Ultra was applied in the cells (figure 02 - MTT). Comparing the irradiated group with the control group, an increase in the mitochondrial activity was observed ( $p < 0.001$ ) by 32%. These results confirm that the LED device used in this assay can stimulate the muscle cells and optimize the performance of athlete with a gain of energy.

Adherent cells detach from cell culture plates during cell death. Cells that undergo cell death lose their adherence and are subsequently lost from the population of cells, reducing the amount of crystal violet staining in a culture and this way may be possible to quantify the cell viability. In the Figure 02 show after irradiation with LED device that the cells C2C12 has a significant ( $p < 0,002$ ) grow up in relation to cells not treated (control) (Figure 2 – Crystal Violet). The number of adherent and viable cells has an increased by 30% and this show the significant effects of LED device on C2C12 muscle cells.

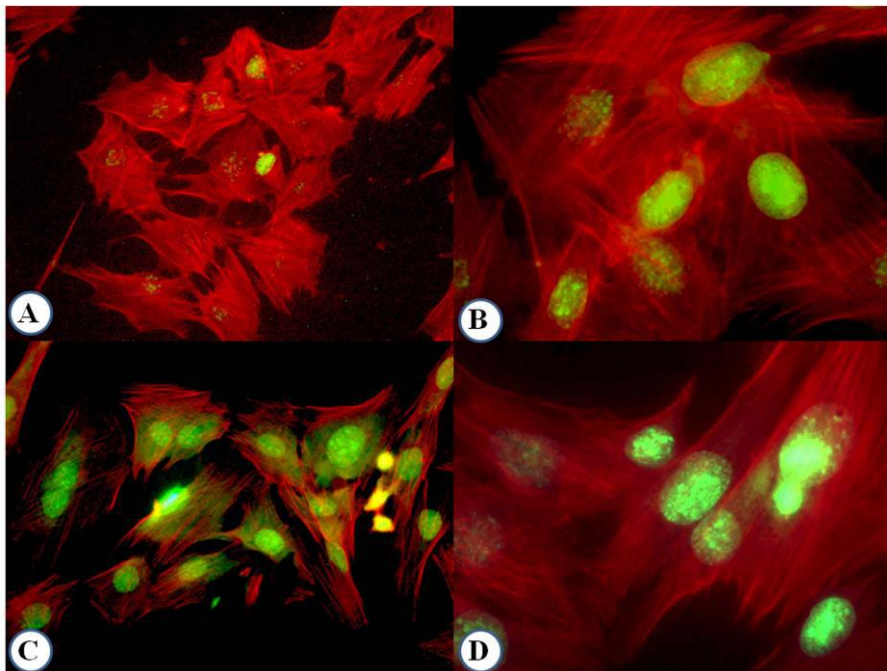
When was done the fluorescence assay for Nucleus (stained with DAPI) and Cytoskeleton (stained with Rhodamine Phalloidin) the results (Figure 03) confirm the effects of LED device observed in MTT and CV assay. The cytoskeleton filaments are unorganized in cells not treated with LED device (Figure 03A and 03B). In cells treated with LED device the cytoskeleton filaments is more organized (Figure 03C and 03D) and this can to help in the contraction force of muscle cells. No change in nucleus was observed in treated or not treated cells with LED device.

**Figure 2** - Mitochondrial activity assay evaluated by the colorimetric MTT and Viability assay evaluated by Crystal Violet solution. Data were compared defining the significance level at 5% ( $p \leq 0.05$ ). All experiments were performed in triplicate. MTT assay \*\*\*  $p < 0,0001$  and Crystal Violet assay \*\*\*  $p < 0,0002$ .



Source: Authors.

**Figure 3** – Nucleus and Cytoskeleton fluorescence assay stained with DAPI (Nucleus) and Rhodamine Phalloidin (Cytoskeleton) analyzed in a fluorescence microscope Leica DMLB equipped with a Leica MPS-30 photographic system. A – Control cells (400x); B – Control cells (1000x); C - Irradiated cells (400x); D – Irradiated cells (1000x).



Source: Authors.

#### 4. Discussion

The light-emitting diode (LED) device, Sportllux Ultra has a significant effect on C2C12 cell culture. Mitochondrial activity and cell viability showed a significative increase in their activities after irradiation. The microscopy fluorescence observations showed an alignment of cytoskeletal components of C2C12 cells after irradiation.

