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# Photobiomodulation Using Amber Led and Infrared Laser to Controlling the Pigmentation and Flaccidity from Skin

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## Abstract

The Photobiomodulation, using different visible light wavelengths, known as phototherapy on past; shows several benefits on heath and aesthetic procedures on skin ageing treatment. The biological effect depends on how this light interacts on the different skin layers in depth and how this light interacts with the different biomolecules, membranes, biomolecules and organelles present in the skin. Shortest wavelengths (high energy), such as violet and blue light, interact more superficially on the skin in contrast to longest wavelengths, such as red light and amber, interact more deeply on the skin; getting to the muscles when we talk about infrared light. The effects of UV radiation (UVA and UVB) on skin pigmentation are known however the effects of blue light are being discussed due to relation with the late and lasting pigmentation on skin either. The visible light induce the bleaching of chromophores on skin (oxidation of melanin); and depending of wavelength and irradiation dose, the oxygen reactive specimens increases and the skin protection decreases. Then, the Inflammation response and the melanin production is consequently Increased (protection mechanism) increasing the pigmentation on skin. The aim of this study is to demonstrate the effects of low level light therapy, using amber LED light and infrared laser light on appropriated dosimetry associated or not, on skin pigmentation control and on skin quality on elasticity and muscle tone. The amber LED light induces the photobleaching of melanin as well as its production on skin since that acts modulating the inflammatory and vascularization response at skin. At same time the infrared laser light also decreases the inflammatory response modulating the skin pigmentation either. The association of both improves the age spots reduction on skin by decreasing of inflammatory response by modulation on melanin synthesis (efficient inflammatory response control) as well promotes face lifting effect induced by collagen production and muscle tone. The studies were performed *in vitro* on cell culture to evaluation the melanin synthesis reduction for follow groups: 1) Amber LED light irradiation, 2) Infrared laser light irradiation and c) Amber LED Light associated to infrared laser light irradiation. The *in vivo* studies were performed using visual and skin elasticity evaluations, using cutometer analyses, before and after 30 days of studies associating both wavelengths on protocol (once time a week during 4 weeks). The results suggest that amber light associated to infrared light improves the decreases of age spots and Tissue and Muscle Flaccidity reduction.

**Keywords:** Photobiomodulation; Amber; LED; Infrared; Laser

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## Introduction

The beneficial properties of light have been revisited over the years, since it is one of the oldest therapies in the treatment of diseases such as, for example, lupus, psoriasis, vitiligo, neonatal jaundice and others and it is currently being applied frequently in several types of treatments in the areas of health and aesthetics [1,2]. The application of light for therapeutic purposes in the past entitled Phototherapy has been replaced by the term Photobiomodulation or Low intensity laser therapy, since the physiological effect can be described as bio stimulus or by bio-inhibition [3].

It can be said that photobiomodulation is based on the interaction of light with tissues, stimulating photophysical, photochemical and photobiological processes. Acting at the cellular level, the light now coming from laser devices and or LEDs promotes biochemical, bioelectric and bioenergetic modifications acting in the increase of cellular metabolism due to interaction with the mitochondrial respiratory chain photoaceptors [1-5].

 

In aesthetics, Photobiomodulation has been applied in the treatment of stretch marks, blemishes, melasma, cellulite, acne, bags and dark circles, against the adverse effects of aging and photoaging (wrinkles, expression lines, elasticity and firmness), as well as in alopecia, in the postoperative period, in the skin healing processes, for analgesia purposes, lymphatic drainage, hydration among others [1,6].

The physiological effects of light on the skin are related to its penetration into target tissues. Wavelengths of greater energy and less depth of penetration into the skin shows effects on superficial layers of the skin, in contrast, longer wavelengths of greater depth in the skin have physiological effects on deeper layers of the skin [1]. Violet LED light is absorbed into the skin superficially and can be used for microbial decontamination as well as for optical diagnosis of skin, where through optical filters the violet light when absorbed in the skin emits distinct green autofluorescence for normal and non-functional or tumor tissues [1,7]. The blue LED light acts superficially in the microbial control since it is absorbed by the porphyrins contained in bacteria such *Propionibacterium acnes*, or *P. acnes* and others [1,8]. In addition, blue light modulates water content and its interaction with the keratin of skin and hair, increasing its hydration [1,6,9-11].

