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Optimizing Fiber Laser Ablation for Enhancing the Tumor Microenvironment in Neuroblastoma

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ABSTRACT Background: Neuroblastoma (NB) is a type of cancer that often affects children. Surgical manage-

to ensure thorough and effective treatment. The primary aim of our study is to develop and optimize a model for laser ablation specifically tailored for small NB tumors. **Methods**: Our research model involves graft tissue to test various characteristics after the fiber optic ablation process. These characteristics include the quality of laser beams, average power levels, and precise pulse timing. **Results**: The heated-laser ablation procedure employed a laser energy of 1.0 mJ and power of 1.0 kW. An optimal setting of a high-quality beam at 60 with 18.5 watts of power and a 2.5 ms pulse duration achieved an impressive tissue ablation rate of up to 95.8%. We also found that heatedlaser is more significant than frozen-laser ablation for the efficiency of enhancements, including an optimal power, a 2-fold decrease of pulse time, and an increase to induce apoptosis (cell death) in cancer cells and alters the collagen structure in the tumor microenvironment. **Conclusion**: The proposed model may help optimize the development of this combined treatment method for solid tumors, particularly in the design parameters of the graft tissue.

ment of smaller tumors (those less than 5 cm in size) poses unique challenges, making it difficult

Key words: thermal ablation, neuroblastoma, fiber laser, laser ablation

INTRODUCTION

Neuroblastoma (NB) is one of the most common solid tumors in infants and young children, which accounts for about 15% of deaths related to tumors in children^{1,2}. The adrenal gland is the most common primary location for NB (50%), while bone is the main metastatic site^{3,4}. About one-third of NB cases in children are smaller than 5 cm⁵. Though small tumors are considered to be less likely to metastasize, NB patients usually have poor prognosis even when they have gone through complete treatment⁶. Current NB treatment strategies include surgery, chemotherapy, radiation therapy, stem cell transplantation, and immunotherapy^{7,8}. However, the role of surgical resection in the treatment of primary tumors smaller than 5 cm remains controversial due to the lack of clear treatment strategies⁶.

Fiber laser is a type of laser that utilizes optical fibers as the active medium to amplify light. In fiber lasers, a laser beam is generated by passing high-intensity light through a specially designed optical fiber, which amplifies the light and produces a combined and focused beam. Fiber lasers have several advantages over traditional gas lasers and solid-state lasers, including higher efficiency, higher beam quality, and greater flexibility in beam shape and output power⁹. These characteristics make fiber optic lasers highly suitable for various industrial, medical, and scientific applications, including thermal tumor ablation ¹⁰.

Thermal ablation is a minimally invasive technique that has been recently used to destroy cancer cells within solid tumors such as liver, thyroid, and kidney tumors^{11,12}. During this procedure, a fiber optic laser beam is delivered into the tumor, and the laser energy is used to heat and destroy cancer cells¹³. Fiber optic lasers are particularly suitable for this application as they can deliver precise and controlled power to the tumor, minimizing damage to surrounding healthy tissues^{10,12}.

The high efficiency and beam quality of fiber lasers also enable faster and more effective tumor ablation ¹⁰. Additionally, fiber optic lasers can be used in conjunction with other imaging technologies, such as magnetic resonance imaging (MRI) or computed tomography (CT), to precisely guide the laser beam to the tumor and monitor the ablation process in realtime ¹⁴.

Generally, fiber laser is a promising technique for tumor thermal ablation, providing higher accuracy, efficiency, and flexibility than traditional laser systems. However, the optimization of this technique has not been extensively studied in pediatric tumors

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such as brain tumors, hepatoblastoma, neuroblastoma, Wilms tumor, etc. This study aimed to cultivate and create artificial pediatric brain tumor (PBT) models using actual pediatric PBT cell lines to investigate the properties of fiber optic lasers and the effectiveness of combined laser and cryo- thermal ablation methods in conjunction with the impact of modified collagen in the tumor microenvironment of PBT. The research primarily focused on optimizing the fiber optic laser technology on PBT tissues (measuring beam quality, high average power, and accurate pulse duration) to develop a new ablation system that is precise and less risky than surgery. The expected outcomes include minimal damage to the surrounding area and precise measurement of temperature and tumor injury level.

MATERIALS AND METHODS

Tissue preparation

Proper tissue preparation can help optimize the ablation process. For example, appropriate hydration of the tissue can improve thermal conductivity and, therefore, the efficiency of the thermal laser ablation process. An important characteristic to enhance is better thermal conductivity. Tissue preparation can improve the thermal conductivity of the tissue, which can enhance the effectiveness of the thermal laser ablation process. Dry tissue has lower thermal conductivity, which can result in uneven ablation and an increased risk of thermal damage to the surrounding tissues. Preparing the tissue with a saline solution can help improve thermal conductivity and minimize thermal damage.

