




Optimizing Settings for Office-Based Endoscopic CO₂ Laser Surgery Using an Experimental Vocal Cord Model

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Objectives/Hypothesis: To provide insight in the thermal effects of individual laser settings in target tissues to optimize flexible endoscopic CO₂ laser surgery treatment.

Study Design: Experimental laboratory study.

Methods: Thermal effects of the CO₂ laser using a fiber delivery system were visualized using the color Schlieren technique in combination with a polyacrylamide gel tissue model. Variable settings were used for emission mode, power, laser fiber distance, and laser duration, which were evaluated in every possible combination. Collateral thermal expansion and incision depth were measured. To validate the model, the results were compared to histology after CO₂ laser irradiation of ex vivo human vocal cords, and the intraclass correlation coefficient was calculated. Thermal damage and incision depth were measured by a blinded pathologist.

Results: Of all parameters studied, duration of laser irradiation had the greatest effect on thermal expansion. Increased distance between laser tip and target tissue resulted in significantly reduced incision depth and increased thermal expansion. Pulsed emission modes led to increased incision depths. The intraclass correlation coefficient for consistency between the model setup and the ex vivo human vocal cords was classified as "fair."

Conclusions: By using high-intensity pulsed lasers at minimal distance to the target tissue, exposure times and subsequent damage to surrounding tissue can be reduced. If an evaporation technique is used, lower power in continuous wave at a larger distance to the target tissue will lead to superficial but broader thermal effects. The model setup used in this study is a valid model to investigate laser-induced thermal effects in vocal cord tissue.

Key Words: Flexible endoscopic CO₂ laser, vocal cords, thermal effects.

Level of Evidence: NA

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INTRODUCTION

Office-based flexible endoscopic laser surgery using a CO₂ laser for laryngeal or pharyngeal lesions is a procedure that is increasingly performed.¹ The advent of distal chip-on-tip flexible laryngoscopes and the development of flexible instruments (e.g., flexible laser fiber delivery systems and biopsy forceps) has made it possible to perform several

laryngeal procedures in an office setting. This development has shortened procedure duration, reduced delay in diagnosis and treatment, lowered costs, and reduced complication risks compared to surgery under general anesthesia.² Laryngeal laser surgery is one of the procedures traditionally performed under general anesthesia but can now be performed under local anesthesia using a flexible endoscope. Laser irradiation of soft tissue inherently produces thermal effects on surrounding healthy tissue in the form of both reversible (altered tissue that will heal) and irreversible (carbonization and necrosis) damage.³

Ideally, lasers used in vocal cord surgery cause minimal collateral thermal damage to surrounding tissues, penetrate only superficially, are able to be used for excision and coagulation, and have a flexible delivery system.⁴ A recent review reported that, currently, no scientific evidence exists on the optimal laser settings in laryngeal procedures; recommendations are usually provided by the laser manufacturer based on empirical evidence.² Few studies were published investigating thermal effects of CO₂ lasers, either using a fiber^{5–7} or an articulated arm delivery system.^{3,8–13} However, none of these studies performed a systematic and complete analysis of the effects of individual CO₂ laser settings. Hence, the exact effect of adjusting laser settings on thermal energy produced by the laser, the depth of laser incision, and the thermal damage to surrounding tissue remains unknown.

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Because office-based laryngeal laser surgery is a novel technique, and experience in tissue effects is still limited, the aim of this study was to compare the collateral thermal expansion and incision depth after irradiation using a CO₂ laser delivered through a fiber with different laser settings. These results can be used to adjust settings depending on the desired surgical effect. For evaporation, a more superficial and broad thermal effect is required, whereas for cutting, a concentrated beam with minimal collateral thermal damage is appropriate. Experiments were performed on a human tissue model, and thermal effects were visualized using the dynamic Schlieren imaging technique.¹⁴ The ability of this imaging technique to predict laser-induced thermal damage in vocal cord tissue was evaluated by comparing the results with conventional histological evaluation of fresh ex vivo human vocal cords irradiated using the same laser settings.

