Journal of Lasers in Medical Sciences

J Lasers Med Sci 2023;14:e61

Original Article

http://journals.sbmu.ac.ir/jlms

doi 10.34172/jlms.2023.61

Histologic Evaluation of Effect of Three Wavelengths of Diode Laser on Human Gingival Margins

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Received: August 1, 2023
Accepted: October 20, 2023
ePublished: December 11, 2023

Abstract

Introduction: Available evidence suggests that the response of the intervened tissue is directly linked to the effects generated by the cutting instrument used. To determine the histological findings in gingival tissue margins excised through gingivectomies performed using 450 nm, 940 nm, and 980 nm diode lasers. The present study aimed to determine the histological findings in gingival tissue margins excised through gingivectomies performed using 450 nm, 940 nm, and 980 nm diode lasers.

Methods: Gingival tissue samples were collected from 30 patients who had undergone gingivectomy procedures. Each study group comprised 10 patients who willingly provided their samples after providing informed consent. The visualization of histological findings was facilitated through Hematoxylin-Eosin staining. Additionally, variables related to pain and hemostasis were assessed during the intraoperative period.

Results: The incision quality was categorized as irregular across all three wavelengths. Histological examination of the epithelial tissue revealed the absence of carbonization and the preservation of cell morphology in over 50% of the resection margin in samples obtained with the 450 nm and 940 nm wavelengths. In the connective tissue, observations included carbonization, collagen coagulation, and basophilia, with the 980 nm wavelength demonstrating the highest percentage of samples displaying collagen coagulation in more than 50% of the resection margin. Conversely, the 450 nm wavelength exhibited the highest degree of preservation of the fibroblast structure.

Conclusion: Based on a comprehensive analysis of the study results, it can be inferred that the 450nm and 940nm wavelength lasers tend to produce less thermal damage and better cell preservation when compared to the 980nm wavelength.

Keywords: Diode lasers; Gingivectomy; Histology.

Introduction

Since the introduction of laser technology in medicine, over ten laser devices have become available in the dental field for various treatments.[1,2] These devices have demonstrated their ability to provide excellent intraoperative and postoperative hemostasis, diminish postoperative pain, and lower the risk of infection, thereby overcoming the drawbacks associated with traditional scalpel-based procedures.[3-5]

The diode laser stands out as one of the most widely utilized types in dentistry, boasting high power. The wavelengths suitable for dental applications typically span from 800 to 980 nm. Comprising a solid active medium, this laser relies on a semiconductor, often incorporating a combination of elements like gallium, arsenic, aluminum, or indium to convert electrical energy into light energy. These diode lasers can emit in continuous or pulsed modes, easily transporting their energy through optical fibers. When applied to soft tissues, they yield the desired surgical effects, including ablation, incision, and excision.[2]

The clinical application of diode lasers offers several advantages. One notable benefit is their ability to provide excellent hemostasis, attributed to the photothermal effect that effectively seals blood vessels and expedites the healing and coagulation processes. Additionally, postoperative pain and inflammation are reduced as a result of the cells receiving biostimulation from the residual energy transferred during the cutting process. This enhanced energy input improves mitochondrial metabolism and stimulates cell regeneration, contributing to a more comfortable and swifter recovery.[6-10]

The photothermal effect of the laser leads to an elevation in tissue temperature, and if not properly controlled, it can result in tissue alterations and thermal damage. These effects can significantly impact the wound-healing process and may distort the histopathological interpretation when

Please cite this article as follows: Flórez PB, Maz HHA, Domínguez JA, Tost AJET, Páez JO. Histologic evaluation of effect of three wavelengths of diode laser on human gingival margins. *J Lasers Med Sci.* 2023;14:e61. doi:10.34172/jlms.2023.61.

biopsies are conducted.[11,12] Consequently, it is essential to assess the histological changes that arise in connection with the thermal impact of the laser.

This study aimed to investigate and compare the histological findings in gingival specimens obtained through gingivectomy procedures performed using diode lasers at wavelengths of 450 nm, 940 nm, and 980 nm, all of which were operated under similar parameters.

Materials and Methods

A comparative clinical study was conducted, involving the samples of gingival tissue margins voluntarily provided by patients undergoing cosmetic gingival surgery with diode lasers.

The study participants were divided into the following groups: Group 1, which comprised the samples obtained using a 450 nm diode laser; Group 2, consisting of the samples obtained with a 940 nm diode laser; and Group 3, which included the samples obtained using a 980 nm diode laser.

