

8-30-2019

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Recommended Citation

Ghidini, G., Vescovi, P., Meleti, M., Giacomo, S., Alessandro, S., & Roberto, S. Absorption Capability of a 645-nm Diode Laser on Swine Soft Tissue Samples: a Preliminary Study in an Ex-Vivo Model. *J Dent Indones.* 2019;26(2): 75-80

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Absorption Capability of a 645-nm Diode Laser on Swine Soft Tissue Samples: a Preliminary Study in an Ex-Vivo Model

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ORIGINAL ARTICLE

Absorption Capability of a 645-nm Diode Laser on Swine Soft Tissue Samples: a Preliminary Study in an Ex-Vivo Model

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ABSTRACT

Most published articles have highlighted several positive effects of low-level laser therapy on tissues; however, to our knowledge, no studies have investigated the depth to which a light beam penetrates cell layers of an irradiated tissue. To date, it is unclear if the laser reaches cells and how many of the cells can be reached in the tissues underlying the oral mucosa. This is of paramount importance as the effect of a laser at a particular depth (e.g., the periosteum and/or bone) should be measurable and predictable to some extent. **Objectives:** The present preliminary *ex vivo* study aimed to assess the possible interaction between complex biological systems and laser light through the irradiation of different soft tissue samples. **Methods:** A 645-nm wavelength diode laser was used for evaluation. Owing to known similarities with human tissues, swine tissue samples harvested from the mandibular oral mucosa were used. One sample of periosteum (0.45-mm thick), two mucosal samples (0.5- and 1-mm thick), and three samples, including both the mucosa and periosteum (1-, 1.3-, and 1.65-mm thick), were used as target soft tissues. Measurements were recorded with a power meter located under the irradiated tissues. **Results:** The mean absorption values for the 0.5-mm mucosa sample; 0.45-mm periosteum sample; 1-, 1.3-, and 1.65-mm mucosal and periosteum samples; and 1-mm mucosal sample were as follows: 70.64 ± 20.14 mW; 90.75 ± 42.87 mW; 93.40 ± 18.68 mW, 101.93 ± 13.60 mW, and 102.80 ± 18.54 mW; and 111.40 ± 13.22 mW, respectively. **Conclusion:** The red-light laser with a 645-nm wavelength can reach cells in each layer of the measured tissues.

Key words: low-level laser therapy, photobiomodulation, absorption, 645nm, swine soft tissues

How to cite this article: Ghidini G, Vescovi P, Meleti M, Giacomo S, Alessandro S, Roberto S. Absorption capability of a 645-nm diode laser on swine soft tissue samples: a preliminary study in an *ex vivo* model. J Dent Indones. 2019;26(2):75-80

INTRODUCTION

Mesenchymal stem cells have emerged as a popular and versatile tool in the field of regenerative medicine, and there has been increasing interest in their use in tissue engineering in recent decades.¹ There have been numerous publications about stem cell applications in several medical fields, ranging from cosmetic to post-surgical rehabilitation.²

Laser devices consist of an optical cavity associated with an active medium in which the generation of an electric current produces and emits light. This type of irradiation is characterized by specific properties, including polarization, coherence, collimation, and monochromaticity, which differentiates it from other light sources.³

Although lasers are generally divided into categories based on their physical property (gas, liquid, solid, or semiconductor), type of medium (e.g., helium, neon, He/Ne; erbium: yttrium, aluminum garnet, Er:YAG), and degree of exposure hazard (classes I–IV). However, they are more often grouped on the basis of their medical applications and energy level (high-, medium-, and low-level lasers).

Low-level laser therapy (LLLT) has been applied on *in vitro* and *in vivo* models to stimulate cell proliferation and differentiation. LLLT has a variety of biostimulatory effects, including the promotion of wound healing^{4,5}, fibroblast⁶⁻⁸ and chondral proliferation⁹, collagen synthesis^{10,11}, anti-inflammatory activity¹², and nerve regeneration¹³. Among the effects induced by LLLT on hard and soft tissues, it stimulates a variety of

osteogenesis-inducing factors, which may promote the osteoblastic phenotype, biomineralization, and induction of bone-like organization.^{14,15}

Most published articles have highlighted the positive effects of LLLT on tissues. However, to the best of our knowledge, no studies have investigated the depth to which the laser can reach the cell layers of an irradiated tissue. Therefore, the following questions are not fully answered: “How deep does the laser light penetrate?” and “Are all the targeted cells in the irradiated tissues reached by the laser light?”

