

RESEARCH ARTICLE

Thermal Effects of 445 nm Laser Irradiation on the Bovine Tongue Tissues Ex Vivo



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Abstract: The purpose of this study was to employ the use of thermographic techniques to compare temperature changes and thermal heat transfer patterns in soft tissues when using the 445nm-diode laser with initiated and non-initiated tips. Methods: Bovine tongue slices measuring 5 mm in thickness were placed in between two microscopy glass slides at a distance of 11 cm from a thermographic camera. Fifteen 2 cm long incisions were made along the surface of the soft tissue parallel to the glass slides and the camera capture field. Incisions were performed using a 445nm diode laser (continuous wave at 2 watts) with 320 μ m-thick glass initiated and non-initiated fiber tips for a total 30-second irradiation period. The maximum temperature changes in oC (ΔT) in the soft tissues, as well as the vertical and lateral heat transfer (in mm), were recorded at 10-second intervals for a duration of 30 seconds, using the thermographic images captured using the infrared camera and a special image analysis software. Descriptive statistical analysis occurred to evaluate the temperature changes over the critical temperature threshold. Results: The maximum ΔT in oC for the initiated lasers was 17.48 ± 9.05 and for non-initiated lasers was 10.72 ± 2.29 . The heat transfer in the two groups for vertical/lateral was $7.50 \pm 1.4/18.30 \pm 4.01$ for the initiated lasers and $7.10 \pm 1.82/19.24 \pm 3.86$ for the non-initiated lasers. Conclusion: Initiated tips and non-initiated tips of 445nm laser present deep penetration depths and high temperatures in bovine homogenous soft tissues. The irradiation period and clinical indication must be strictly followed to avoid complications from overheating in soft tissues.

Keywords: 445nm, diode lasers, heat transfer, irradiation, thermography

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1. Introduction

Soft tissue diode lasers are a type of dental lasers that operate in the 810-980 nm wavelength range. They are often used in periodontal therapy, implantology, oral surgery, and cosmetic dentistry, among other areas. One of the main advantages of using soft tissue diode lasers in dental settings is their ability to provide precise, controlled cutting and ablation of soft tissues, without damaging adjacent tissues. Additionally, soft tissue diode lasers offer several other benefits, including significant hemostasis, minimal postoperative pain, and improved healing times [1]. One of the most common uses of soft tissue diode lasers in dental settings is in periodontal therapy. Lasers can be used to remove diseased or inflamed gingiva, as well as to decontaminate root surfaces and periodontal pockets [2]. By using lasers in periodontal therapy, clinicians can achieve better access to deep pockets, leading to improved outcomes for patients with severe periodontitis. Furthermore, studies have shown that laser-assisted periodontal therapy can result in significant improvements in clinical attachment levels, compared to traditional treatment methods [3]. Lasers offer the distinct advantage of disinfecting the surgical site instantly, allowing for a noncontact procedure that eliminates mechanical trauma to the tissue. Specifically, diode lasers, introduced in the mid-1990s for dental and oral surgery, are favored for their compact size, portability, and cost-effectiveness in comparison to other lasers, making them a popular choice among practitioners. These lasers operate at wavelengths such as 810, 940, and 980 nm, and when appropriately selected and

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applied, they prove to be safe and effective for various soft tissue surgeries [4]. Research has further supported the potential benefits of diode lasers, with studies indicating that they cause lower thermal damage to tissues compared to other laser types and therefore have been used in medicine and dentistry [5, 6]. Despite these advantages, some studies have pointed out disadvantages such as delayed repair in larger lesions and the risk of charring in smaller ones, similar to issues faced with other laser types [7]. During surgical procedures, employing a laser can lead to bleeding, discomfort after the operation, and a sensation of burning. Without adequate cooling while emitting laser beams, there's a risk of necrotizing both hard and soft tissues [8]. Additionally, concerns regarding laser plume inhalation and its potential to cause respiratory lesions in operators have been raised [9]. The main objective of our study is to identify the thermographic effects of the 445nm blue light diode laser with initiated- and non-initiated glass fiber tips, to better understand the influence of tip type in laser energy in an ex vivo application using a simulant of clinical setting.