MATERIALS AND METHODS

Laboratory Laser Setup

A CO₂ laser (AcuPulse Duo; Lumenis, Yokneam, Israel) with a wavelength of 10.6 μm was used to perform the experiments. The laser was fitted with a hollow fiber wave guide (500 μm, FiberLase; Lumenis). The laser was tested in various settings: in continuous wave (CW), pulsed (P), and super pulsed (SP) modes, using a power of 4, 6, 8, and 10 W, a laser duration of 1, 2, and 3 seconds, and a distance between laser tip and tissue of 2, 5, 10, and 15 mm. These settings were chosen as they were deemed to be representative of the intraoperative conditions.

Dynamic Color Schlieren Imaging Technique

The model used in this study is based on the color Schlieren technique, which allows for the visualization of a temperature gradient in a transparent medium.¹⁴ The gradient seen is the result of

changes in refraction index of the medium as the temperature of the medium rises. In this study, a polyacrylamide (PA) phantom gel was used as the transparent medium.¹⁴ PA gel was chosen as a model tissue as it has similar thermal, optical, mechanical, and electrical properties compared to human tissue.¹⁴ A schematic figure of the model is shown in Figure 1. At one end of the setup, a light-emitting diode (LED) light source was positioned. The light beam emitted by this LED was concentrated using a pinpoint aperture before traveling through a telephoto lens (>400 mm, infinite focus) creating a parallel beam. The parallel light rays passed through the PA gel placed on a plateau perpendicular to the light beam. The flexible laser fiber was positioned directly above the gel using a weighted stand with a wire holder enabling it to be set at variable distances to the gel. After the light rays passed through the gel, a second, decollimating lens (135 mm) focused the light onto a rainbow Schlieren filter consisting of multicolored concentric rings. The filtered light was captured by a digital single-lens reflex (DSLR) camera at 50 frames per second with 400-mm zoom and infinite focus.

The experimental model is based on the premise that, if the gel has a constant temperature, and thus a constant refraction index, the light beam will remain parallel and most of the light will be blocked by the dark center of the filter. However, irradiation of the gel leads to a temperature gradient away from the incision, causing a gradient in refractive index of the heated gel. As a result, the parallel light beams passing through the gel are refracted at different angles and are no longer blocked by the dark center of the Schlieren filter. Instead, light beams are deflected through different color rings of the Schlieren filter, resulting in a color gradient in the image captured by the DSLR camera. In this model, a large temperature difference, and thus a large refraction index, results in orange- and red-colored images (outer rings of the filter), whereas small temperature changes produce blue or green images (inner rings of the filter).

Experiments in PA Gel

Real-time thermal Schlieren images of the gels were obtained using the aforementioned settings (Fig. 2). Each combination of