To date, whether the laser light reaches cells in the tissues underlying the oral mucosa and to which degree it reaches the tissues remain to be fully elucidated. This is of paramount importance as the effect of the laser at a deep level (e.g., the periosteum and/or bone) should be measurable and predictable to some extent.

The present preliminary *ex vivo* study aimed to assess the possible interaction between complex biological systems and laser light through the irradiation of different soft tissue samples.

METHODS

A 645-nm wavelength diode laser was used for evaluation (Raffaello, Dental Medical Technologies, DMT srl. Lissone, MB, Italy), thereby allowing further comparison with the most commonly used wavelength in published studies in the literature.

Owing to its known similarities with human tissue samples, swine tissue samples were used. The samples were harvested 24 h before the start of the experiment. The tissue samples were obtained from a swine mandible and were preserved in 500 mL of Medium 199 with Earle's salts and sodium azide (0.05%) at 2°C–8°C.

The samples were harvested from the mandibular oral mucosa using a 15C scalpel and were adapted onto the power meter platform, as shown in Figure 1. The area of irradiation in each sample was in accordance with the spot of the laser beam (0.337 cm²). In this context, the area of the specimen has no consequence of misuration as irradiation only affected the region under the laser beam.

The power output value was set at 220 mW, and measurements were recorded with a power meter located underneath the irradiated tissue. The emission was angled perpendicular to the power meter at a distance of ~2 cm. An actual power output of 168 mW was detected.

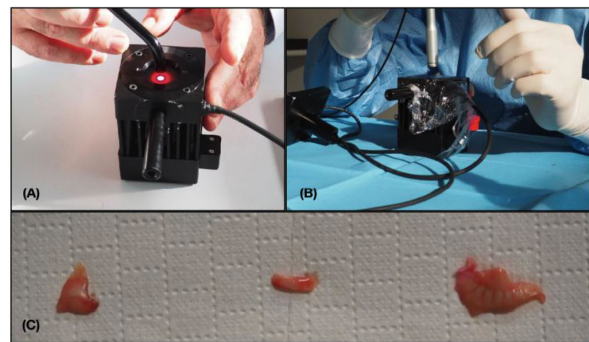


Figure 1. (A) Example of tissue specimens harvested from the mandibular oral mucosa. (B) Power meter. (C) Setting of the experiment

Irradiation was performed for 113 s, six times, and once for each sample. No support system was used; the laser tip measured 0.337 cm² in an area. Periosteum sample measuring 0.45 mm in thickness, two mucosal samples measuring 0.5 and 1 mm in thickness, and three mucosal and periosteum samples measuring 1, 1.3, and 1.65 mm in thickness were used as the soft tissue targets.

Before performing laser irradiation on the swine tissues, irradiation was performed with and without plastic protective devices around the laser tip and surrounding the power meter to reveal possible interferences and/or absorption of the laser beam with plastic protection devices.

RESULTS

Following 10 cycles of irradiation, the data-log (Table 1) was converted into graphics, with time in seconds and on the x-axis and power output in milliwatts on the y-axis (Figure 2). For each sample, the mean absorption and standard deviation were calculated. The calculated values of the mean absorption showed that an increased thickness was in accordance with lower transmissions.

The mean absorption values were as follows: 70.64 ± 20.14 mW for the 0.5-mm mucosal sample; 90.75 ± 42.87 mW for the 0.45-mm periosteum sample; 93.40 ± 18.68 mW for the 1-mm mucosal and periosteum sample; 101.93 ± 13.60 mW for the 1.3-mm mucosal and periosteum sample; 102.80 ± 18.54 mW for the 1.65-mm mucosal and periosteum sample; and 111.40 ± 13.22 mW for the 1-mm mucosal sample. No differences were found between the detected measurements when the laser was used with or without the plastic protective devices. The laser beam penetrated all tissues independent of the tissue type and width of the specimen.

