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Comparison of the Effectiveness of 532 nm and 660 nm Diode Laser on MRSA Viability in Different Tissue Thicknesses in Vitro

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Abstract

Laser-mediated therapeutic approaches have offered multiple advantages in reducing cutaneous microbial complications across different environments along with biological processes. However, the application of such an approach in clinical trials of different tissues has been very difficult and cannot be applied to improve microbial control investigations across different tissue sites. This study aims to provide a comparative perspective to evaluate the analgesic parameters of 530nm and 660nm lasers at MRSA-infected tissue sites under laboratory conditions. To investigate this, the light penetration depths and transport properties will be initially investigated, providing insight into this strategy and its optimization in laser-based microbial decontamination efficiency. This type of treatment facilitates the development of new and untested protocols that take into account the optical properties of MRSA-infected tissues in different clinical settings, to ensure optimal control of microbial pathogens while minimizing the potential for future infection of surrounding tissues. Wavelengths of 660 nm provided acceptable efficiency for controlling pathogens in different 3 mm thick cattle meat samples, confirming the effectiveness of this technique. In contrast, the 530nm wavelengths provided better efficacy in beef tissue samples of 5mm and 10mm thickness, demonstrating the validity of the advantages of laser therapy for diverse tissues compared to standard antibiotics, while indexing new treatment protocols against pathogens in various therapeutic settings. This treatment approach offers several advantages of laser properties over conventional therapies while reducing the concerns expected with laser-based interventions. Thus, this technique can be calibrated in the laboratory to ensure light transmittance considerations are matched to target site characteristics to reduce microbial risk and ensure patient safety through optimal tissue conditions.

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1. Introduction

The effectiveness of laser therapy is one of the advantages and at a very high level by directly interacting with microbial biological processes [1, 2]. The eradication of methicillin resistant Staphylococcus aureus is a fundamental goal in clinical medicine since such treatments cannot be treated with conventional antibiotics and therefore is a complex problem that poses complex methods for laser therapy standards [3]. Specifically, the effects of photothermal and photochemical processes on bacterial cells are most pronounced at wavelengths of 532 nm and 660 nm [4,5]. Although there is increasing interest in these applications, healthcare providers know little about precisely diode lasers and their response to tissue thickness variation [6]. Although the introduction of lasers as a mechanism for microbial decontamination shows an overall increase in its adoption, there is still a need to understand the specific effects that different wavelengths on MRSA within tissues of different thicknesses [7]. Selecting the appropriate laser

wavelength is imperious because it decides the degree of photochemical and photothermal effects in the biological tissues [8]. Furthermore, tissue thickness is a significant parameter, which affects the efficiency of diode-laser-based interventions [9]. This study seeks to explore these therapeutic parameters by comparing the similarities and differences of the effects of lasers at 530 nm and 660 nm wavelengths on the microbial contamination threshold, especially MRSA. In contrast, considering the investigation of different tissue thicknesses, ranging from 3 mm to 10 mm, is the aim of this study to provide more important details about the resistance of MRSA to specific wavelengths in different biological environments. Not only this, but this study also focuses on challenging the concerns and obstacles facing clinical users and enhancing the prospects of public health.

2. Materials and Methods

i. Tissue Collection

The study obtained different tissues of beef and chicken breast through agreement with local suppliers. These samples were prepared in the laboratory, according to the applicable health guidelines and ethical standards. These samples were examined to ensure that they were free from any deformities, defects or signs of spoilage. Initially, chicken breast and beef tissues were sectioned to simulate different biological tissue environments using calipers and scalpel including 3 mm, 5 mm and 10 mm, to measure the maximum estimated light penetration in those different tissues including (1, 3, 5, 10 and 20) mm (details in Figure 1). In order to include a better choice of application of the experiments, a range of light penetration depths were measured to include a suitable threshold for antimicrobial therapy. In this way, shorter wavelengths can be considered less penetrating, while longer wavelengths can be considered more penetrating into tissues and more deeply as reported in several reports [1]. This insight reinforces the theory of differences in the statistical ranges of microbial killing and may provide a more explanatory understanding of how tissue properties influence treatment outcomes [2]. Accordingly, the thickness of each tissue class was strictly standardized to avoid confounding factors that might alter the results. The tissue samples were treated so as not to cause any damage or prior contamination. All instruments were sterilized before starting, and all laboratory procedures were performed in a laboratory environment free from external microbial influences. In this manner, all tissues were stored and handled in environments immediately after sampling. To prevent any random colonization or damage to tissue quality, they were stored under a refrigerated temperature of 4°C. Tissue samples were carefully stored and spaced to ensure no cross-contamination, and then stored in sealed plastic tubes. Throughout the experiment, a random sample was designated as a blank sample for periodic monitoring to assess the quality and integrity of the tissue from injury or damage. Damaged, defective, or otherwise degrading samples were replaced immediately to ensure the accuracy of the experimental results. Thus, these procedures and management provide accuracy of results and preservation of tissue integrity, proving the importance of accurate and reproducible experimental results.

