PULSED EXPOSURE MODE OF THE 445 NM SEMICONDUCTOR LASER IN PHONOSURGERY: AN EXPERIMENTAL STUDY

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Abstract

The study presents the results of an experimental study devoted to the choice of the most optimal mode of pulsed contact laser exposure of semiconductor laser with a wavelength of 445 nm in phonosurgery, which implies maximum preservation of anatomically and functionally significant structures of the larynx combined with a radical approach to the pathological process. From the standpoint of the mucoundular theory of voice formation, wave-like oscillations of the vocal folds are possible due to the mobility of the cover layer of the vocal fold (epithelium, superficial layer of the lamina propria) relative to its body (deep layer of the lamina propria, vocal muscle). Thus, any injury at the level of the integumentary layer is associated with the risk of excessive scarring and loss of the ability to wave-like sliding. Pig vocal folds, according to a number of authors, have a structure similar to human ones in terms of both histological structure and acoustic parameters, which justifies the rationality of their use as an experimental model. In a series of experiments using a 445 nm laser, contact pulsed impacts on a biological model were carried out with pulse durations of 10, 20, 50, and 100 ms, followed by evaluation of the following parameters based on the data of histological sections: the depth and width of the ablation crater, the width of the zone of lateral thermal damage. Thus, the most optimal for phonosurgical interventions modes of pulsed laser exposures with a wavelength of 445 nm are described.

Key words: phonosurgery, laser 445 nm, dysphonia, laser surgery, laryngology.

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ИМПУЛЬСНЫЙ РЕЖИМ ВОЗДЕЙСТВИЯ ПОЛУПРОВОДНИКОВОГО ЛАЗЕРА С ДЛИНОЙ ВОЛНЫ 445 НМ В ФОНОХИРУРГИИ: ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ

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Резюме

В работе представлены результаты экспериментального исследования, посвященного выбору наиболее оптимального режима импульсного контактного лазерного воздействия полупроводникового лазера с длиной волны 445 нм в хирургии голосовых складок (фонохирургии). Эндоларингеальная фонохирургия подразумевает собой максимальную сохранность анатомически и функционально значимых структур гортани в сочетании с радикальностью в отношении патологического процесса. С позиции мукоундулярной теории голосообразования волнообразные колебания голосовых складок возможны за счет подвижности покровного слоя голосовой складки (эпителий, поверхностный слой собственной пластинки) относительно ее тела (глубокий слой собственной пластинки, голосовая мышца). Таким образом, любая травматизация на уровне покровного слоя сопряжена с риском его избыточного рубцевания и потерей способности к волнообразному скольжению. Голосовые складки свиньи, по данным ряда авторов, имеют схожее строение с человеческими как по гистологическому строению (толщина слоев, соотношение коллагеновых и эластических волокон), так и по акустическим параметрам, что обосновывает рациональность их использования в качестве экспериментальной модели. В серии экспериментов с использованием лазера 445 нм проведены контактные импульсные воздействия на биологическую модель с длительностью импульсов 10, 20, 50 и 100 мс с последующей оценкой по данным гистологических срезов следующих параметров:

глубина и ширина кратера абляции, ширина зоны бокового термического повреждения. Таким образом, описаны наиболее оптимальные для фонохирургических вмешательств режимы импульсных воздействий лазера с длиной волны 445 нм.

Ключевые слова: фонохирургия, лазер 445 нм, дисфония, лазерная хирургия, ларингология.

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Introduction

Benign neoplasms of the larynx are the most common pathology of the larynx, leading to persistent dysphonia, which leads to a decrease in the quality of life and often a loss in the productivity of professional activity. Treatment of this pathology is possible only with the use of surgical techniques.