Amber LED light is absorbed into the skin by cytochrome C oxidase (maximum absorption in thiswavelength), also acting on mitochondrial respiration, thus accelerating the rate of ATP production, promoting a large release of nitric oxide (NO), which is responsible for due to vasodilation and neuro-transmission, being important in tissue repair [12]. In addition, recent studies demonstrate that this wavelength acts decreasing the inflammatory response reducing the erythema [13,14], decreases the melanin synthesis, induces the autophagy on melanocyte cells as well as stimulating an important local vasoconstriction (management of the vascularization) on skin [15]. In addition, the amber light acts on skin increases collagen and decreasing the metalloproteinases (MMP 1) activity showing better results on aging treatment [16,17]. Other clinical studies suggest that increases of the viscoelasticity of the hair by increases of collagen and other biomolecules on hair follicle on dermal papilla [6].

The red light has multiple functionalities; since the increase in vasodilation by production of nitric oxide promoting increased oxygenation and blood nutrition (systemic laser therapy), promotes increased proliferation and cell differentiation of fibroblasts in the dermis, increasing the production of biomolecules such as collagen, hyaluronic acid, proteoglycans, elastin among others acting on tissue repair and dermal structuring [1]. The effects on red light on pigmentation control were described recently acting on tyrosinase inactivation [18]. Also the red light promotes analgesia and can be an adjunct in various types of medical and aesthetic protocols, being an adjunct to infrared light on treatments [1-4].

Infrared light interacts with cell membranes, changing its polarity, promoting an increase in the flow of $Ca^{++}$, $Na^{+}$ and $K^{+}$ ions, important in stimulating the release of chemical mediators such as endorphins, encephalins and bradykinin inhibition, as well as the activity of C fibers conducting painful stimuli, leading to the analgesic effect. This wavelength acts to relieve acute and chronic pain, on the lymphatic drainage and bone, adipose and neural repair [19,20]. In addition, when the infrared light interacts with the membrane proteins, mainly with the aquaporins, increases the amount of water molecules in the skin promoting hydration on the different skin layers [1-4,11]. Also, It is also important to mention that infrared light acts directly on the muscle improving higher muscle tone [1-4].

The Photobiomodulation (PBM) using different wavelengths on the same protocol can be useful on Skin ageing treatment modulating the Oxidative Stress [21]. In a previously studies; the authors discussed about the photobleaching of melanin by visible light, mainly by shorter wavelengths, increasing the oxygen reactive species and consequently the skin pigmentation. Other authors discussed about the skin pigmentation effects from visible light comparing the UVA and UVB radiation on skin. In View of this many authors advice about the protection of skin from UV and blue light. The effects of UV radiation (UVA and UVB) on skin pigmentation are known however the effects of blue light are being discussed due to relation with the late and lasting pigmentation on skin either. The visible light induce the bleaching of chromophores on skin (oxidation of melanin); and depending of wavelength and irradiation dose, the oxygen reactive specimens increases and the skin protection decreases. Then, the Inflammation response and the melanin production is consequently Increased (protection mechanism) increasing the pigmentation on skin [22-25].

The amber light effects are common related on inflammatory response control as the infrared light. The association of amber and infrared light improves the age spots reduction on skin by decreasing of inflammatory response; modulating the melanin synthesis [1,13,14,16].

The association of amber LED light and infrared laser light was done previously in a paper *in vitro* on fibroblast culture cells and in a deal with the results this association of 75/25% irradiation dose ratio of 590/870 nm bring optimal results on gene expression to fibroblast gene expression [17]. None other paper showed clinical results on decreasing the pigmentation and flaccidly treatment at same time.