2. Literature Review

The effects of lasers into four primary categories based on their biological interaction: Electromechanical effects; Non-thermal effects including photochemical reactions, photophysical processes, and photobiostimulation / biophotomodulation [10]. The study of laser-tissue interactions must consider the thermal properties of tissues, which are intrinsically linked to how temperature is distributed within them. The study of thermal energy transport in biological tissues, such as conduction, convection, radiation, metabolic processes, and phase changes, noted that lasers can produce a spectrum of effects from coagulation to melting, depending on the laser's peak power and wavelength. Water vaporization occurs at 100°C, leading to the formation of gas bubbles and subsequent thermal decomposition of tissue, and without water, carbon atoms create smoke in a process termed carbonization; beyond 300°C, melting can ensue. This area of study, including the principles of photophysical, photochemical, and photobiological interactions and other laser-tissue dynamics, is extensively documented in the literature [11]. Soft tissue diode lasers are also commonly used in implantology, where they can be used to expose implant sites, remove excess tissue, and contour the gingival tissue around implant restorations. By using lasers in implantology, clinicians can achieve a more precise and predictable outcome, with reduced bleeding and swelling compared to traditional methods. Furthermore, laser-assisted implant decontamination has been shown to result in better re-osseointegration of ailing implants in vivo. Similar applications of laser energy have shown that Low-Level Laser Therapy (LLLT) has shown to the osseointegration of biomaterials, boosting their functionality. It has been shown to substantially promote cell adherence to the surfaces of implants, which positively affects the long-term stability and effectiveness of grafted materials [12]. In addition to periodontal therapy and implantology, soft tissue diode lasers are also used in oral surgery, cosmetic dentistry, pediatric dentistry, and other areas of dentistry [13]. Lasers can be used to treat oral lesions such as aphthous ulcers, fibromas, and hemangiomas, with reduced discomfort and faster healing times compared to traditional methods [14]. Lasers can also be used to perform frenectomies, gingivectomies, and other soft tissue procedures, with minimal discomfort and rapid healing times [15]. The diode laser is recognized as an extremely effective technique for performing excisional biopsies on benign oral soft tissue growths, offering benefits during and after the operation that surpass those of traditional scalpel-based surgery [16]. The application of lasers in pediatric dental procedures is favorably received by both the young patients and their parents. The minimally invasive nature of this approach often results in more cooperative behavior from children during dental treatments [17]. The use of diode laser therapy in conjunction with graphite paste has shown to greatly reduce dentinal sensitivity after irradiation therapy, a therapy that was also deemed safe for pulpal health [18]. Lasers have also been observed to drastically decrease bacteria load in multiple dental applications [19]. Despite the many benefits of soft tissue diode lasers, there are also some limitations to their use in dental settings. One limitation is their variably high cost over traditional scalpels, which may limit their accessibility to some dental practices [20]. Additionally, lasers require specialized training and expertise to use effectively, and may not be suitable for all patients or all clinical situations. Finally, lasers may pose some potential risks to patients, such as accidental damage to adjacent tissues, and the potential for thermal damage to tissues. Diode lasers offer substantial benefits across a range of dental interventions, yet practitioners should remain aware of potential adverse outcomes, including the excessive heating of adjacent tissues and implants within the mouth.

The 445 nm wavelength laser presents a promising tool for oral soft tissue surgeries, including cutting, removing tissues, and sterilizing areas, by leveraging its high absorption rates in key biological components like melanin and hemoglobin, as well as collagen under blue light conditions. Unlike conventional near-infrared (NIR) diode lasers, the 445 nm laser's effectiveness is not significantly diminished by water, allowing for precise and immediate incisions without pre-heating. This specific wavelength ensures that energy is efficiently absorbed by the targeted tissue areas, minimizing thermal damage to surrounding areas and enhancing surgical precision [21]. The unique biophysical characteristics of the 445 nm laser, including its transmission through tissue and its thermographic behavior, suggest superior performance in tissue ablation from the outset, with minimal thermal impact at lower power levels and controlled temperature increases at higher intensities. These findings highlight the potential of the 445 nm laser as a more effective and safer alternative for oral soft tissue surgeries and caries ablation compared to traditional IR lasers, offering a blend of high absorption and reduced side effects [22, 23]. Although the advantages are well documented, there are raised concerns over the use of 445nm diode lasers to decontaminate implant surfaces due to overheating,