Real-time tracking

Real-time monitoring of the tissue throughout the process can help adjust laser parameters and ensure optimal tissue ablation. Imaging techniques such as ultrasound or MRI can be used to guide the procedure and monitor the ablation process.

Operational principles of thermal generation in fiber laser

Understanding the mechanisms of cryo-ablation and thermal laser ablation and how they affect the treated tissue is crucial. This knowledge can help choose the appropriate technique for a specific tissue type and optimize the treatment parameters. The laser power can impact the speed and extent of tissue damage. Higher laser power can result in faster and deeper tissue ablation but may also increase the risk of thermal damage to the surrounding tissues. The appropriate laser power depends on the tissue type and the desired extent of tissue ablation. In laser devices, the power is typically maintained at a constant level. The selection of laser parameters: For thermal laser ablation, choosing the laser wavelength, power, and pulse duration can affect the depth and extent of tissue damage. Optimizing these parameters can help achieve the desired level of tissue ablation while minimizing damage to the surrounding tissues. Similarly, for cryoablation, optimizing laser parameters such as pulse duration, laser power, and cryogen spray parameters can help achieve optimal tissue ablation. The selection of laser parameters is an important aspect of optimizing the cryo-ablation and thermal laser ablation processes. The selection of laser parameters can impact the level and depth of tissue damage, as well as the efficiency and effectiveness of the ablation process. The wavelength of the laser beam can affect the depth of tissue penetration and the extent of tissue damage. Different wavelengths interact differently with different types of tissues, and choosing the appropriate wavelength depends on the tissue type and desired outcome of the procedure. For example, a wavelength of 532 nm is more absorbed by cancerous tissues, while a wavelength of 1064 nm is more absorbed by water-containing tissues.

RESULTS

High-quality beam optimization

We examined various configurations for the highquality beam (QB), gauging average power in watts. For each setting, we noted the percentage of tissue ablated. Throughout the study, we maintained a consistent pulse duration of 2.5 ms. Our findings (Table 1) suggest that elevating the QB settings correlates with an increase in average power and tissue ablation percentage. However, pinpointing the ideal configuration requires considering the specifics of the thermal ablation process and may necessitate further experimentation and assessment. To regulate the heat in the laser QB, the laser beam was fine-tuned to showcase variations in temperature distribution both with and without laser application. This revealed pronounced disparities, particularly along a diagonal trajectory, as depicted in (Figure 1). Amplifying the QB settings appeared to boost both the average power and the percentage of tissue ablated. Significant temperature distribution contrasts were evident between samples treated with and without the laser, with the former exhibiting a marked diagonal temperature pattern.



Figure 1: Comparative Heatmaps Illustrating Tissue Temperature Distributions in the Absence and Presence of Laser Ablation. Absence of Laser Ablation (left) and Presence of Laser Ablation with Gradient Laser Firing (right). Both the tissue depth and width are represented on the Y and X axes, respectively, with a range from 4 to 96 units.

Table 1: Optimization of high-quality beam with afixed pulse duration of 2.5 ms for thermal ablation onneuroblastoma tissue with n = 25

QB	Power (W)	Tissue ablated (%)
10	12.5	44.5
20	15.2	50.9
30	16.8	61.4
40	17.5	73.6
50	18.1	80.1
60	18.5	95.8

Real-time optimization

To measure the amount energy delivered to tissue and the depth of tissue penetration, we measure the pulse duration. The duration of the laser pulse could affect the amount of energy delivered to the tissue and the depth of tissue penetration. A shorter pulse duration results in higher peak power and may lead to more precise tissue removal, while a longer pulse duration can result in more thermal damage to the surrounding tissue. The appropriate pulse duration depends on the type of tissue and the desired level of tissue ablation (Table 2).

The fixed QB is set to 40, and the pulse duration is varied from 2 to 4 ms. The cell destruction depth and survival rate were recorded for each pulse duration, with a sample size of 25. The data show that shorter pulses lead to higher peak power and more precise tissue removal, while longer pulses can cause more thermal damage to surrounding tissues.