The aim of this study is to demonstrate the effects of low level light therapy, using amber LED light and infrared laser light on appropriated dosimetry associated or not, on skin pigmentation control as well as improves of the skin elasticity and muscle tone.

# Materials and Methods

## Equipment

The laser and LED light treatment was done using commercial equipment called Venus (MMOptics - São Carlos-Brazil). The equipment shows red and infrared laser light on 630 nm and 850 nm respectively and blue and Ambar LED light on 450 nm and 590 nm. In **Figure 1** the irradiation procedure at skin can be observed using different wavelengths.

**Figure 1** **(A)** Photobiomodulation procedure on different face areas application. **(B)** The Photobiomodulation procedure on skin using amber LED light associated to infrared laser light with total fluency of 30J/cm². The equipment used on study is called Venus (MMOptics – São Carlos- Brazil).

## *In vitro* study

The studies *in vitro* on cell culture were performed to evaluation the melanin synthesis reduction for follow groups:

- Amber LED light irradiation (Group 1),
- Infrared laser light irradiation (Group 2) and
- Amber LED Light associated to infrared laser light irradiation (Group 3).

The A-375 human melanoma cells line was obtained from the American Tissue Culture Collection (ATCC), and was maintained in Iscove's Modified Dulbecco's Medium (IMDM) in 10% FCS in a humidified atmosphere of 5% $CO_2$ at 37ºC.

# Experimental Procedure

The experiments were done firstly discovering the ideal irradiation dose (J/cm²) through Cytotoxicity assay to evaluate later the melanin synthesis reduction on cell culture. The *cell viability determination* was measured using the colorimetric thiazolyl blue tetrazolium bromide (MTT) and reported to be reliable for evaluation of light induced cytotoxicity for follow fluencies: 1, 2, 5, 10, 25 J/cm².

## Cytotoxicity assay

Culture dishes (35 mm, 9 cm² growth area) were seeded with 9.4 × $10^3$ cells/cm² (8.5 × $10^4$ cells/dish) in 1 ml growth media (cell number was determined from preliminary results to optimize MTT detection) and grown at 37ºC in an atmosphere of 5% $CO_2$ in humidified air for 24 h. After 24 h post-seeding the treatment of the cells with the drug in the concentration of 2 mg/ml and 4 mg/ml for 18 h of incubation was done. After this time, the drug in the medium was removed and 1 ml of Dulbecco's phosphate buffered saline was added to each dish for irradiation in different fluencies with the light device. Post irradiation, 1 ml of medium was added to each dish and the cultures were incubated for an additional 48 h. The cytotoxicity was measured with the MTT assay. Triplicate wells were analyzed for each concentration. Data were collected on a MCC 340 plate reader at 540 nm and the survival rate of cell growth was calculated using the following formula:

Survival rate (%)=$A_{540}$ (drug)/$A_{540}$ (control) × 100.

The results of the MTT tests were used for statistical analysis. Statistical analyses were performed with one-way ANOVA (test ($p<0.05$) of ORIGIN 5.0 (Scientific Graphing and Analysis Software, Northampton, MA) [26,27]. The median inhibitory concentration ($IC_{50}$) was determined using the Calcusyn program [28] and the cell survival (%) was assessed as a function of **fluencies**. For choosing the adequate fluencies to evaluate the melanin amount for 3 groups of conditions (amber light irradiation, infrared light irradiation and association of both), the viability in MTT test was considerate around 80% of cells viability comparing to control (cells on dark) for the experiments.