Figure 1. Tissue preparation into slices of different thicknesses

ii. Microbial Strain

The MRSA strains were carefully selected with expert knowledge to serve as the experimental microbiological contaminants in this investigation. One of the most controversial issues in the field of microbial decontamination has been the study of MRSA as one of the major Gram-positive strains resistant to antibiotics [10]. These strains were identified as antibiotic-resistant and as a model that is most susceptible to the effects of lasers, especially diode lasers, on microbial growth. MRSA was cultured on agar growth media for 24 hours before the experiment at 37°C in a carefully sterile environment. These culture conditions were calibrated to enhance the metabolic pathways of MRSA and promote bacterial growth and survival [11]. By measuring the optical density the concentration of the bacterial suspension was estimated as 5 McFarland (1.5×109 CFU), obtaining a standardized concentration [12]. The same result

was also shown while performing other microbiological analyses. Sample backs from various localities on each tissue sample enabled the verification of homogenous MRSA spread across the specimen [12]. This method was able to fulfill dual objectives including being a perfect representation of real situations and providing an atmosphere for the accurate evaluation of the outcome of diode lasers on MRSA of different tissue thicknesses.

iii. Experimental Groups

The experiment included two control groups that were conducted to examine baselines and MRSA survival rates without laser treatment, as follows:

• Blank (unstained) tissue sample group

The unstained tissue samples were the reference values and were used to determine the starting measurements. While keeping the sample free of any intentional spreading of MRSA infections. The function behind the application of this control group was to provide a point for comparison of intrinsic tissue features devoid of the external context of the microorganisms. The samples received the same processing, storage, and testing protocols as with the control and the experiment group, but without being intentionally contaminated or exposed to the laser treatment.

• Contaminated tissue samples without laser treatment (viability control(

MRSA-contaminated tissue samples, that were not treated, were included to determine the natural survival of MRSA under experimental conditions. According to previous theories, the comparison of MRSA survival in control groups was intended as a reference to examine their behavior and growth without the interaction of the diode laser. In contrast, these control groups were intended to examine the survival of the main MRSA and to determine the basic characteristics of the uninfected tissues. However, by studying the effects of the control groups, it was shown that the diode laser affected MRSA survival to a greater extent than the experimental groups within the different tissue layers [13]. To prevent bias, all control groups were randomly assigned during the trial by duplicating each control group (n = 3) to increase the validity and reliability of the data from that group as is the practice in many clinical contexts. Therefore, the efficacy of the laser was considered more reliable and relevant than what was reported in the literature reporting information on MRSA viability and was considered an unprecedented benchmark for understanding the parameters of laser treatment within different tissue environments in the vitro study.

• Analysis of laser-treated MRSA residues

This study investigated the interaction of laser parameters using a diode laser of two different wavelengths (532 nm and 660 nm) and tissue samples infected with MRSA. The energy density for both wavelengths was 167 J/cm² for 532 nm and 142 J/cm² for 660 nm. Two lasers of different wavelengths were systematically applied to evaluate the penetration of distinct tissues at each wavelength and the impact on their properties. In addition, the study investigated the factors contributing to the negative outcomes reported in many clinical trials, including how wavelength differences affect penetration depth, which in turn affects the efficacy of antibiotics against MRSA persistence across different tissue thicknesses. Furthermore, the study controlled for several aspects such as power settings, exposure time, exposure distance between samples, and the effect of temperature on tissues during and after laser irradiation [14,15]. The ability to understand and regulate these thermal effects has been considered a safety valve to ensure the safety and effectiveness of antimicrobial treatments in medical practice[3]. This strategy is of great importance for the advancement and development of laser-based microbiological disease control technology. To evaluate the viability of MRSA after laser treatment, McFarland turbidity was evaluated at the highest concentration (1.5×109 CFU) [14,15]. MRSA viability results were accurately indexed for experimental and control groups using quantitative data analysis. The CFU/ml approach produced numerical data, which provides a quantitative measure of MRSA colony viability and can therefore be qualitatively interpreted as bacterial survival. The study applied strict correction procedures to ensure accuracy and reliability in collecting several informative data without any restriction in the CFU/ml count procedure, which adhered to standard protocols in all samples studied. This methodology was critical in determining the viability of MRSA under laser irradiation.