indicating a conflicting opinion about the safety of the 445nm laser [24]. Specifically, studies documenting temperature fluctuations within uniform tissues subjected to 445 nm diode laser treatments, distinguishing between initiated- and non-initiated-laser tips, are absent from the literature. Similarly, there's a lack of research utilizing thermographic techniques to capture and quantify tissue thermal profiles during exposure to a 445 nm diode laser, whether using initiated- or non-initiated tips. Our research seeks to employ thermography to both visually and numerically assess the temperature shifts and thermal spread that homogeneous bovine oral soft tissues undergo when irradiated with a 445 nm diode laser, comparing the effects of both initiated- and non-initiated tips.

The energy from the laser interacts with biological tissues in various manners, such as through reflection, transmission, absorption, and scattering processes. Reflection typically has a minimal impact, whereas transmission can reach and affect deeper tissue strata. Conversely, absorption and scattering exhibit a reciprocal dynamic, where scattering can cause heat to diffuse into non-target areas, possibly leading to unintended harm or tissue death. However, the initiation of diode laser tips can mitigate such scattering effects by focusing the laser's energy at the point of contact, thereby diminishing the likelihood of excessive thermal buildup and subsequent tissue damage. This initiation process entails the laser tip's preparation with blue-colored articulating papers prior to performing tissue cuts [25].

3. Research Methodology

Bovine tongue slices from fresh slaughtered young cows measuring 5 mm in thickness were placed in between two microscopy glass slides at 11 cm from a thermographic infrared camera (8640P Series, ICI Infrared cameras, Inc., Beaumont, TX) at room temperature (17oC). Microscopy slides were stabilized with red molding wax. Red molding wax was placed purposefully to not impact the thermography readings of the bovine tissue through the microscopy glass slides. Bovine tongues were sourced from a local market with direct access to slaughtered cows daily. Tongue tissue was stored in the refrigerator unit at 5oC overnight and allowed to warm to room temperature over the course of 4-6 hours. All tissues were inspected to be moist, non-spoiled, with visible heme prior to the initiation of the incisions. In this study, forty incisions, each 2 cm in length, were executed with a 445 nm SiroBlue diode laser by Sirona, based in Bensheim, Germany, utilizing both initiated and non-initiated tips. The cuts were applied across the top of the homogeneous tissue, in alignment with the microscope slides and within the visual field of the thermographic camera. This camera was strategically positioned at a right angle to the slides' length to monitor thermal shifts across the tissue via the slides, capturing temperature data at specific intervals: at the outset, 10 seconds, 20 seconds, and 30 seconds into the procedure (Figure 1). These thermal readings and images were documented in real-time using specialized thermographic software. The incisions were conducted in a contact mode using a 320 nm glass fiber. Initiation of the laser tip involved a brief touch to blue articulating paper from Bausch [26], which was used to coat the fiber tip with pigment. Bausch's articulating papers are known to be a blend of oil, pigments, and wax, with specific proportions being 10% each of blue and red pigments, 50% white mineral oil, and 10% natural waxes, ensuring the absence of hazardous materials and no extraordinary risks under standard application conditions. After initiation, the glass fiber tips exhibit a distinct blue/purple marking on the cutting edge. The ideology behind initiating the tips is to allow the articulator coloring to absorb and concentrate the 445nm diode laser energy as it travels through the glass fiber rod, allowing a more controlled application of wavelength energy at the cutting edge where the initiated markings are present. During these procedures, all personnel were equipped with protective eyewear suited for the 445 nm wavelength.