Endolaryngeal phonosurgery implies the maximum preservation of anatomically and functionally significant structures of the larynx, combined with a radical approach to the pathological process. The maximum preservation of structures involves, first of all, the integumentary layer of the vocal fold. From the standpoint of the biomechanics of the vibrational oscillations necessary for the emergence of a voice, the structure of the vocal folds is divided into the so-called "integumentary layer" and "body". The mucous membrane (the epithelium and the surface layer of the lamina propria) is the "integumentary layer" and represents a single morphofunctional unit capable of self-sustaining oscillations relative to the "body", which is formed by a deep layer of the lamina propria lying on the vocal muscle, while the middle layer of the lamina propria is designated as "transition zone". Later, some different divisions were proposed, the differences in which mainly relate to the position of the intermediate layer of the lamina propria, although in any case, the main idea is that the "cover" and "body" have different biomechanics [1]. The surface layer of the lamina propria contains the least amount of fibrillar proteins (collagen and elastin), which determines its high mobility. Thus, any trauma at this level and stimulation of fibroblast activity can lead to excessive scarring and limitation of mobility of the vocal fold integumentary layer.

In phonosurgery for benign lesions of the vocal folds, it should always be taken into account that surgical tissue incision, excision, or ablation may themselves cause excessive tissue scarring as a consequence of its healing [2]. Thus, the surgeon is always faced with the task of minimizing trauma to the tissues of the vocal folds during surgery, which confirms the validity of the search and introduction into practice of new, more gentle, methods of phonosurgical interventions [3].

Materials and methods

In the experimental part of the study, an assessment was made of the impact of 445 nm semiconductor laser radiation on a biological tissue model. The *ex vivo* vocal folds of pigs (Sus scrofa domesticus) were used as a biological model, and were collected within 2 hours after the humane death of animals in the slaughterhouse and then stored at a temperature of 2°C to avoid biological tissue degradation until the experiment.

For laser irradiation, an IPG Photonics laser system (Russia) with a wavelength of 445 nm was used. A reusable nonsterile fiber instrument IPG Surgical Fiber Reusable (Russia) with a core diameter of 400 μm was used as an optical fiber.

The experiment was carried out after preliminary natural warming of the biological tissue to room temperature. During the experiment, a semiconductor laser with a wavelength of 445 nm was used in a pulsed mode by applying individual point laser effects in the contact mode at a maximum power of 13 W with individual pulse durations of 10, 20, 50, and 100 ms. For each pulse mode, 10 separate laser exposures were performed along the medial edge of one vocal fold. Thus, 5 swine larynxes were used in the experiment: 4 of which were for the experimental part, and 1 – as a control.

After irradiation was completed, the biological tissue samples were dissected (Fig. 1), then the material was fixed in 10% buffered neutral formalin solution for 48 hours (the ratio of the fixative and the studied samples was 10:1). Afterwards the biological material was cut into plates 5 mm thick and fit into histological cassettes. Next, the sections were decalcified in an acid solution (the ratio of the decalcifier and the samples under study was 50:1) and standard alcohol wiring was embedded in paraffin according to the generally accepted method [4]. Then, sections were made from the blocks with a thickness of 2 μm on a Leica DM1000 light microtome (Germany), followed by their staining with hematoxylin and eosin according to the standard method. Then, histological sections were digitized using an Aperio AT2 scanning microscope (Germany). Morphometry was performed using an Aperio ImageScope 12.4.6.5003 image analyzer. Due to the high power density in the zone of contact between the fiber and the tissue surface, a tissue destruction (ablation) site (crater) is formed, the diameter and depth of which were measured in the experiment, as a result of which ranges of damage values were obtained for each pulse duration. The area of laser exposure was considered to be an optically void area in which evaporation of normal histological structures occurred and interruption