As a result, each experimental group for both wavelengths was separated into three subgroups (n = 3) according to tissue thickness. These subgroups were exposed to laser irradiation in a vertical position in a continuous position for 10 min in the dark, after which they were compared to unirradiated controls.

This treatment period was chosen to maximize antibacterial activity while minimizing potential tissue damage caused by high heating, as documented in multiple published papers. Longer exposures may improve bacterial killing but increase the risk of tissue damage, while shorter periods may alter microbial reduction. In addition, the continuous mode ensures a constant supply of laser energy, which promotes long-lasting thermal effects and uniform treatment throughout the tissue thickness. It also improves repeatability and simplifies data analysis and experimental methods. Subdivision allows for a detailed examination of how tissue thickness affects laser treatment response, aiding in understanding the effects of light laser on antimicrobial efficacy across different tissue depths.

- Experimental design

Before undergoing irradiation, 36 tissue samples were classified into three distinct test groups: the blank, control, and irradiated groups, each comprising three samples (n = 3), as shown in Figure 2. The tissue samples, consisting of a control and a treatment group, were placed into an aluminum box specially made for irradiation. The box was designed to include, with great precision, a wooden platform that was installed together with proper barriers to control the scattering or reflection of the laser. So far it has been proven that scattering and reflection of the laser light can break down the energy concentrated in one point which can decrease the treatment effect if the energy is spread to a larger area and thus, the intensity of the light is reduced [14,16]. To ensure the maximum transmission of the laser light in different tissues, the power meter recorded the intensity of the light transmitted through the tissue for various tissue thicknesses, namely 1, 3, 5, 10, and 20 mm before any staining or treatment begins. This process was aimed at detecting the efficiency of light transmission through the tissue which is a primary factor in determining treatment efficiency and preventing inconsistent results across different tissue thicknesses [17,18]. This measurement process is represented by the Equation:

$$T = \frac{I}{I_o} \times 100\,\% \quad \ldots (1)$$

As can be seen in these results, I refer to the transmittance intensity (W/cm2) on the tissue surface, T is the light transmittance, and I_0 refers to the intensity of incident light penetrating the layers of these tissues. The scattering or reflection of these waves may hinder their transmission to such depth that microbes will not receive sufficient lethal doses, potentially allowing them to find refuge in deeper layers of the skin [19]. To mitigate this problem, the laser device was directed vertically into the center of each sample to achieve a uniform sample-spot distance of 1 to 2 cm, as shown in Figure 3. This positioning enhanced the efficiency of the treatment and decreased any problems that may arise from the laser's penetration or scattering into different mediums [4]. This approach improves treatment effectiveness by ensuring consistent and focused laser energy delivery while reducing the potential for scattering or energy loss caused by reflection [5]. Each sample received an MRSA injection before irradiation. A 30 µl microbial suspension with a 5 McFarland (1.5 × 109 CFU) concentration was pipetted onto the tissue samples surfaces. After that, these inoculation samples were left to incubate for half an hour, allowing the bacteria to effectively penetrate the tissue surfaces. MRSA bacteria have enough time during this incubation period to stick to the tissue surface, which is comparable to how bacteria typically colonize biological tissues before therapy. A 10-minute exposure time was chosen to achieve the intended treatment outcomes for all samples while maintaining consistent treatment duration. After that, many decimal dilutions were set to compare with the zero dilution to conduct a comparative statistical analysis [6]. A smear layer of 150 µl was blotted onto mannitol salt agar to achieve a 0.5 McFarland standard concentration [20,21]. Then, control and irradiated samples were incubated at 37°C for 24 h to enumerate the microorganisms growing on the agar layer, which represented the CFU/ml remaining after laser irradiation for the control, experimental, and blank groups under tightly controlled experimenter conditions.

- Statistical Analysis

Two-way ANOVA was performed to evaluate potential interaction effects between laser wavelength and tissue thickness on MRSA viability. The results of all tests were compared and presented as mean ± SD. This analysis aimed to separate whether the complex effect of different wavelengths and tissue thickness resulted in synergistic or antagonistic effects on bacterial survival. The significance of differences for each treatment was differentiated by calculating the calculated p-value. A p-value less than 0.05 was considered statistically significant, while a p-value greater than 0.05 was not statistically significant.