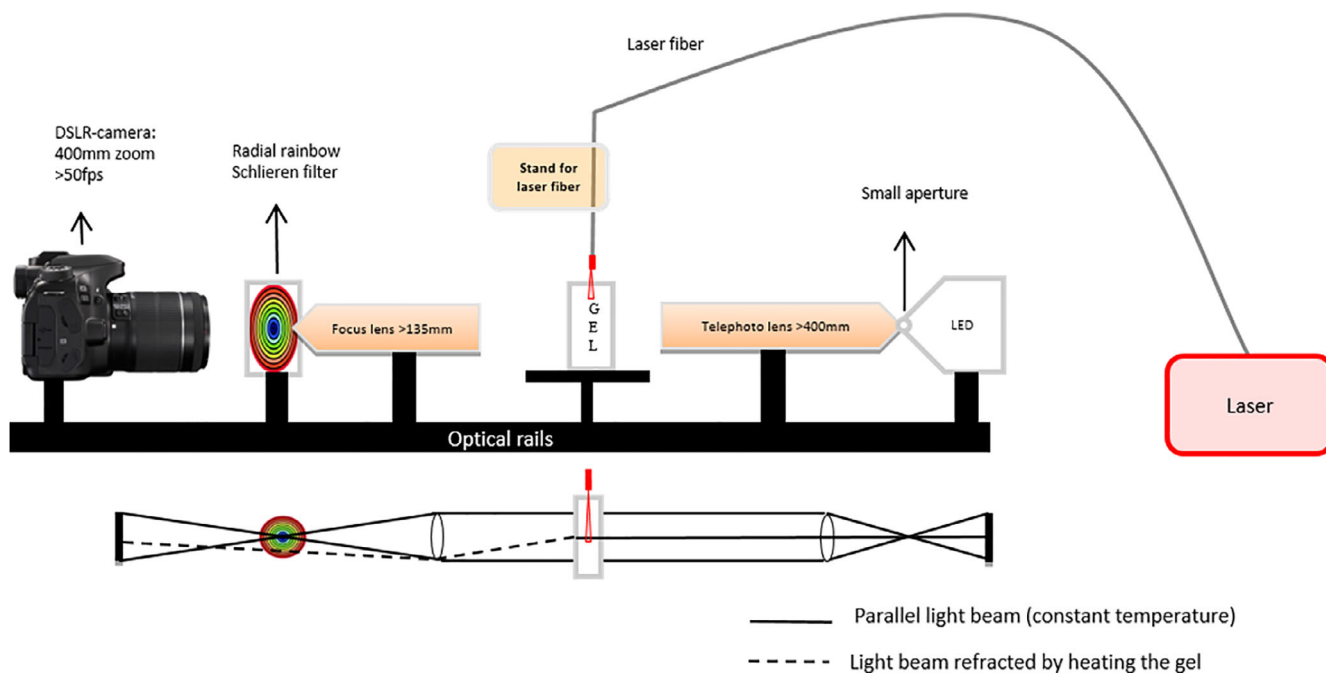


Fig. 1. Schlieren model and path of light-emitting diode (LED) light beam. DSLR = digital single-lens reflex.

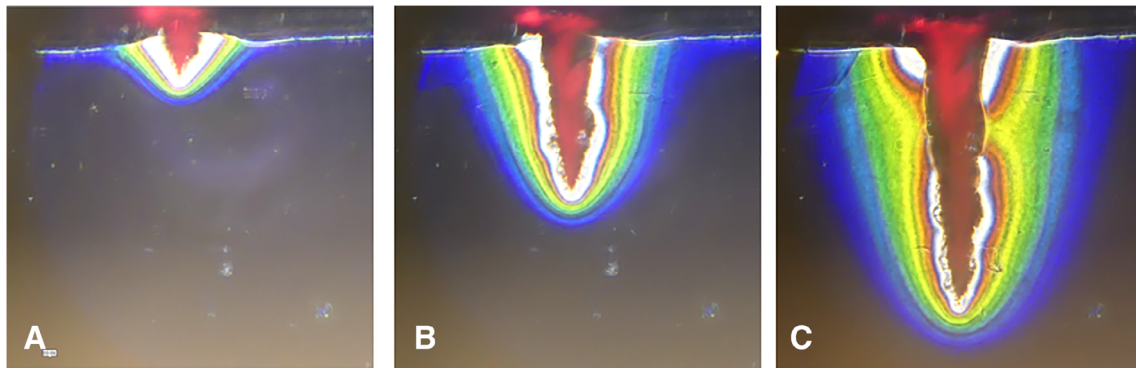


Fig. 2. Schlieren image during irradiation of the polyacrylamide gel using the AcuPulse CO₂ laser. Settings: continuous wave, 10-mm distance laser tip to gel, 4 W. (A) One second. (B) Two seconds. (C) Three seconds.

settings was measured in triplicate. After 1, 2, and 3 seconds, the recording was interrupted and FLJI imaging software (ImageJ; National Institutes of Health, Bethesda, MD)¹⁵ was used to measure the thermal effects caused by the laser beam (i.e., the extent of thermal expansion surrounding the incision as well as incision depth). The thermal expansion in the gel was defined as the distance from the edge of the incision to the end of the color gradient measured at a depth of 1 mm. In images with an incision depth of <1 mm but with visible thermal effects at 1 mm beneath the gel surface, thermal expansion was measured as the distance from the center of the color gradient to the end of the gradient in the horizontal axis. The incision depth was measured as the distance between the surface of the gel and the deepest point of the incision. If the incision depth exceeded the limits of image captured by the camera, the incision depth was not recorded, as no accurate measurements could be made for these settings.