Figure 1
Experimental Setting



3.1. Experimental setting

In this controlled ex vivo experiment, the investigative team sought to assess the peak temperature fluctuations (ΔT) within uniform slices of bovine tongue when incised using 445 nm diode lasers equipped with both initiated and non-initiated tips. Specimens from bovine tongues, standardized at 5 mm in width and cut to a precise length, were uniformly prepared by a single researcher using a consistent method, then anchored between glass slides positioned 11 mm from a thermographic camera. Each piece featured dual horizontal planes suitable for incisions, limited to a pair per plane, to guarantee the use of unaltered tissue for every incision, thereby avoiding the influence of residual thermal diffusion. Utilizing 445 nm diode lasers, the study executed a total of 40 incisions with 320-micron glass fiber tips in a steady wavelength setting, calibrated to a 2-watt power level, achieving a power intensity of 2,488 W/cm². All incisions were consistently made to a depth of about 2 mm across a span of 30 seconds. Twenty incisions were conducted with an initiated tip, while the remaining twenty involved a non-initiated tip, all by a singular surgeon (G.R.) who possesses substantial expertise in laser surgeries. Thermographic imaging captured by an infrared device throughout the 30-second laser exposure allowed for the evaluation of maximal temperature shifts at specific intervals, alongside the real-time observation of thermal propagation during the incision process.

During each trial, four thermographic images were captured using an infrared camera. Three members of the research team were necessary to ensure the accurate and consistent record of time throughout the experiment. The experienced dental surgeon (G.R.) performed all the incisions using the blue light 445nm diode lasers in the soft tissues. Prior to the non-initiated experimental group, articulation paper was not used to initiate the tip. Prior to the initiated experimental group, G.R. initiated the tip with articulating paper briefly. The used 320-micron-thick glass fiber tips were discarded after each experimental trial. Each bovine soft tissue was incised with a new glass-fiber tip. One members of the team recorded the time and took snapshots of the images at the correct times, while the other member of the team recorded the peak temperatures of each snapshot taken at specific time intervals. The first image was taken before the 30-second irradiation period, and the remaining three images were taken at 10, 20, and 30 seconds.

The IR FlashPro Software© served to evaluate these thermographic images, logging initial and peak temperatures for each specific interval. Temperature variation (ΔT) was determined by the difference between the baseline temperature (equivalent to ambient room conditions) and the highest temperature recorded. Using the thermographic software, both lateral and vertical heat diffusion (measured as the height and breadth of thermal expansion into the tissue) were quantified to discern thermal energy dispersion discrepancies between initiated- and non-initiated glass fiber tips. Measurements for the thermal spread were obtained from the on-screen digital scale with a ruler, and a mathematical equation was applied to translate these digital dimensions into precise soft-tissue measurements. To quantify the spread of thermal energy within the tissue, an equation was formulated to rectify the scaling disparities: $A1/S1=A2/S2$, where 'A' signifies the true size of the tissue in millimeters, and 'S' denotes the on-screen size in millimeters. The area impacted by thermal energy was gauged by measuring the length and breadth at approximately two-thirds of the radius from the center of heat loss, which was the point of highest temperature. This two-thirds segment was selected due to its reproducibility and clarity within the thermographic analysis software. Ensuring consistent energy loss measurement across each experiment was crucial due to the variability in initial and maximum temperature readings. The IR11 filter was utilized to homogenize the assessment of radiation impact within the soft tissue. The dimensions of both lateral and vertical thermal dispersion were meticulously documented in millimeters.

3.2. Statistical analysis

Descriptive statistical analysis occurred to evaluate the temperature changes over the critical temperature threshold. A comparative analysis of the temperature changes over a period of time for initiated- and non-initiated tips was performed using ANOVA.

4. Results

Thermal Variations with Initiated Tips for 445 nm Diode Laser

At the 10-second interval, the average peak temperature variation recorded with initiated tips at a 445 nm diode laser wavelength was $12.00 \pm 10.06^\circ\text{C}$. Progressing to the 20-second interval, this average peak temperature elevation was noted as $18.12 \pm 12.79^\circ\text{C}$. By the 30-second interval, the recorded average peak temperature rise using the same laser setup was $17.48 \pm 9.05^\circ\text{C}$ (refer to Table 1).