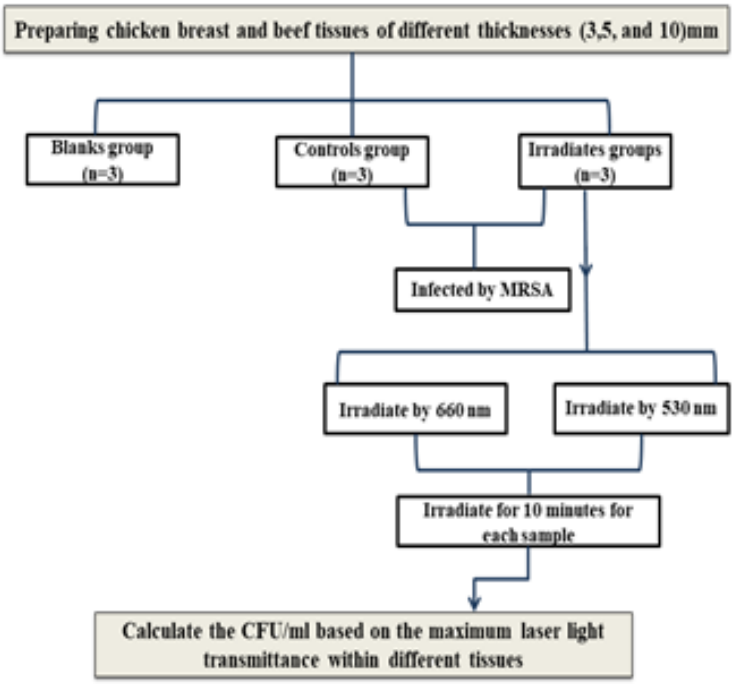

Figure 2. Experimental work processes.

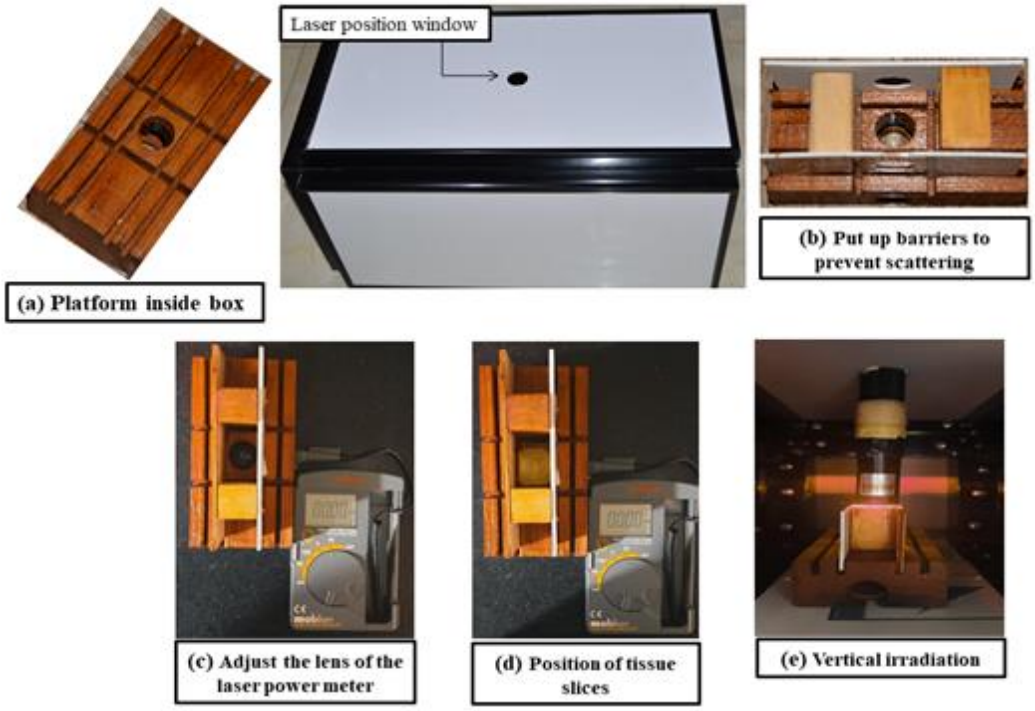

Figure 3. Preparation of the experimental setup involves the following components: (a) Arranging a movable wooden platform within the box to accommodate the samples (b) Establishing the placement of barriers around the samples (c) Installing the lens for the laser power meter (d) Identifying the specific location for the tissue samples (e) Implementing a vertical irradiation mechanism.