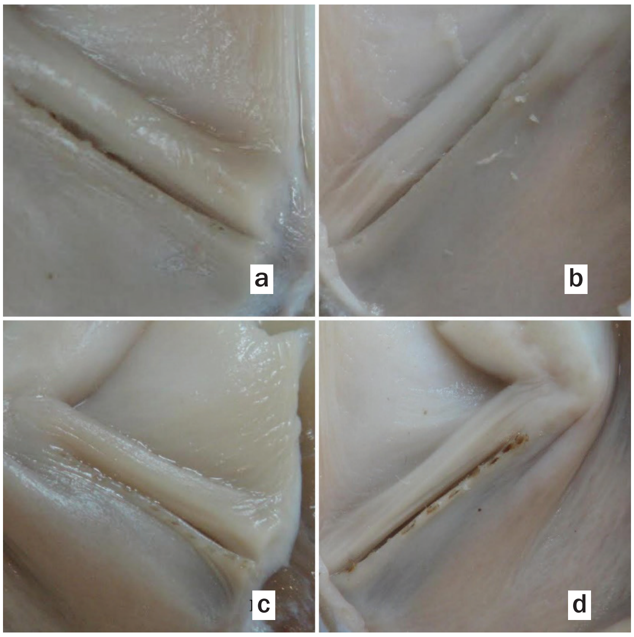


Рис. 1. Макроскопические препараты голосовых складок свиней после лазерного воздействия (a – 10 мс, b – 20 мс, c – 50 мс, d – 100 мс).
Fig. 1. Macroscopic preparations of the pig vocal folds after laser exposure (a – 10 ms, b – 20 ms, c – 50 ms, d – 100 ms).

of normal stratified squamous nonkeratinized epithelium with the formation of a specific ablation crater was objectively noted, as well as tissue adjacent to the crater (lamina propria, vocalis muscle) with signs of thermal damage in the form of a violation of the nuclear structure and disorientation of the course of elastic and collagen fibers.

Statistical processing of the results was carried out on the Jupyter Notebook platform using Python 3.9 with correlation calculation using Spearman's (r) coefficient.

Results and discussion

The gold standards of phonosurgery are interventions using cold microinstruments and CO_2 laser. Speaking about classical "cold" phonomicrosurgery, despite the active introduction of minimally invasive techniques (microflap, mini-microflap), it is worth noting that for many benign lesions of the vocal folds, the volume of surgery is calculated in micrometers, which makes it difficult for the surgeon to calculate the accuracy of his actions using microsurgical scissors, scalpel, and other instruments. Thus, the trauma of the tissue of the vocal folds associated with the "cold" intervention is potentially more pronounced due to the large size of the instruments compared to the necessary sizes in the micron range for radicalization in relation to the pathological process.

As mentioned above, with regard to the most gentle restoration of the vibrational oscillations of the vocal fold during phonosurgery, it is important to perform intervention within the epithelium and the surface layer of the lamina propria, which will subsequently avoid excessive scarring and impaired mobility of the cover layer of the fold relative to its body. According to a number of publications, the surface layer makes up about 30–40% of the entire depth of the lamina propria, the thickness of which, in turn, is 1 mm on average [5, 6, 7]. The depth of the epithelium of the true vocal fold, in this case, is about 80–100 µm [8] and, thus, the depth of the cover layer of the vocal fold is on average 400–500 µm, within which phonosurgical intervention is allowed to avoid binding of the scar tissue to the body of the vocal fold and mobility restrictions.

Several researchers believe that in pigs it can be distinguished a tendency to a similar three-layered structure of the lamina propria with a similar ratio of collagen and elastic fibers throughout the entire depth of the lamina propria. The thickness of the mucous membrane of the vocal folds of pigs is on average 0.9 mm [9]. According to the acoustic analysis of the natural phonation of animals, it was found that the range of phonation frequencies in pigs is the closest to that in humans [10,11].

Despite the anatomical and histological advantage of porcine vocal fold models and the rationality of their use as a scientific model for testing surgical techniques (due to their easy availability in the slaughterhouse, without the need to sacrifice animals for research purposes), there are currently a small number of publications in which this model is evaluated from the point of view of laser exposure, which is used in human vocal fold phonosurgery.

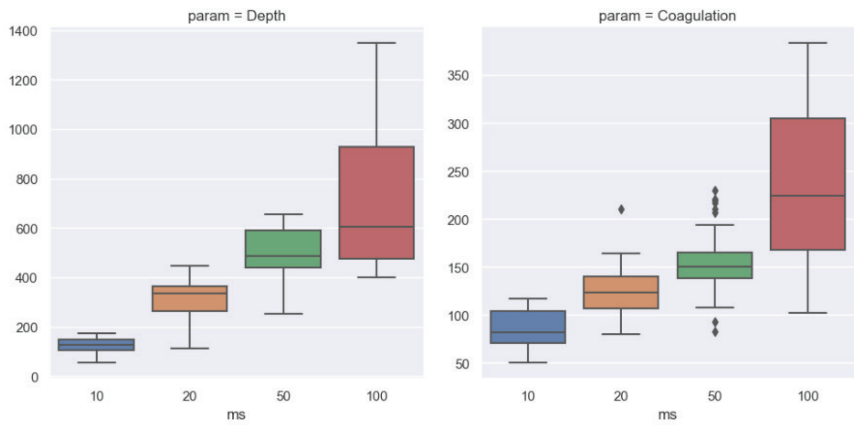
In none of the articles, we found a histological assessment to analyze point laser effects on biological tissue using laser radiation with a wavelength of 445 nm on the vocal folds of laboratory biological models, which determines the relevance of our experimental work.