Experiments in Vocal Cord Tissue

To validate the aforementioned model, experiments were conducted on ex vivo tissue samples using the CO₂ laser with a fiber delivery system. Twelve larynges obtained from fresh-frozen human cadavers of donors who in life had bequeathed their bodies to the Anatomical Institute at the Radboudumc for academic purposes with a valid handwritten testament, were used to carry out this study. The cadaver specimens were obtained according to the Dutch Body Donation Program for Science and Education (Wet op de lijkbezorging, 1991). The larynges were stored at -5°C to prevent tissue degradation. One day before the experiments took place, larynges were thawed to room temperature. We decided not to warm the larynges to body temperature (37°C), as this led to tissue degeneration. On the day of the experiments, the vocal cords were dissected from the larynges and pinned on a paraffin plate while retaining their natural shape. Subsequently, the vocal cords were irradiated in CW, P, and SP mode, with a power of 6 W and 10 W, a duration of 1 and 3 seconds, and a distance of the laser tip of 2 and 10 mm to the tissue. A total of 24 incisions were made in 12 pairs of vocal cords.

Histology

After irradiation, the incision was stained with black ink, and the tissue was fixed in 4% formaldehyde solution. After 72 to 96 hours, the tissue was serially dehydrated in ethanol baths. The samples were embedded in paraffin and sectioned into 6- μ m coronally cut microsections. The sections were stained with hematoxylin and eosin. Thermal expansion in each section, considered the distance from the edge of the incision to the end of the thermal

damage measured at a depth of 0 mm to 1 mm below the epithelium, was evaluated by a pathologist blinded to the laser settings (Fig. 3). Incision depth was also measured and recorded. A photographic file was compiled with microscopic images.

Statistical Analysis

The effect of the four laser settings investigated in all possible combinations in the gel on collateral thermal expansion and incision depth was analyzed using multivariable linear regression. Both unstandardized β coefficients (B) and standardized β coefficients (β) were calculated. B coefficients represent the increase or decrease in dependent variable (i.e., incision depth in millimeters when an independent variable such as laser duration is increased by one unit). β coefficients (minimum value -1 and maximum value +1) were calculated to compare the relative effect of the different independent variables. A β of -1 represents a direct negative correlation, whereas a β of +1 represents a direct positive correlation. To evaluate the ability of our model to correctly predict laser-induced thermal damage in vocal cord mucosa, the intraclass correlation coefficient (ICC) for both incision depth and thermal expansion was determined between the

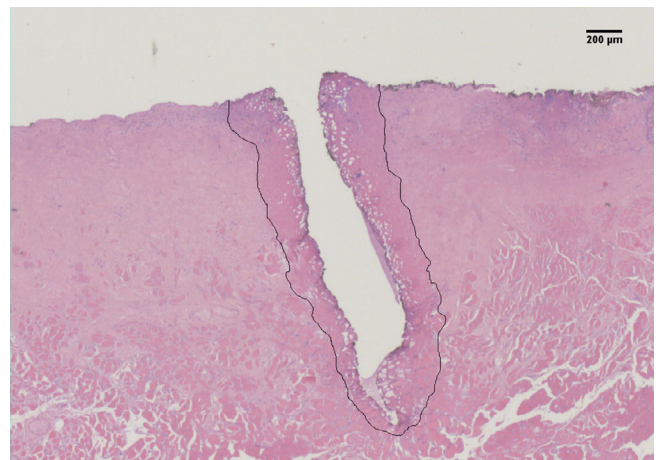


Fig. 3. Hematoxylin and eosin-stained histological vocal cord sample after irradiation (AcuPulse CO₂ laser, CW, 5 mm, 6 W, 1 second). The black line denotes the extent of collateral thermal damage.