iv. Statistical Analysis

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3. Results and Discussion

3.1. Light Transmission Measurement

Analysis of the results revealed differences in light transmission efficiency across different tissue thicknesses for beef tissue, which is presented in Table 1 and Figure 4 and for chicken breast tissue which is presented in Table 2 and Figure 5 other factors can count for composition shape and optical properties. Beef tissue has also shown differences in the optical transmissions depending on the different thicknesses of the tissue by the mean of the measured transmission spectra in terms of 660 nm and 532 nm wavelengths. These differences are most significant from the time of laser irradiation till the end of the 10th minute of incubation and suggest that the type of tissue also determines how the laser beam is absorbed and acts upon the tissue. Likewise, by comparing mean transmission light, mean transmission light for the various thicknesses of chicken breast and beef tissue also displays largely contrasted initial and final permeations —which produces a strong message of how tissue thickness affects laser beam penetration. In general, the observed differences in light transmission efficiency across different tissue thicknesses underscore the importance of considering tissue properties when designing laser-based therapies. Understanding these differences is critical to improving treatment outcomes and ensuring antimicrobial efficacy against pathogens such as MRSA.

3.2. MRSA Viability Count

Based on what was reviewed in the introduction, all irradiated groups showed greater efficacy in beef ($P < 0.05$) in killing MRSA than the control groups. The groups that were exposed to lasers at 532 nm and 660 nm had mean p-values of 0.003 and 0.002, respectively. This compares with the mean p-values of 0.146 and 0.190 for the exposure at 532 nm and 660 nm for all control groups. Specifically, when treated with the 532 nm laser, 3 mm tissue showed a more substantial reduction in MRSA viability (P value: 0.003) as compared to 5 mm and 10 mm. Furthermore, when exposed to 660 nm, tissues with a thickness of 3 mm showed a higher decrease in MRSA viability (P value: 0.001) than those with a thickness of 5 mm and 10 mm, as are shown in Table 3 and Figure 6. Conversely, a highly significant ($P < 0.05$) reduction of the MRSA viability index was observed in all the treatment groups to the control chicken breast tissue. The 532 nm laser and 660 nm laser treated groups had mean p-values of 0.001 and 0.002, respectively, while the mean p-values for the 532 nm and 660 nm wavelengths for all control groups were 0.626 and 0.136. Specifically, 3 mm tissue showed a higher decrease in MRSA viability (P value: 0.001) after being exposed to the 532 nm laser as opposed to 5 mm and 10 mm. However, when tissues with a thickness of 10 mm were exposed to a 660 nm laser, there was a notable decrease in MRSA viability (P value: 0.006) as opposed to tissues with a thickness of 5 mm and 3 mm. Table 4 and Figure 7 provide documentation of these results.

4. Discussion

The diverse viabilities of MRSA in wavelengths of 500 to 600 nm revealed the wavelength-specific impacts of the diode laser on the pathogen. This evidence fits in with previous studies which showed that different wavelengths have specific photochemical and photothermal effects on bacterial cells [22], [23]. The 532 nm wavelengths which are located in the green visible spectrum and the 660 nm wavelength which are located in the red spectrum, have different effect on MRSA cells. As a result, it affects their survival rates. However, the pronounced MRSA survival in the presence of different tissue depths calls for thoroughness in the tissue properties of laser-based microbial killing [24]. Literature evidence has shown thicker tissue reduces the depth of light penetration and its absorption [25]. The results suggest that laser effectiveness is not only related to wavelength, but the layer of the target tissue can also affect the treatment [26]. This information will help determine the use of laser in situations where microbial contaminants are present at different levels of tissues [7]. It has been realized that the type of wavelength used was of great importance to the light depth that could penetrate the tissue. Firstly, it was observed that the shorter wavelength 532 nm has less transmission within the