Thermal Variations with Non-initiated Tips for 445 nm Diode Laser

At the initial 10-second measurement, the average peak temperature rise using the 445 nm diode laser wavelength with non-initiated tips stood at $3.87 \pm 1.94^\circ\text{C}$. Moving to the 20-second measurement, the observed average peak temperature increment was $7.40 \pm 2.49^\circ\text{C}$. Finally, at the 30-second measurement, the average peak temperature escalation with non-initiated tips reached $10.72 \pm 2.29^\circ\text{C}$ (Table 1).

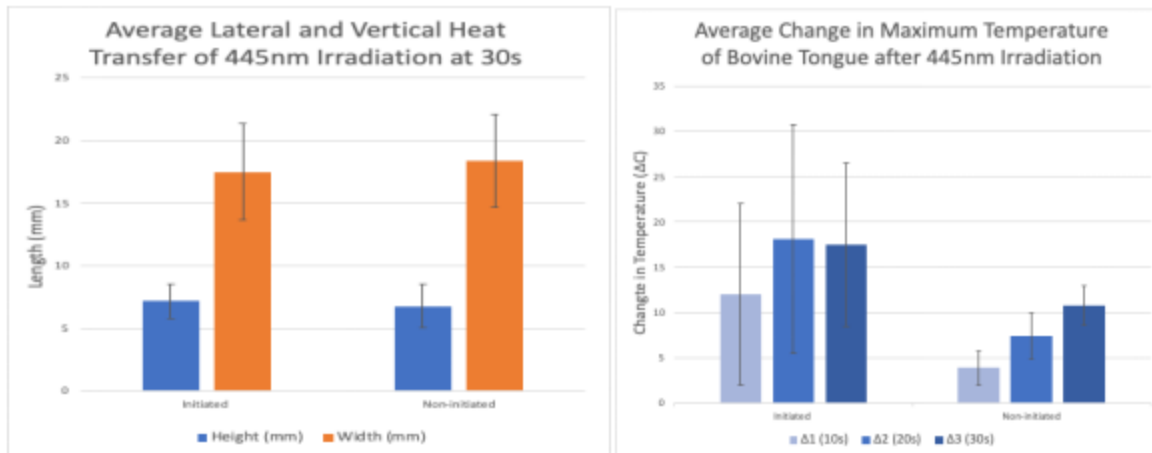
Thermal Energy Displacement Patterns – Initiated Versus Non-initiated Tips

When utilizing initiated tips, the 445 nm diode laser showed a vertical heat transfer (indicating depth of penetration) of 7.50 ± 1.40 mm and a lateral heat spread (indicating width) of 18.30 ± 4.01 mm. In contrast, with non-initiated tips, the laser exhibited a vertical heat migration (penetration) of 7.10 ± 1.82 mm and a lateral extent of heat distribution (width) of 19.24 ± 3.86 mm (depicted in Figures 2-4).

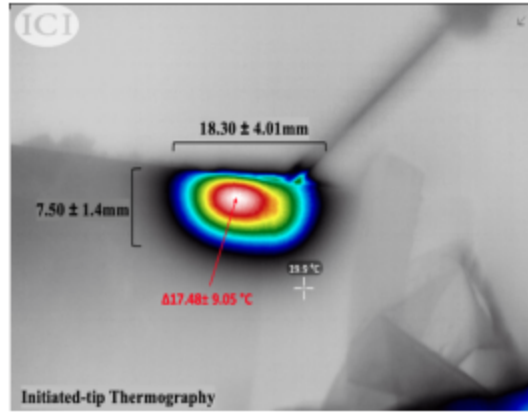
Table 1
Temperature change over irradiation period during homogenous bovine oral soft tissue irradiation and heat distribution after 30 sec of irradiation in depth (height) and width (lateral heat distribution), where $\Delta 1$ is 10s of irradiation, $\Delta 2$ is 20s of irradiation, and $\Delta 3$ is 30s of irradiation