mean of the results acquired using the model and the results from histological analysis. Both the ICC for absolute agreement and the ICC for consistency between the model setup and histological evaluation were obtained. Incisions through the entire vocal cord were not included in the analysis, as no accurate measurements could be made for these settings. The data analysis was performed using SPSS version 25 (IBM, Armonk, NY) for Windows (Microsoft, Redmond, WA). *P* values <.05 were considered to be statistically significant.

RESULTS

Laser Parameter Evaluation

A total of 432 experiments were performed (three pulse modes, three laser durations, four wattages, and four distances, with three repetitions per combination of settings). Incision depths exceeding the Schlieren image frame captured by the camera were observed in 153 of the experiments, and these were excluded from the analysis.

Linear regression analysis (Table I) demonstrated that prolonged laser duration was strongly related with increased thermal expansion ($B = 0.279$, $\beta = 0.848$, $P < .005$). A B of 0.279 represents an increase in thermal expansion of 0.279 mm in the tissue, when laser duration is increased by 1 second. Furthermore, increased laser power and distance between laser tip and gel led to a small but significant increase in thermal expansion ($B = 0.020$, $\beta = 0.167$, $P < .005$ and $B = 0.009$, $\beta = 0.170$, $P < .005$, respectively); increasing laser power by 1 W would result in an increase in thermal expansion of 0.02 mm, whereas increasing laser distance by 1 mm would result in an increase of 0.009 mm. Additionally, using the CO₂ laser in P and SP modes would result in an increase in thermal expansion by 0.043 and 0.046 mm, respectively, compared to CW mode ($B = 0.043$, $\beta = 0.076$ and $B = 0.046$ and $\beta = 0.082$, $P < .005$, respectively).

Effects of different settings on incision depth were evaluated by linear regression analysis. The parameter with the greatest effect on incision depth was laser distance; increasing the distance between laser tip and surface of the gel by 1 mm would result in a decrease of incision depth by 0.241 mm ($B = -0.241$, $\beta = -0.722$, $P < .005$). Increased power and laser duration resulted in significantly deeper incisions

	Laser Setting	B	β
Thermal expansion (mm)	Power (W)	0.020*	0.167*
	Laser duration (sec)	0.279*	0.848*
	Laser distance (mm)	0.009*	0.170*
	Pulse	0.043*	0.076*
	Super pulse	0.046*	0.082*
Incision depth (mm)	Power (W)	0.445*	0.612*
	Laser duration (sec)	1.031*	0.531*
	Laser distance (mm)	-0.241*	-0.722*
	Pulse	1.728*	0.471*
	Super pulse	0.793*	0.239*

* $P < .005$.

B = unstandardized β coefficient; β = standardized β coefficient.