tissue boundaries compared to 660 nm. However, that is a very crucial factor determining the path of laser energy through tissue, therefore, affecting the survivability of MRSA. The absorption and scattering of biological tissues are multi-layered with different wavelengths on both sides of the parallelism [26], which leads to distinct ways the MRSA interacts with the lasers and subsequently influences the detected variation in MRSA viability. The research aims further to find out that different wavelengths can generate different photobiological effects in biological systems. There is a difference in the processes of operation of both the 660 nm and 532 nm wavelengths on the bacterial cells of MRSA which includes penetration into the bacterial cell and the resultant cell damage and viability rate [27]. An organism's cellular and molecular reaction to light appears to be not uniform but dependent on wavelengths: while the internal structure of MRSA and its targets reacts differently to the power supplied by the 660 nm and 532 nm lasers. However, the results of the current study are consistent with existing literature on microbial decontamination using lasers, providing additional evidence of wavelength and tissue thickness dependencies [28]. Comparisons with studies investigating similar parameters revealed light transmission and penetration depth as critical factors for direct assessment of the effect on microbial cells within tissue boundaries [29,30], providing insight into microbial membrane damage and healing mechanisms, further supporting the generalizability of the in vivo findings. Based on these results, future research can explore the effect of additional laser parameters, such as pulse duration and energy density, on MRSA viability. In addition, evaluating the applicability of these findings in more complex biological settings or in vivo models would contribute to a more comprehensive understanding. However, incorporating diode laser impact on MRSA viability in clinical trials can give an account of in vivo results and consequently assist in translation into viable clinical applications. In vivo models offer a more complete and detailed perspective of the biocontrol phenomenon where many factors interact in the real host environment.

Table 1. Differences in the penetration depths of 660nm and 532nm laser light through varying thicknesses of beef tissue

Light transmission measurement	Red diode laser/ first moments	Red diode laser/within 10 consecutive minutes	Green diode laser/ first moments	Green diode laser/within 10 consecutive minutes
0	31.17±0.30	32.45±0.47	37.33±0.36	36.42±0.19
1	16.33±0.53	13.12±0.64	29.15±0.52	9.41±0.42
3	12.24±0.28	8.31±0.58	4.36±0.17	3.04±0.19
5	10.29±0.18	6.93±0.64	0.90±0.28	0.42±0.22
10	4.47±0.26	3.33±0.08	0.20±0.14	0.11±0.07
20	1.10±0.06	0.90±0.04	0.04±0.0023	0.0003±0.0015
$LSD_{0.05}$	0.507	0.826	0.732	0.331

Table 2. Differences in the penetration depths of 660nm and 532nm laser light through varying thicknesses of chicken breast tissue

Light transmission measurement	Red diode laser/ first moments	Red diode laser/within 10 consecutive minutes	Green diode laser/ first moments	Green diode laser/within 10 consecutive minutes
0	31.57±0.60	32.53±0.23	35.52±0.72	37.08±0.41
1	27.30±0.52	21.6±0.60	20.42±1.10	11.98±0.41
3	9.79±0.17	8.24±0.26	6.44±0.52	4.97±0.38
5	4.69±0.28	2.28±0.51	2.12±0.24	1.27±0.23
10	2.23±0.14	1.14±0.44	1.20±0.13	0.85±0.14
20	1.47±0.23	0.21±0.33	0.12±0.05	0.07±0.03
$LSD_{0.05}$	0.642	0.725	0.574	0.415

Table 3. Multiple comparisons of laser effect between/within groups treated across various thicknesses of beef tissue

	Thickness	N	Mean	Standard deviation	Mean P- value (532 nm)	Mean	Standard deviation	Mean P- value (660 nm)
Irradiated	3	3	104385	13831	0.003	132233	17488	0.001
	5	3	47033	4190	0.001	85611	11943	0.010
	10	3	36222	20669	0.001	54834	16262	0.014
	Total	9	62547	34140	0.003	90893	36293	0.002
Control	3	3	113718	9542	0.124	154966	13397	0.139
	5	3	117983	7330	0.217	147798	4780	0.227
	10	3	136212	9693	0.193	162335	3495	0.080
	Total	9	122638	12913	0.146	155033	9657	0.190

* Means ± SE
*Means with the same difference in the same column are not significantly different

Table 4. Multiple comparisons of laser effect between/within groups treated across various thicknesses of chicken breast tissue

	Thickness	N	Mean	Standard deviation	Mean P- value (532 nm)	Mean	Standard deviation	Mean P- value (660 nm)
Irradiated	3	3	49000	5727	0.001	13821	3094	0.001
	5	3	17166	1595	0.004	24319	4247	0.010
	10	3	24243	6111	0.002	35931	2634	0.006
	Total	9	30136	15090	0.001	24690	10019	0.002
Control	3	3	89330	7388	0.778	38710	2476	0.139
	5	3	91479	4518	0.450	51306	5737	0.227
	10	3	88094	1958	0.450	63267	4934	0.080
	Total	9	89634	4680	0.626	51095	11355	0.136

* Means ± SE
*Means with the same difference in the same column are not significantly different.