| | $\Delta 1$ ($^\circ\text{C}$) | $\Delta 2$ ($^\circ\text{C}$) | $\Delta 3$ ($^\circ\text{C}$) | Height (mm) | Width (mm) |
|---------------|---------------------------------|---------------------------------|---------------------------------|-----------------|------------------|
| Initiated | 12 ± 10.06 | 18.12 ± 12.79 | 17.48 ± 9.05 | 7.50 ± 1.40 | 18.30 ± 4.01 |
| Non-initiated | 3.87 ± 1.94 | 7.40 ± 2.49 | 10.72 ± 2.29 | 7.10 ± 1.82 | 19.24 ± 3.86 |

Figure 2
Average lateral and vertical heat transfer after 30 s of irradiation with a 445 nm laser (a) and changes in the tissue temperature within 30 sec of irradiation in the homogenous muscle of the cow tongue (b)



Figures 3
Thermographic images of initiated 445 nm laser tips, respectively. Single image snapshots used as a representative of the mean data collected



Figures 4
Thermographic images of non-initiated (right) 445 nm laser tips, respectively. Single image snapshots used as a representative of the mean data collected

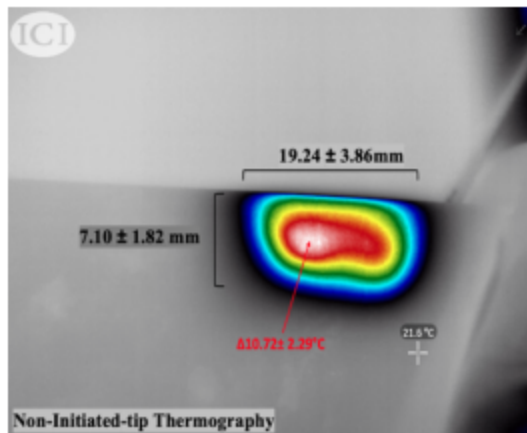
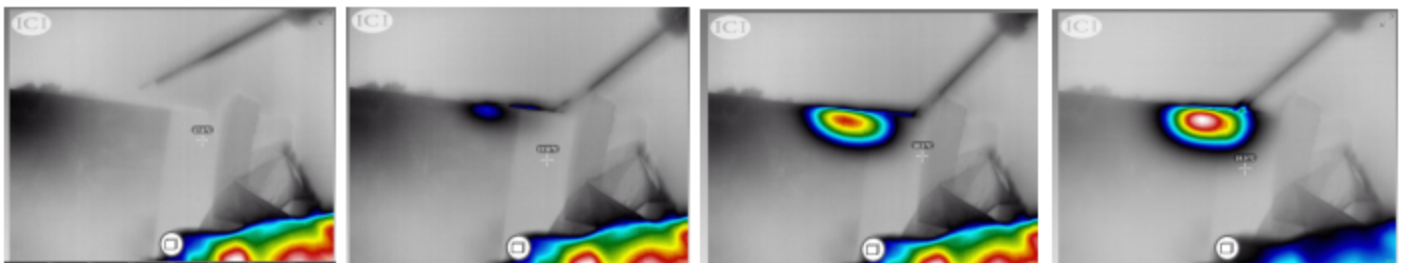


Figure 5
Thermographic images at 0s, 10s, 20s, 30s, of irradiation period



Images have respective average calculations for penetration depth, lateral heat spread, and maximum change in temperature at 30 seconds (Figure 5). The non-initiated tip is responsible for lower energy concentration compared to the temperature increase utilizing the initiated tip to concentrate the energy at the irradiation spot.

The comparison between temperature changes utilizing the non-initiated- and initiated tips showed a statistical significance ($p < 0.05$) over period of 10, 20 and 30 sec of irradiation period (Table 2).