Testing on the high-quality beam model for thermal ablation

There's a consideration about whether escalating QB settings results in a higher cell mortality rate within tumor formations. Our study utilized a QB where we gauged the average power in watts and recorded the percentage of tissue cell mortality. We maintained a constant pulse duration of 2.5 ms throughout the experiment. Our data (Table 3) suggests that amplifying the QB setting doesn't necessarily correlate with an uptick in cell mortality within the tumor. Furthermore, the cooling duration is relatively extended, spanning between 5 to 10 seconds. Consequently, it's essential to deploy multiple cycles of the cryo-beam. Subsequently, we assessed the vitality of regular cells, newly acquired NB tissue, and 3D NB tissue derived from these cells by determining the proportion of living cells relative to the overall cell count. We computed the mean cell vitality and its standard deviation for each procedure and kind of tissue. Notably, cryo-ablation tends to result in enhanced cell vitality in comparison to thermal ablation for both standard and NB tissues. This distinction is particularly more noticeable in cryo-ablation concerning NB tissue and 3D cellular formations. Furthermore, the vitality rate of cells in fresh NB tissue appears to be lesser than that in 3D NB tissue when subjected to thermal laser procedures. It's also evident that the duration required for cryo-ablation exceeds that of high-temperature laser ablation (Table 4).

The findings suggest that the fiber laser thermal ablation technique surpasses the cryo-ablation approach when it comes to tissue damage. On average, the

Pulse Time (ms)	Power (W)	Average cell destruction depth (mm)	Tissue ablation diameter (mm)
2.0	50	4.2	8.9
2.5	40	4.0	8.5
3.0	35	3.8	8.1
3.5	30	3.5	7.5
4.0	25	3.2	6.8

Table 2: Optimization of pulse time with a fixed high-quality beam of 40 for thermal ablation on neuroblastoma tissue, based on average cell destruction depth and tissue ablation diameter (n = 25).

Table 3: Optimization of the high-quality beam with a fixed pulse duration of 2.5 ms for cryo-ablation on neuroblastoma tissue (n = 25).

QB	Cooling time (s)	Icing Size (cm)	Cell death (%)
20	10	1.5	30.5
30	8	1.2	35.7
40	6	0.9	39.2
50	5	0.7	43.8

 Table 4: Comparison of cryo-ablation and laser ablation on normal tissues (n=5), fresh neuroblastoma tissues (n=20), and 3D neuroblastoma tissue (n=5)

	Normal tissue		Fresh tissue		3D tissue	
Ablation technique	Laser	Cryo-	Laser	Cryo-	Laser	Cryo-
Average power (W)	50	50	50	50	50	50
Pulse time (ms)	2.5	2.5	2.5	2.5	2.5	2.5
Live cell (%)	9.2 ± 0.6	90.3 ± 0.6	5.3 ± 2.1	75.3 ± 2.1	3.9 ± 1.5	33.9 ± 1.5
Cell destruction (%)	56.7 ± 1.2	1.5 ± 1.0	75.7 ± 3.4	5.7 ± 3.4	81.3 ± 4.2	1.3 ± 4.2
Treatment time (s)	2.4 ± 1.6	15 ± 1.5	3.8 ± 0.7	18.8 ± 0.9	2.0 ± 1.03	14.6 ± 1.1

thermal procedure takes 2.6 seconds to ablate the tissue, eliminating the need for extra rounds for necrosis processing. In contrast, the cryo-ablation approach demands an extended average duration of 14.35 seconds and additional cycles to yield comparable outcomes (Table 5). Nevertheless, expanded studies with a broader set of samples are essential to validate these specifications.

Finally, we analyzed the characteristics of three varied cancer tissues, spanning from soft to rigid. The next trio of heatmaps exhibit the consequences of laser ablation, with distinct yellow ovals highlighting increased temperatures. The second heatmap showcases a temperature surge reaching 75° C, implying a heightened or extended laser application compared to its counterparts. The third and fourth maps, though bearing resemblance to each other, peak at 70° C, showcasing a marginally reduced intensity compared to the second one. Collectively, the last three heatmaps portray varied intensities or spans of laser ablation effects on tissue (Figure 2).

DISCUSSION

Modern tumor ablation methods aim for localized, selective cancer cell destruction. The thermal ablation approach is especially beneficial when surgical interventions pose significant risks. Our focus was on optimizing fiber laser ablation for neuroblastoma (NB) tissues. The advancement of tumor ablation methods through the localized destruction of cancerous tissue using thermal, mechanical, electrical, or high-intensity focused ultrasound energy has demonstrated selective destruction of cancer cells in targeted areas¹⁵. The thermal ablation method is often employed in cases where cutting tissues with a scalpel poses risks or difficulties, such as when the tissues

Technique	Average time (s)	Ablating cycles	Destruction
Cryo-	14.35	3.5	Low
Laser	2.6	1	High





are located near vital organs or major blood vessels¹⁶. This method can also be utilized when complete removal of the tumor is not feasible or when the patient is not medically fit to undergo major surgery¹⁷. We tried to optimize the use of thermal ablation, especially fiber laser ablation, in NB tissue in this study. Until now, the use of thermal or cryo-ablation methods for cancer treatment based on 3D tumor models remains an actively researched field 18-20. The thermal ablation method utilizes heat to directly destroy target tissues and can be performed at different wavelengths. Tissues smaller than 5 cm, including small tumors and other pathological tissues, can be effectively destroyed by applying high temperatures directly to these tissues, resulting in efficient destruction of the internal cells. However, the crvo-ablation method has shown less effectiveness due to the need for longer cutting depths and the use of inappropriate cutting probes. Further in-depth studies are needed to determine the optimal approach for using the cryoablation method.