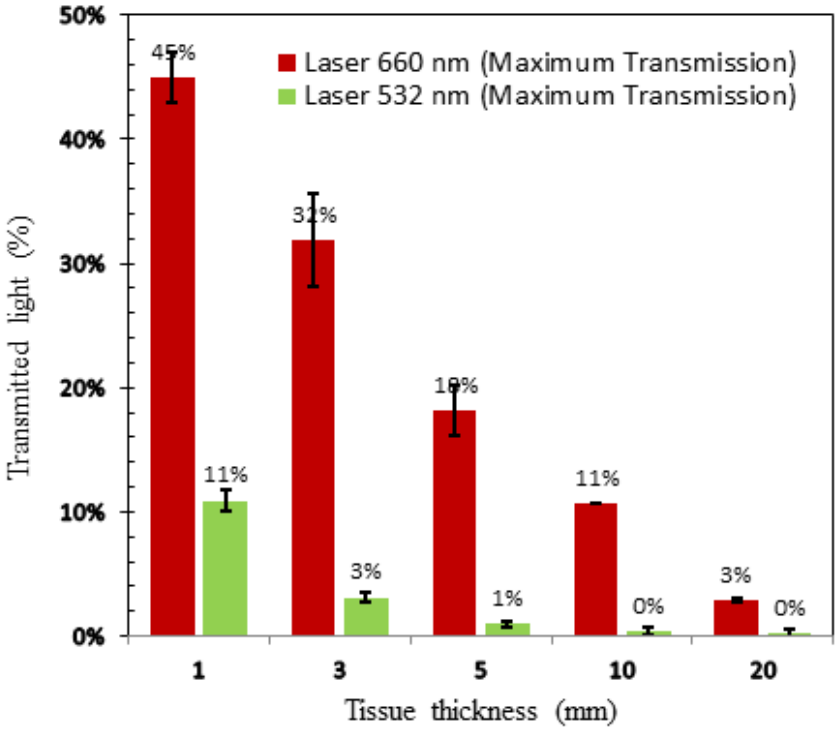

Figure 4. The maximum transmittance of laser light at 660 nm and 532 nm across various thicknesses of beef tissue for more than 10 consecutive minutes

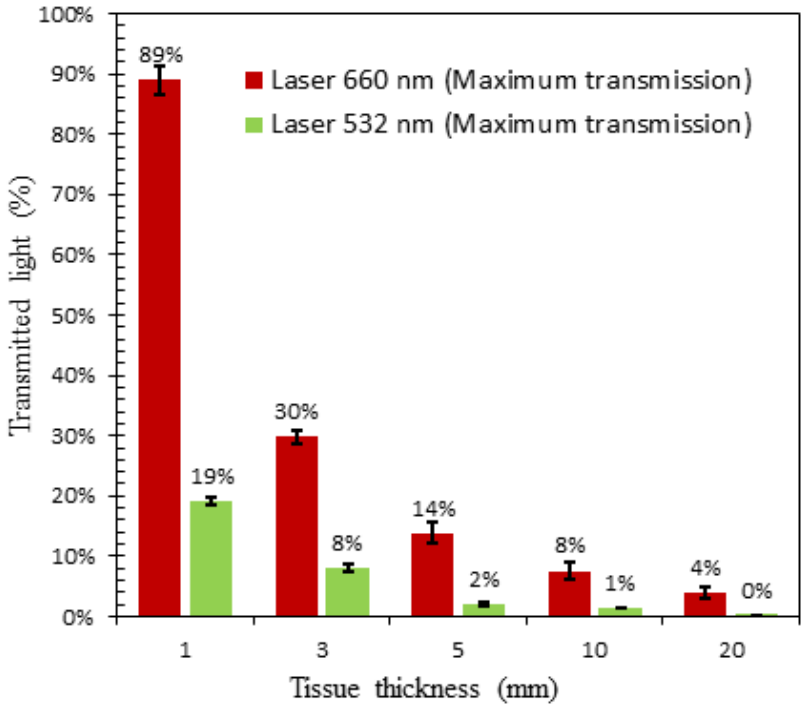

Figure 5. The maximum transmittance of laser light at 660 nm and 532 nm across various thicknesses of chicken breast tissue for more than 10 consecutive minutes.