Table 1. Values of transmitted power (mW) and absorption (power [mW]) at different time

Specimen	Time (seconds)					
	30		60		90	
	T	A	T	A	T	A
Periostium (0.45mm)	95	66	95	66	94	67
Mucosa (0.5mm)	81	76	83	74	85	71
Mucosa (1mm)	44	107	42	109	42	109
Mucosa + Periostium (1mm)	85	78	75	88	73	90
Mucosa + Periostium (1.3mm)	26	121	24	123	24	123
Mucosa + Periostium (1.65mm)	69	94	72	91	64	99

*T=transmitted; A=absorbed

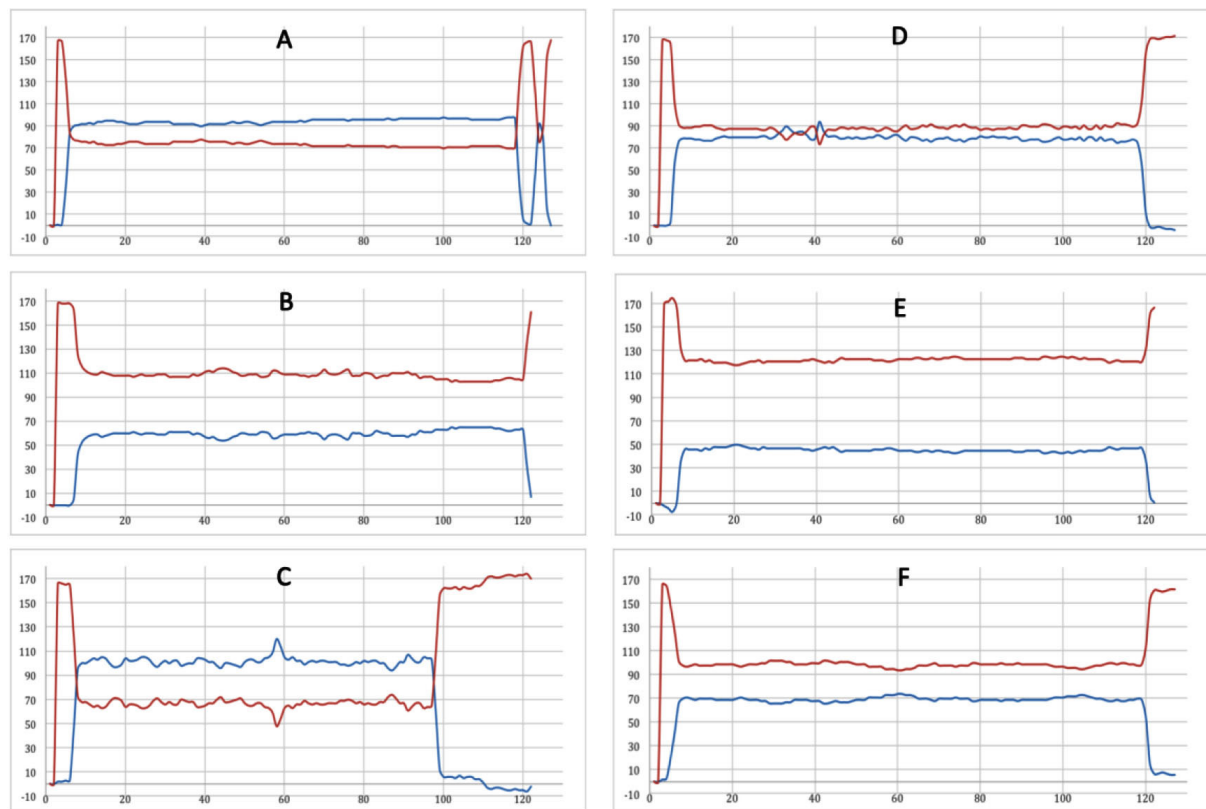


Figure 2. Graphics of transmission (blue) and absorption (red) of the soft and hard tissue samples. x-axis: time (s), y-axis: power output (mW). (A) Mucosa sample. Thickness: 0.5mm. Absorption mean = 70.64 ± 0.14 ; (B) Mucosa sample. Thickness: 1mm. Absorption mean = 111.4 ± 13.22 ; (C) Periosteum sample. Thickness: 0.45mm. Absorption mean = 90.75 ± 42.87 ; (D) Periosteum plus mucosa sample. Thickness= 1mm. Mean absorption = 93.40 ± 18.68 ; (E) Periosteum plus mucosa sample. Thickness= 1.3mm. Mean absorption = 101.93 ± 13.60 ; (F) Periosteum plus mucosa sample. Thickness= 1.63mm. Mean absorption = 102.80 ± 18.54 .