When performing point pulsed laser actions using a laser with a wavelength of 445 nm, an increase in the pulse duration leads to an increase in the depth of the ablation crater and an increase in the thickness of the lateral thermal damage zone (Fig. 2), while there was no significant increase in the width of the ablation crater (Fig. 3).

The data of the statistical analysis of the measurements are presented in Table. 1.

The Spearman correlation coefficient for the relationship between the pulse duration and the depth of the ablation crater was 0.81, for the thickness of the lateral thermal damage zone it was 0.74, and for the width of the ablation crater – 0.45 ($p<0.05$). It follows from the presented data that there is a relationship between the pulse duration and each of the estimated parameters and it is statistically significant. At the same time, this relationship is less typical for the width of the ablation crater, which can be explained by the physical properties of laser radiation, when, in the contact mode of exposure, radiation absorption mainly occurs deep into the biological tissue.

The presented histological sections clearly demonstrate an increase in the depth and width of the zone of



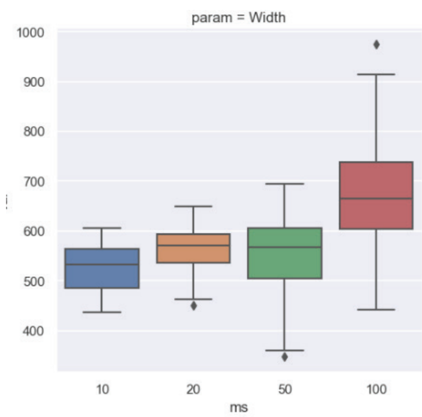


Рис. 2. Зависимость глубины кратера абляции в мкм (слева, ось y) и толщины зоны бокового термического повреждения в мкм (справа, ось y) от длительности импульса лазерного воздействия в мс (ось x).
Fig. 2. Dependence of the ablation crater depth in μm (left, y-axis) and the thickness of the lateral thermal damage zone in μm (right, y-axis) on the laser exposure pulse duration in ms (x-axis).

Рис. 3. Зависимость ширины кратера абляции в мкм (ось y) от длительности импульса лазерного воздействия мс (ось x).
Fig. 3. Dependence of the ablation crater width in μm (y-axis) on the laser exposure pulse duration in ms (x-axis).

Таблица 1
Зависимость глубины, ширины кратера абляции и зоны бокового термического повреждения от длительности импульсного лазерного воздействия с длиной волны 445 нм.
Table 1
Dependence of the depth, width of the ablation crater and the zone of lateral thermal damage on the duration of pulsed laser exposure with wavelength of 445 nm.

		Глубина кратера абляции, мкм The depth of the ablation crater, μm	Зона бокового термического повреждения, мкм The lateral thermal damage zone, μm	Ширина кратера абляции, мкм The width of the ablation crater, μm
10 мс 10 ms	Среднее значение (стандартное отклонение) Mean value (standard deviation)	124,8 (32,9)	85,2 (20,0)	526,6 (55,2)
	Мин; макс min; max	104; 148	71; 104	485; 563
20 мс 20 ms	Среднее значение (стандартное отклонение) Mean value (standard deviation)	312,5 (83,8)	126,8 (27,8)	564,0 (51,7)
	Мин; макс min; max	262; 365	107; 140	536; 593
50 мс 50 ms	Среднее значение (стандартное отклонение) Mean value (standard deviation)	498,6 (93,7)	152,4 (30,6)	548,7 (90,1)
	Мин; макс min; max	440; 592	138; 165	504; 604
100 мс 100 ms	Среднее значение (стандартное отклонение) Mean value (standard deviation)	730,3 (313,9)	235,7 (82,6)	673,4 (115,7)
	Мин; макс min; max	475; 926	168; 304	602; 737