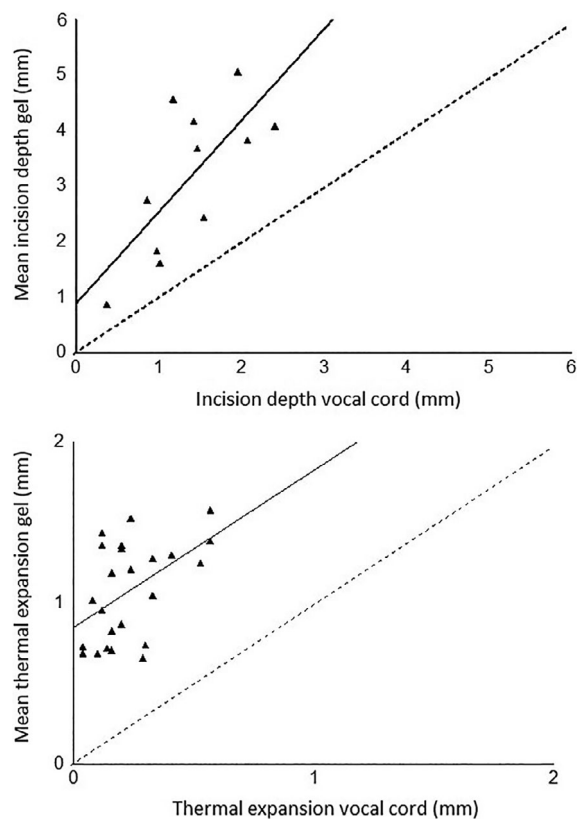


Fig. 4. Scatter plots comparing the incision depth and thermal expansion in gel and vocal cord tissue. The dashed line represents $y = x$, the solid line represents the trend line through the measurements.

($B = 0.445$, $\beta = 0.612$, $P < .005$ and $B = 1.031$, $\beta = 0.531$, $P < .005$, respectively); increasing laser power by 1 W would result in an increase in incision depth of 0.445 mm, whereas increasing laser duration by 1 second would result in an increase of 1.031 mm. P and SP modes would result in an increase in incision depth of 1.728 mm and 0.793 mm, respectively, compared to CW mode ($B = 1.728$, $\beta = 0.471$, $P < .005$ and $B = 0.793$, $\beta = 0.239$, $P < .005$, respectively).

Gel Model Evaluation

The incision depth and collateral thermal expansion in both the gel model and the vocal cords are plotted in Figure 4. The ICC for absolute agreement and consistency for incision depth were 0.221 and 0.534 ($P = .037$), respectively. The ICC for absolute agreement and consistency of thermal expansion were 0.058 and 0.462 ($P = .010$), respectively. The values for consistency of incision depth and thermal expansion were classified as fair, using Cicchetti's classification system for interpreting ICC.¹⁶

DISCUSSION

Office-based, flexible endoscopic laser surgery is a promising procedure for the treatment of laryngeal lesions with many potential benefits as an alternative to surgery under general anesthesia.^{1,2,4,17-20} As the use of

this technique slowly increases, it is essential to have knowledge of the thermal effects that individual laser parameters have on vocal cord tissue. An understanding of these effects can assist surgeons in deciding which laser parameter to adjust to achieve the intended surgical effect. This is the first study to systematically describe the thermal effects of a CO₂ laser using a flexible fiber delivery system. By comparing the experimental gel model we used in our study to histological evaluation of *ex vivo* irradiated vocal cords, we demonstrated that, although there does not seem to be absolute agreement, there is a fair level of agreement for consistency. This suggests the tissue model can be used as an alternative to human or porcine vocal cords to interpret the influence of laser settings on thermal effects observed in target tissues.

The systematic experiments performed using this model revealed that of all studied parameters, laser duration had the greatest influence on collateral thermal expansion. Additionally, laser duration was significantly related to incision depth. Our study results further indicate that of all laser parameters, power intensity had the greatest positive effect on incision depth. The effect of increasing power on thermal expansion appeared to be relatively small, but significant. Previously, two studies assessed the thermal effect of flexible CO₂ laser delivery systems, albeit in only a small selection of setting combinations, in which a trend of increasing thermal damage was seen when using the laser at higher power.^{3,7} For incision depth, Wang et al. did not find a difference by altering power settings,⁷ whereas Wilder-Smith et al.³ did find increasing incision depths at higher power. The effects of the other parameters have never been evaluated using a flexible fiber delivery system.