Table 5: Comparison of the two thermal ablation techniques

The primary objective of this study was to investigate the physical and mechanical effects on NB cells, evaluating the safety, efficacy, and potential outcomes of the thermal ablation model (Figure 3). To substantiate these findings and ascertain clinical relevance, further research involving NB tissue samples in animal models is imperative. Furthermore, our study underscores the potential of thermal laser agents in eliminating small NB tumors. Nevertheless, future research should delve into comprehending the intricate interactions within the peripheral region, encompassing the extracellular matrix, cancer-associated fibroblasts, infiltrating immune cells, and inflammatory cytokines, to establish optimal therapies for NB and other solid tumor malignancies.

CONCLUSION

Our research on in vitro testing using pediatric NB tissue samples showed that in comparison to thermal ablation methods, the hot thermal ablation method resulted in higher tissue destruction levels. Additionally, it required fewer ablation cycles and had a shorter average tissue removal time compared to cryo-ablation. However, since this was an in vitro study with a small sample size, further extensive research is still needed in this field in the future.

LIST OF ABBREVIATIONS

NB: Neuroblastoma PBT: Pediatric brain tumor QB: High-quality beam

COMPETING OF INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

Q-G Nuyen, C-B Bui, S-B Nguyen drafted the article; acquired, analyzed and interpreted data; D-K Nguyen and T-Q Nguyen, CN Pham and S-B Nguyen critically



Figure 3: The in vitro experimental setup model for the development of a neuroblastoma tumor thermal ablation system incorporates a probe consisting of two essential components: 1) a thermal sensor and 2) a laser emitting head. This model demonstrates that the thermal sensor head plays a crucial role in assessing the tumor's location, size, and density by measuring the temperature differential between the tumor and the adjacent healthy tissues. Furthermore, the application of thermal ablation via a fiber laser has the potential to impact the tumor microenvironment.

revised the manuscript for important intellectual content, and approved of the submitted manuscript All authors are approved of the submitted manuscript.

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Tối ưu hóa quá trình cắt hủy nhiệt bằng laser sợi quang để tăng cường vi môi trường khối u trong u nguyên bào thần kinh

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TÓM TẮT

Đặt vấn đề: U nguyên bào thần kinh (NB) là một loại ung thư ảnh hưởng đến trẻ em thường gặp. Việc phẩu thuật để điều trị và quản lý các khối u nhỏ (dưới 5 cm) đã đặt ra những thách thức và gây khó khăn cho việc đảm bảo điều trị triệt để và có hiệu quả. Mục đích chính của nghiên cứu này là phát triển và tối ưu hóa mô hình cắt hủy bằng laser thiết kế riêng cho các khối u NB nhỏ. **Phương pháp:** Mô hình nghiên cứu của chúng tôi sử dụng mô ung thư nuôi cấy để kiểm tra các đặc điểm khác nhau trước và sau quá trình cắt hủy laser. Những đặc điểm này bao gồm chất lượng của chùm tia laser, mức công suất trung bình và thời gian phát xung chính xác. **Kết quả:** Quá trình cắt hủy nhiệt bằng laser sử dụng năng lượng laser 1,0 mJ và công suất 1,0 kW. Mức tối ưu của chùm tia chất lượng cao là 60 với công suất 18,5 watt và thời lượng xung 2,5 ms đã đạt được tỷ lệ cắt hủy mô ấn tượng lên tới 95,8%. Chúng tôi cũng phát hiện ra rằng cắt hủy nhiệt nóng bằng laser có hiệu quả hơn so với cắt hủy nhiệt lạnh bằng laser về hiệu quả tăng cường vi môi trường, thể hiện qua việc giảm công suất tối ưu, thời gian phát xung 2 lần, tăng khả năng gây ra quá trình chết theo chu trình trong tế bào ung thư và gây ra các biến đổi cấu trúc collagen trong vi môi trường khối u. **Kết luận:** Mô hình này có thể giúp tối rư hóa việc phát triển phương pháp điều trị kết hợp cho các khối u rắn, thông qua các thông số thiết kế của mô ung thư nuôi cấy.

Từ khoá: cắt hủy nhiệt, u nguyên bào thần kinh, laser sợi quang, cắt hủy nhiệt bằng laser

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