## Measurement of melanin amount

**Melanin extraction:** For melanin extraction, the cell pellet was resuspended in 500 µL of PBS (Dulbecco's Phosphate Buffered Saline 10x, D1408, Sigma, diluted in distilled water to 1x) and centrifuged for 5 minutes, at 6000 rpm, the supernatant was dispensed, 200 µL of NaOH 1 M was added with 10% DMSO, vortexed for 5 minutes and incubated at 80ºC for 1 hour, vortexed for another 5 minutes, centrifuged for 5 minutes. The absorbance was read at 470 nm. The results were evaluated with the softmax pro 5.4 software and the percentage of melanin content in percentage in relation to the non-irradiated control. The results can be observed on **Table 1 and Figure 2.**

**Table 1:** Melanin amount obtained on cell culture after irradiation procedure.

| % Melanin Amount | | | |
|---|---|---|---|
| **Sample** | **Control** | **% Melanin** | **% Depigmentation** |
| Irradiation - Amber LED Light (25 J/cm²) | 100 | 62.6 | 37.4 |
| Irradiation - Infrared laser Light (25 J/cm²) | | 87.4 | 12.6 |
| Irradiation - Amber LED Light + infrared laser light (30 J/cm²) | | 36.4 | 63.6 |

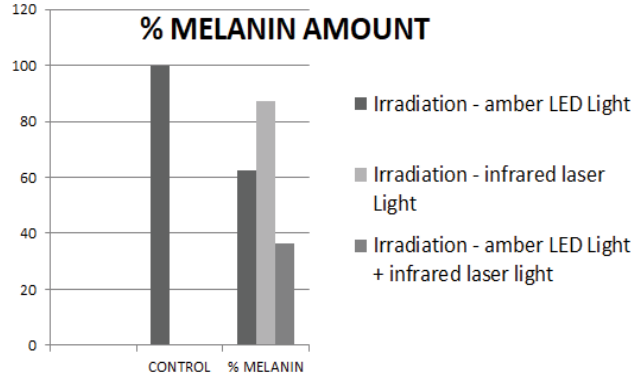


**Figure 2** Melanin amount obtained on cell culture after irradiation procedure.

***In vivo* studies:** The study was carried with 5 female volunteers with age from 30 to 40 years old, healthy (without any concomitant disease), skin phototype III and IV, with skin flaccidly and spots characterized by elevated pigmentation. The volunteers were clarified about the study and read and signed the informed consent, authorizing the accomplishment of the procedures.

## Professional clinical procedure

The irradiation was done using amber LED light irradiation 25 J/cm$^2$ associated to infrared laser light irradiation 5 J/cm$^2$ at same time (Total fluency=30 J/cm$^2$). The application of both wavelengths at same time is useful modulating mainly the inflammatory response. The proportion of irradiation dose ratio was 75%/25% to 590/850 nm as suggest in previously paper [17]. The irradiation procedure was realized once a week (4 folds on months) and the instrumental quantifications for skin elasticity by cutumeter analyses was evaluated before and after 30 days. Visual evaluations were done also as we can see in **Figures 3, 4 and 5.** The protocol follows the steps:


**Figure 3** Results obtained before (A-A1, B-B1, C-C1) and after 30 days (D-D1, E-E1, F-F1) decrease of pigmentation and the face lifting effect on skin before and after 30 days. On picture black and white the spots are more evident.


**Figure 4** Results obtained before (A-A1, B-B1, C-C1) and after 30 days (D-D1, E-E1, F-F1) decrease of pigmentation and the face lifting effect on skin before and after 30 days. On picture black and white the spots are more evident.

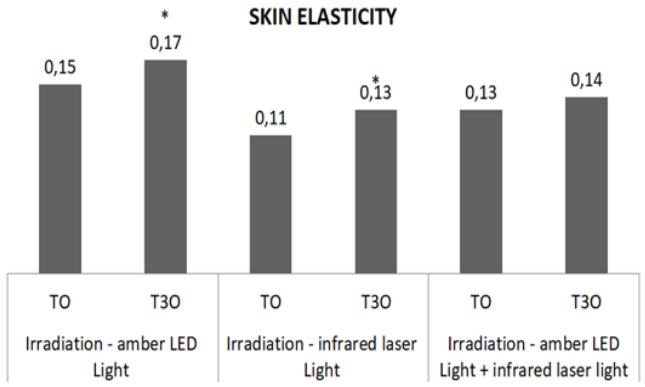


**Figure 5** Skin elasticity parameter (R8) before and after 30 days of treatment.

1) Hygienization of skin,

2) Application of light on skin (irradiation procedure of total face) and the final step

3) Sunscreens application.