By comparing our results of a flexible fiber delivery system with results of previous studies testing CO₂ lasers with a conventional articulated arm or handpiece, we found comparable outcomes. Although in previous studies on this topic the extent of the tested laser setting combinations was limited, a correlation between laser power and incision depth had been described earlier.³ Additionally, three studies^{3,8,12} also revealed a positive correlation between laser power and thermal damage. In contrast, two other studies did not find a relation between laser power and thermal damage.^{10,11} In a study evaluating the surgical margin after CO₂ laser surgery in T_{1s}, T1, and T2 glottic carcinoma, Buchanan et al. even observed an inverse relationship between power and thermal tissue damage.⁹ Remarkably, none of these studies considered laser duration. Buchanan et al. proposed that this inverse correlation could be an indirect effect; using laser irradiation at higher power intensities led to an increase in cutting speed and therefore shorter procedural time, thereby leading to less thermal damage.⁹ The results of our study emphasize laser duration is a very important parameter to consider in thermal effects of CO₂ lasers.

This was the first study to assess the influence of the distance between laser and target tissue on thermal effects. Laser distance is a parameter that is of interest only in flexible laser surgery, as laser light emitted by an articulated arm or handpiece is a parallel beam that

delivers energy to target tissue independently of the distance. A strong, negative correlation was observed between the incision depth and the laser distance to the gel. This implies using the laser closer to the target tissue results in significantly deeper incisions. This is important to consider when performing office-based procedures using a flexible fiber, as the distance to the target tissue can vary with the movements of the larynx.

Furthermore, CO₂ lasers in (super) pulsed modes led to significantly deeper incisions compared to irradiation in continuous wave mode, with only a minimal effect on thermal expansion. As laser duration was found to have the most prominent effect on thermal expansion of all laser parameters, it may be beneficial when excising vocal cord lesions to use (super) pulsed modes with high-power intensities to reduce exposure times and thereby reduce unnecessary thermal damage. Similar advice is given in the study by Yan et al., who recommended using the shortest possible time pulse together with the highest possible power to accomplish a procedure with minimal lateral thermal spread.²¹

Due to the limited amount of larynges available for the comparison between the model setup and vocal cord tissue, we chose only to investigate the CO₂ laser, as this is currently the most frequently used laser in our center. Because other lasers, such as the potassium titanyl phosphate laser, have different wavelengths and absorption characteristics, our results cannot be extrapolated directly to these laser types. Additional research must be done using other lasers to compare the effects observed using this model.

One of the limitations of the current study is the large amount of missing data for the gel experiments caused by the depth of the incision exceeding the Schlieren image frame, which could not be resolved by adjusting the setup. Furthermore, the number of experiments on human vocal cords was limited due to scarcity of available larynges and due to the fact that several incisions penetrated the entire vocal cord depth, making it impossible to acquire exact measurements. Had our measurements been complete, ICC values might have been higher. For this study, human fresh frozen vocal cords were used to mimic the clinical setting as accurately as possible. However, the tissue properties of these *ex vivo* samples that were frozen at -5°C and subsequently thawed might differ from *in vivo*, well-perfused vocal cord mucosa with a temperature of 37°C, possibly affecting the results. The water content of the vocal cord samples may also differ, which could influence the absorption rate of the CO₂ laser and therefore reduce the ablation rate. Despite these limitations, this study remains the first to systematically investigate laser settings to this extent, and the results can be used to gain understanding into the thermal effects caused by these settings.

CONCLUSION

Laser duration was found to be the leading parameter influencing collateral thermal expansion in the human tissue model when using a CO₂ laser with a flexible fiber delivery system. Depending on the desired surgical effect (i.e., evaporation or cutting), laser parameters can be adjusted on the basis of our results. Office-based laryngeal

laser surgery is mainly used for lesions that require evaporation as opposed to excision. The results suggest that this can best be achieved by irradiating at greater distance to the tissue, with low-power intensities and in CW mode, thereby reducing the incision depth and accomplishing broader thermal effects. In contrast, when the laser is applied for cutting thicker lesions, using high-power (super) pulsed modes at minimal distance to the target tissue can reduce exposure time and subsequent damage to surrounding tissues. Effects seen in the dynamic color Schlieren setup in combination with the tissue model used in this study demonstrate a fair level of consistency with results in ex vivo vocal cords. This indicates that this model can be used to investigate the influence of individual laser parameters on the thermal effects caused by flexible CO₂ laser therapy in vocal cord tissue.

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