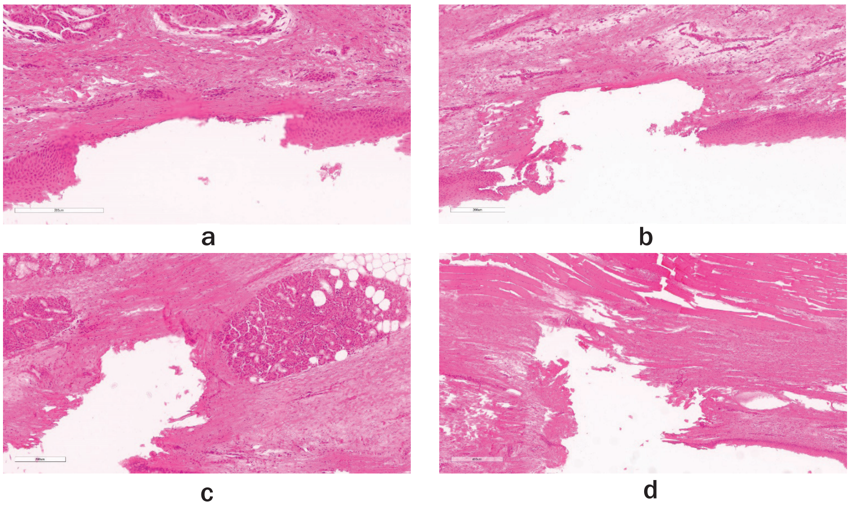


Рис 4. Микрофотографии гистологических срезов голосовых складок свиней после нанесенных лазерных воздействий, окраска гематоксилином и эозином: a – длительность импульса 10 мс, масштаб 200 мкм; ареактивный (без воспалительного инфильтрата) язвенный дефект с некрозом всех слоев плоскоклеточного эпителия; b – длительность импульса 20 мс, масштаб 200 мкм; очаговый некроз ¼ собственной пластинки слизистой оболочки; c – длительность импульса 50 мс, масштаб 200 мкм; очаговый некроз голосовой складки с распространением на 2/3 глубины собственной пластинки слизистой оболочки; d – длительность импульса 100 мс, масштаб 400 мкм; увеличение очага некроза за счет вовлечения большей площади эпителия и некроза 1/3 мышечных волокон голосовой складки.

Fig. 4. Micrographs of histological sections of the vocal folds of pigs after laser exposure, stained with hematoxylin and eosin: a – pulse duration 10 ms, scale 200 µm; areactive (without inflammatory infiltrate) ulcerative defect with necrosis of all layers of the squamous epithelium; b – pulse duration 20 ms, scale 200 µm; focal necrosis of the lamina propria and ¼ of the muscle fibers of the vocal fold; c – pulse duration 50 ms, scale 200 µm; focal necrosis of the vocal fold extending to 2/3 of the depth of the mucosal lamina propria; d – pulse duration 100 ms, scale 400 µm; an increase in the focus of necrosis due to the involvement of a larger area of the epithelium and necrosis of 1/3 of the muscle fibers of the vocal fold.

lateral thermal damage (Fig. 4, a-d). At the same time, it is noticeable that with a pulse duration of 100 ms, the damage zone overcomes all layers of the lamina propria and most of the muscle fibers of the vocal fold.

Conclusion

Thus, the pulse duration during phonosurgical interventions should be chosen directly by the surgeon, depending on the pathological formation of the vocal fold. In our opinion, the most optimal modes of laser exposure are radiation with a single pulse duration of 10 ms and 20 ms, which, with a high probability, will effectively remove epithelial or subepithelial formations within the surface layer of the lamina propria of the vocal fold. In cases of mass lesions on a wide base, a mode with a single pulse duration of 50 ms can be recommended, while a pulse duration of 100 ms should be avoided during phonosurgical intervention, given the high probability of laser radiation penetrating the entire thickness of the vocal fold proper.

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