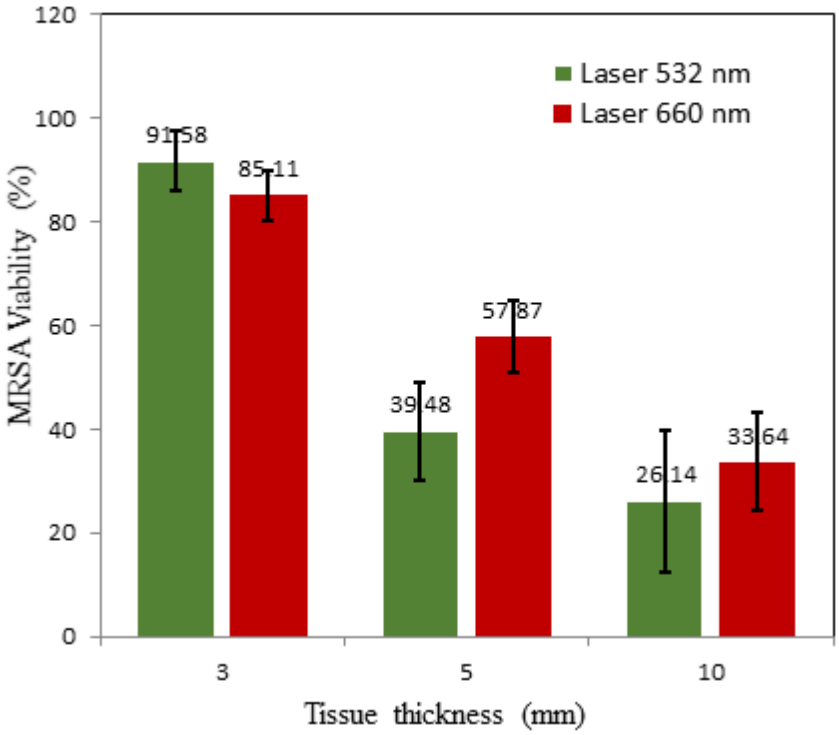

Figure 6. The percentage of MRSA viability after irradiation with wavelengths of 660 nm and 532 nm for various beef tissue thicknesses

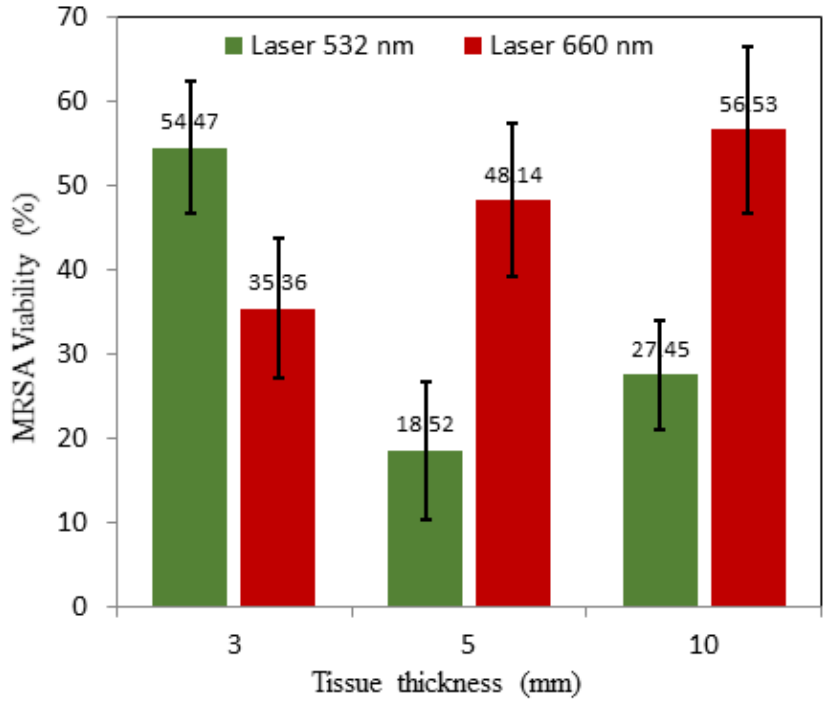

Figure 7. The percentage of MRSA viability after irradiation with wavelengths of 660 nm and 532 nm for various chicken tissue thicknesses.

5. Conclusions

This study provided a contrast between the effects of 532nm and 660nm diode lasers on reducing MRSA contamination across different levels of skin thickness, making a significant contribution to understanding the processes involved in laser-mediated microbial control. The wavelength-specific effects, coupled with tissue thickness considerations and specific interaction effects emphasize the need for precision in the design of laser interventions. Moreover, considerations of penetration depth and transmission properties are essential to optimize laser-based microbial control strategies, ensuring precise targeting of pathogens while minimizing collateral tissue damage. These findings contribute to advancing understanding of optimal strategies for microbial decontamination, emphasizing the importance of a multifaceted approach in clinical practice.

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Conflict of Interest: The authors declared no potential conflicts of interest.

Data Availability Statement: The corresponding author can provide the data described in this study upon request

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