The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells (Vistica et al., 1991)

The irradiation of C2C12 cells with Sportllux Ultra LED device induce an increased of mitochondrial activity. The cellular response to interaction with light energy (Laser or LED) is determined by the absorption of energy by photoacceptor molecules present in mitochondria, with Cytochrome C Oxidase present in complex IV of the mitochondrial chain being the main one, which converts photons into signals that stimulate cellular processes, modulating signaling pathways, production of reactive oxygen species, ATP, Ca<sup>2+</sup> and other metabolic events in cells (Sommer, 2019). This mitochondrial stimulation reflects in an increase in ATP synthesis, a reduction in the release of ROS and an increase in creatine kinase activity when the gastrocnemius muscle of rats is irradiated (Avni et al., 2005).

The results of cell viability with Crystal Violet assay are in agreement to Turrioni et al. (2015) and Li et al. (2010) where the obtained data show an increase of number of cells after LED irradiation. The increased cell proliferation and viability seem to be related to cytochrome c oxidase activation, which enhances levels in the respiratory chain and adenosine triphosphate (ATP), and these biochemical changes led to macroscopic effects such as increased cell proliferation (Gao et al., 2009). Rohringer et al. (2017) in your research about the impact of of LED light-therapy on endothelial cells show that LLLT and LED light increased migration and proliferation of endothelial cells consistently in several independent models.

Our results demonstrate that the synergism of LED irradiation (660nm and 850nm) induced the proliferation of C2C12 cells and with this we can infer that the application of the Sportllux Ultra LED device may help in the recovery of injuries in athletes in a faster way. Light therapy (Laser or LED), at wavelengths within the visible and near-infrared electromagnetic spectrum between the bands of 650 to 1060 nm, is a biomedical tool in areas of human and animal medicine (Zabeu, 2021), leveraged for the advancement of scientific research in the basic sciences for knowledge of photobiology and bioenergetics. According to Huang et al. (2011), the review carried out on the photophysical effects of light on biological tissue basically involves two very important parameters, which are the wavelength and the penetration of light into the tissue, and this is quite variable between each cell type and the complexity of each one.

Ricci et al. (2009) investigated the effects of laser light on endothelial cell morphology, suggesting that LLLT influences the organization of endothelial cytoskeletons. Lasers, however, are associated with several disadvantages like significant patient discomfort, continue to be expensive, can produce heat, and apply light only on a narrow spot (Teuschl et al., 2015). Light emitting diodes (LED) can be effective alternative light sources, providing advantages like broad beam width and cost-efficiency. LED already moved into the focus of research and have recently been shown to be similarly effective (Rohringer et al., 2017).

The cytoskeleton is a complex, dynamic network of interlinking protein filaments presents in the cytoplasm of all cells, (Hardin et al., 2015). It extends from the cell nucleus to the cell membrane and is composed of similar proteins in the various organisms. In eukaryotes, it is composed of three main components, microfilaments, intermediate filaments and microtubules, and these are all capable of rapid growth or disassembly dependent on the cell's requirements (McKinley et al., 2015).

The cytoskeleton fluorescence assay with Rhodamine Phalloidin labeled cells to study the cytoskeleton based on the



alteration of actin filaments. The results showed an alignment of cytoskeletal components in relation to the non-irradiated cell. This organization will help in the strength of muscle contraction. A multitude of functions can be performed by the cytoskeleton. Its primary function is to give the cell its shape and mechanical resistance to deformation. (Alberts et al., 2008; Herrmann et al., 2008). A large-scale example of an action performed by the cytoskeleton is muscle contraction. This is carried out by groups of highly specialized cells working together. A main component in the cytoskeleton that helps show the true function of this muscle contraction is the microfilament. Microfilaments are composed of the most abundant cellular protein known as actin (Cooper, 2000).

The Sportlux Ultra LED device used for irradiation consists of 84 LEDs, each individual LED emitting in  $660\pm 20$  nm and  $850\pm 20$  nm and after turning on all light emitters (660nm and 850nm) light up together. This synergic irradiation is a differential of Sportlux Ultra LED device. The results obtained from the increase in mitochondrial activity, cell viability and alignment of cytoskeleton components confirm the data obtained by Russell et al (2005) that hypothesized that the synergy of 633 nm and 830 nm wavelength light will combine these effects to enhance fibroblast proliferation and thus increase fibers synthesis. The potential applications of Sportlux Ultra may be numerous but new studies are necessary in others cell culture (in vitro assay) and in animals and humans (in vivo assay) to better understand of results obtained.

## 5. Conclusion

The application of irradiation with the Sportlux Ultra LED device stimulated an increase of energy by mitochondrial activity, number of cells by cell viability assay and alignment of cytoskeleton components by fluorescence assay in C2C12 line cells. Our results suggest that organized cytoskeletal actin filaments normally contribute to cell survival and that induced major cell changes in the cytoskeleton that result in cell shape change. These results suggest that the Sport Lux Ultra LED device can help in the repair of tissue injuries and to collaborate to increase of performance in athletes in a faster way.

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