DISCUSSION

Low-level laser therapy has been widely used in numerous clinical situations to accelerate the regenerative processes of tissues in view of its stimulatory effect on the proliferation of various cell types. LLLT has applications in almost every medical field, from prevention and therapy of oral chemo-induced mucositis to the treatment of androgenic alopecia. The clinical effects and benefits of LLLT have been reported in several published studies; however, the mechanisms underlying these processes remain to be fully elucidated.

The present *ex vivo* study attempted to answer the question “Is the laser beam able to reach every cell layer in the irradiated tissue?” through analysis of the outcomes. The nature of the present study (preliminary) does not allow drawing any conclusive results due to the limited number of observations performed for each sample.

In Figure 1, which presents graphs produced from each sample, the presence of two lines indicates that a part of the laser wavelength is absorbed, whereas the other part is transmitted. Thicker tissue samples had higher values of absorption, whereas thinner tissue samples had higher values of transmission.

The nature of the components of samples did not influence the results; no differences were found between the mucosal, periosteum, or combined mucosal and periosteum samples at similar thicknesses. The thickness of samples may be the only feature that affects absorption and transmission.

Therefore, the results of the present study showed that a red-light laser with a 645-nm wavelength can reach cells in each layer of the analyzed tissues, thereby indicating a possible interaction and the biostimulation of the targeted cells, even at a deeper level. This finding is important considering the fact that light was never completely absorbed by soft tissues in the present model; therefore, part of the energy can reach bone tissues underneath the superficial layer of the epithelium, periosteum, and up to the bone. This finding is particularly relevant due to its possible application in the treatment of patients with impaired healing processes, including those treated with bisphosphonates or other anti-resorptive/angiogenic drugs, those affected by diabetes, or those treated with corticosteroids.^{16,17} Apart from patients with systemic diseases or those taking drugs that may interfere with the healing process, the notion that laser light penetrates beyond the irradiated surface, even when used in a low-level energy mode, has implications in other fields of dentistry.

The post-extraction sockets^{18,19} (even if a first intention closure is performed), periodontal surgery and

implantology²⁰, orthodontics^{21,22}, temporomandibular joints disorders, and neurological conditions may all benefit from superficial irradiation in which part of the laser beam penetrates a deeper level^{23,24}.

During the irradiation of tissues, the laser beam reaches not only pathological cells but also healthy cells. The A study conducted by Hamblin in 2018 has indicated that only cells under oxidative stress can interact with laser light.²⁵ Even if the exact mechanism remains to be fully understood, the primary site of light absorption in mammalian cells is the mitochondria and, more specifically, the cytochrome c oxidase. LLLT may be more effective in diseased or damaged cells and tissues, and it does not remarkably affect healthy cells, as unhealthy or hypoxic cells are more likely to have inhibitory concentrations of nitric oxide.

The results of the present study showed how both soft and hard tissues can benefit from photobiomodulation; LLLT can be performed not only during surgery (when hard tissues are directly exposed to laser light) but also after surgery during the healing process (reaching the bone underneath the surgical wound through the healing mucosa).

Moreover, the present study showed that sterile and non-sterile devices can be used to isolate the laser tip from operative fields during procedures without interfering with the delivered laser energy. This finding is important as it allows the operator to use non-disposable laser tips, thereby preventing further connections between fibers and the laser light source and reducing energy dispersion.

It can be observed that the lack of a support system influenced the first and last seconds of measurements, and these areas of the graphs should not to be considered. Manual support of the laser tip involves the possible micromovements of the operator's hand, which can lead to variations in the emission angle, resulting in the loss of coherence of the laser beam and scattering. It is important for the operator to remember that during the administration of LLLT, the distance between the laser tip and target tissues must remain as constant as possible to deliver the same level of energy to every irradiated area.

CONCLUSION

A red-light laser with 645-nm wavelength can reach cells in each layer of measured tissues. This *ex vivo* experiment on soft swine tissues was conducted with the use of a power meter showing that the tissue layer was reached by the laser beam. As the thickness of swine samples is comparable with that of human tissue samples, protocols based on such laser features can allow targeting of greater depths and lead to possible interactions (photobiomodulation) of human cells.

ACKNOWLEDGMENT

Thanks to all authors, professors and experts for their efforts and contributions to this study.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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(Received March 3, 2019; Accepted June 12, 2019)