It is important to mention that the patients were informed about the importance of sun protection after procedure to avoiding the hyperpigmentation post inflammatory. The volunteers used sunscreen with minimum protector factor of sun (FPS 50) during all study.

### Skin elasticity measurements

Skin elasticity was evaluated by a noninvasive suction skin Cutometer® MPA 580 (Courage+Khazaka, Germany) on every assessment. The measurement setting was a time/strain mode for 18 s, followed by a relaxation period of two seconds, and measuring probe of 2 mm was used which applied a constant suction pressure of 350 mbar. The values for the Cutometer® elastic parameters (R0-R9) were obtained from the skin deformation curves, as previously described [29]. Statistical analysis was performed by adjusting the linear models by time and treatment using the MIXED procedure of the SAS program. After evaluation of treatment effect, multiple comparisons were analyzed.

## Results and Discussion

In **Figure 1** the Photobiomodulation procedure on skin using laser and LEDs systems was performed on patient to decrease the spots and flaccidity of skin. On **Table 1 and Figure 2** is possible to see the values of Melanin amount obtained on cell culture after irradiation procedure. Also the percentage of depigmentation can be observed. On **Figure 3** is possible to see the pigmentation and the face lifting effect on skin before and after 30 days.

As we can see the PBM procedure decreases the pigmentation and the flaccidly on skin (D-D1, E-E1, F-F1) after 30 days. The irradiation using amber LED light decreases the pigmentation on skin; since that decreases the inflammation and vascularization of skin as well as increases the degradation of melanin at skin.

Many studies discusses about skin pigmentation by visible light, on shorter wavelengths, due to increasing of oxygen reactive species which takes to increases of the skin pigmentation [24]. The results suggest that after irradiation procedure the skin pigmentation decreases by bleaching of the melanin (amber light) and the pigmentation doesn't comes back because of inflammation modulation by amber and infrared laser light associated on procedure.

In **Figure 4** either is possible to see the pigmentation and the face lifting effect on skin before and after 30 days. In the **Figure 5** the measurements to parameter of skin elasticity (R8) can be observed.

According to the results presented, the treatment with amber LED and infrared laser and its combined use maintained the elasticity of the skin after 30 days of use, when compared to $T_0$. According to the statistical analysis, there was a significant difference between the initial time ($T_0$) and the final time T 30 days for the treatments using irradiation with associated amber LED and infrared laser (Group 3) and amber LED irradiation (Group 1) in relation to the R8 parameter. There were also differences between treatments where treatment using associated amber LED and infrared laser irradiation (Group 3) showed improvement in skin elasticity after 30 days of treatment. In a deal with the results on **Figure 5** the amber LED light associated to infrared laser light increases the skin elasticity.

As mentioned before and in a deal with the results the amber LED light induces the photobleaching of melanin as well as its production on skin since that acts modulating the inflammatory and vascularization response at skin. At same time the infrared laser light also decreases the inflammatory response modulating the skin pigmentation either avoiding the feedback positive response, to increasing the amount of melanin on skin by protection mechanism, as common on Post-Inflammatory Hyperpigmentation (PIH). Consequently, the depigmenting process on skin will be happens. Also the association of both wavelengths decreases the flaccidly of skin by amber light (improves the collagen production) and improves the muscle tone by infrared laser light promoting the lifting effect on face.

## Conclusion

The results suggest that the amber LED light acts decreasing the melanin amount due to photobleaching as well as acts on decreasing the flaccidly. The association of amber LED light with infrared laser light improves the melanogeneses control since that acts mainly on inflammation modulation. Also the effects on skin elasticity increases promoting the facial lifting. Here we suggest the importance to use at same time both wavelengths for strengthening dermis and muscle; improving the facial lifting promotion; sustaining the whole face.

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