Table 2
Temperature tissue changes utilizing the non-initiated and initiated tips showed a statistical significance ($p < 0.05$) over the 10 sec (change 1), 20 (change 2), and 30 sec (change 3) irradiation period

| Group Statistics | | | | | | |
|------------------|--------------------|---------------|----|-------|----------------|-----------------|
| time | | initiated | N | Mean | Std. Deviation | Std. Error Mean |
| change 1 | temperature_change | non-initiated | 20 | 3.87 | 1.94 | .43 |
| | | initiated | 20 | 12.00 | 10.06 | 2.25 |
| change 2 | temperature_change | non-initiated | 20 | 7.40 | 2.49 | .56 |
| | | initiated | 20 | 18.12 | 12.79 | 2.86 |
| change 3 | temperature_change | non-initiated | 20 | 10.72 | 2.29 | .51 |
| | | initiated | 20 | 17.48 | 9.05 | 2.02 |

Table 3
Independent samples test of significance between vertical and lateral heat transfer

| Independent Samples Test | | | | | | | | | |
|---|------|------|------------------------------|----|-----------------|-----------------|-----------------------|---|--------|
| Equal variances assumed | | | | | | | | | |
| Levene's Test for Equality of Variances | | | t-test for Equality of Means | | | | | | |
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| width | .300 | .587 | -.779 | 38 | .441 | -1.800 | 2.311 | -6.479 | 2.879 |
| height | .055 | .816 | .771 | 38 | .445 | 4.250 | 5.511 | -6.907 | 15.407 |

However, the comparison of the lateral heat transfer (width) and vertical depth (height) showed no statistically significant differences ($p > 0.05$) between the non-initiated- and initiated tips (Table 3).

5. Discussion

Our research indicated significant differences in temperature change when using initiated- versus non-initiated laser tips with a 445 nm diode laser wavelength during oral soft tissue surgeries. Initiated tips, which concentrate energy more effectively, resulted in a higher temperature change in bovine tongue tissue over a 30-second irradiation period (reaching an average of $17.48 \pm 9.05^\circ\text{C}$) compared to non-initiated tips (reaching $10.72 \pm 2.29^\circ\text{C}$). Despite this, both types of tips exhibited similar patterns of heat transfer in terms of depth and width, suggesting that the primary difference lies in the amount of energy delivered to the tissue rather than the pattern of energy distribution. The standard deviations between the temperatures was explained by the effect

that the incisions were performed by a surgeon's hand and not by a calibrated device, representing these variations a simulation of the clinical settings.

There is no doubt that a better analytical solution would be the utilization of the hyperbolic Pennes bioheat equation *ex vivo*. This model would be an additional information obtained from this study because we tested homogeneous oral tissues [27]. However, the constant movement of the laser (heat source) was not the same and approximates the clinical intraoral movement. This form of evaluation could be applied in future studies to estimate better the thermal distribution in living tissue compared to the present thermometric/thermographic model.

Also, in a similar experimental setting in our lab, we were able to show that different near-IR lasers (970 and 980nm) present differences in lateral heat and tissue penetration, using initiated or noninitiated fibers [28], and due to these differences, power settings and irradiation period must be considered to avoid risks due to overheating.

The importance of these findings lies in the potential for improved surgical outcomes. The use of initiated tips could enable clinicians to perform procedures with greater precision due to the higher temperatures achieved, which may translate to more efficient cutting and coagulation of tissue. Additionally, the lack of significant differences in heat transfer patterns between initiated- and non-initiated tips suggests that the risk of collateral thermal damage remains similar, allowing surgeons to select the appropriate tip based on the desired temperature profile without compromising the safety profile. However, the wider variability in temperature change with initiated tips indicates a need for careful management of laser parameters to avoid excessive thermal effects. These findings contribute to the optimization of 445nm laser-assisted surgeries, balancing efficacy, and safety for better patient outcomes.

Recommendations

The present study investigated the safety threshold and heat distribution within the homogeneous bovine tongue muscle during the irradiation with a 445 nm diode dental laser. Our findings revealed that the use of a contact 445 nm diode laser using these laser parameters and utilizing initiated or non-initiated glass fiber tips can lead possibly to tissue overheating and damage, highlighting the importance of understanding the safety threshold and heat properties of these lasers in periodontal applications especially when tissue is very thin (thin phenotypes). The clinical use of diode lasers can also result in adverse effects, such as tissue damage, necrosis, and pain, if the laser parameters are not properly controlled and monitored. Therefore, it is crucial to determine the safety threshold and heat properties of diode lasers to avoid these undesirable outcomes. Our results showed that the temperature increase in soft tissues during irradiation with 445nm diode lasers was dependent on the power density, utilization of initiated- or non-initiated tip, and exposure time of the laser. Specifically, higher power densities and longer exposure times led to greater temperature increases, which can potentially cause irreversible tissue damage. It is significant the information obtained from this study, that the heat distribution due to the laser irradiation is over 15 mm laterally and approximately 7 mm in depth (with approximately 2cm incisions). This thermal radiation can have irreversible tissue damage especially when tissue is very temperature sensitive and when the temperature increase is over the 100C within one minute. Based on our findings, the initiated tip in contact increases the tissue temperature very fast providing excellent cutting efficiency for the specific tissue, at a very high rate of heating (homogenous tissue with great vascularization; bovine tongue). Therefore, it is important to use appropriate laser tips (with or without initiator) and monitor the temperature changes in real-time during dental procedures to ensure safe and effective treatment outcomes. Moreover, our study may indicate the use of cooling techniques, such as air or water spray, may be necessary to reduce the temperature increase in soft tissues during 445 nm diode laser irradiation. These cooling methods can effectively dissipate the heat generated by the laser light and prevent tissue damage. However, the optimal cooling parameters, such as flow rate and distance, need to be determined for each laser system and tissue type. Our findings emphasize the need for a better understanding of the safety threshold and heat properties of 445 nm diode lasers for oral soft tissue applications. Clinicians should carefully select the laser glass fiber and laser settings, monitor the temperature changes in real-time, and use appropriate cooling techniques to ensure safe and effective treatment outcomes. Future studies can investigate the efficacy of different methods and parameters providing better safety protocols in reducing tissue overheating and damage during diode laser irradiation for better clinical outcomes in oral soft tissue surgery.

While our study has provided valuable insights into the thermal effects of a 445 nm diode laser on bovine tongue tissues with initiated- versus non-initiated tips, it is imperative to note that these findings may not be directly extrapolatable to *in-vivo* clinical trials. Bovine tissues, while a useful analogue due to their availability and similarity to human tissues in terms of thickness and vascularization, possess different biochemical and histological properties compared to human oral mucosa [29]. The implications of these differences are twofold: firstly, the thermal behavior observed in *in-vitro* bovine tissues might differ from human tissues, potentially leading to different safety thresholds. Secondly, the responses to thermal stress can vary between tissue types (bovine tongue vs human mucosa), which could affect the thermal laser effects. Acknowledging this limitation, the logical progression for our research is to conduct *in vivo* studies in a controlled clinical setting. Such studies would enable us to observe the effects of the 445 nm diode laser on human tissues, considering the complex interplay of factors present in a live oral

environment. Variables like saliva flow, blood perfusion, and the unique thermal conductivity of human mucosa would provide a more accurate representation of the laser's effects. Moreover, it would be beneficial to explore the differences in outcomes between initiated and non-initiated tips in a clinical setting. While our study indicates a difference in thermal effects between the two, the actual impact on treatment outcomes, patient comfort, and tissue healing needs to be assessed in vivo. This would also allow for the evaluation of real-time temperature monitoring and the effectiveness of cooling techniques in preventing overheating, thereby optimizing laser parameters for safe and efficacious periodontal treatments.

Our study serves as a preliminary investigation, highlighting the potential for tissue overheating using initiated laser tips and the importance of understanding laser-tissue interactions. The transition to in vivo human studies is crucial for the development of evidence-based protocols that ensure the safe use of diode lasers in dentistry. Clinicians must remain cognizant of the findings from such research to refine their practice and safeguard against adverse outcomes while harnessing the benefits of advanced laser technology.

Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

Data available on request from the corresponding author upon reasonable request.

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