Patient selection for gingiva margin donation was carried out using non-probabilistic convenience sampling based on predefined inclusion and exclusion criteria.

Inclusion Criteria

- Gingival samples were obtained from patients aged 18 or older who willingly contributed specimens for the study.
- The patients had a gummy smile and were candidates for gingivectomy.
- The patients were both periodontally and systemically healthy.
- Samples were collected following the same surgical protocol used for gingivectomy.
- Patients with an O'Leary dental plaque index of ≤ 15% were included.

Exclusion Criteria

To mitigate potential biases, we excluded the following patients from the study:

- Individuals with orthodontic appliances.
- Patients with a history of periodontitis.
- Those with a history of smoking.
- Pregnant individuals.
- Patients who had been on prolonged courses of anti-inflammatory drugs, anticonvulsants (such as phenytoin, valproic acid, carbamazepine, phenobarbital, vigabatrin), immunosuppressants (like cyclosporine A), and calcium channel blockers (including nifedipine, diltiazem, verapamil, amlodipine, and felodipine) for the preceding six months or at the time of surgery.
- Patients who did not provide voluntary consent to participate in the study were also excluded.

Evaluated Variables

The study examined several variables, including sociodemographic factors such as gender (male or female) and age (measured in years lived).

For the assessment of incision quality, a rating scale ranging from 0 to 4 was employed. In this scale, a score of 0 denoted the lowest regularity, while a score of 4 signified the highest regularity. A rating of 4 indicated that the incision edges were smooth and linear for over 90% of the total resection margin.[13]

The histological findings were evaluated using a scale that categorized them into three groups: Absent, present in less than 50% of the resection margin, and present in more than 50% of the resection margin.

Only histological findings related to the thermal effects of the laser were assessed. These findings encompassed the following aspects: Epithelium: carbonization and preservation of the epithelial cell structure; connective tissue: carbonization, collagen coagulation, basophilia, and preservation of the fibroblast structure.

The study also evaluated intraoperative clinical effects, which included assessing hemostasis (categorized as absent bleeding, slight bleeding, moderate bleeding, or severe bleeding) and intraoperative pain, measured using the visual analog pain scale (categorized as absent, mild pain, moderate pain, or severe pain).

Stages of the Procedure

The execution of the study encompassed several methodological stages:

Patient Selection

Each potential patient was thoroughly briefed on the objectives of the project, the significance of the study, and the forthcoming histological analysis of each tissue sample. The participants who willingly agreed to participate in the study confirmed their involvement and consented to sample donation by signing an informed consent form.

Sampling

Gingival samples were acquired via gingivectomy. Initially, the treatment area was anesthetized using a topical solution (Master Formula, Laboratorios Anfer Ltda, Colombia; components: lidocaine, prilocaine, tetracaine TCH). To determine the height and extent of gingival tissue to be excised, we conducted periodontal probing using a color-coded Michigan periodontal probe. All samples adhered to the same protocol, executed by the same operator, and followed these laser settings:

- 450 nm Diode laser: 1w, tip 400 um x 5 mm activated
- 940 nm Diode laser: 1w, tip 400 um x 4 mm activated
- 980 nm Diode laser: 1w, tip 400 um x 5 mm activated

Gum margins obtained during the surgical procedure were preserved in a tube with 10% formalin for subsequent processing.

Sample Processing and Analysis

From each paraffin block, 5 µm serial sections were generated. Three consecutive slides were selected from the same block for each patient, and hematoxylin-eosin (HE) staining was performed. A specialist in oral pathology with extensive experience conducted the visualization of histological findings.

Data Analysis

The collected data were organized in an Excel table and subsequently imported into SPSS® statistical software for analysis. Descriptive statistics were employed to present qualitative variables as frequencies and percentages, while quantitative variables were represented using measures of central tendency and dispersion. To compare variable behaviors between the study groups, we applied the Kruskal-Wallis test with a significance level set at $P < 0.05$.

Results

The study included a total of 30 participants, comprising 22 females (73.3%) and 8 males (26.7%). Participants' ages ranged from a minimum of 18 years to a maximum of 42 years, with a mean age of 30.2 ± 7.4 years.

Histological Findings

Regularity of the Incision

The results indicated that incisions made with all wavelengths were predominantly irregular. Nevertheless, it is noteworthy that two incisions made with the 940 nm wavelength demonstrated exceptional regularity, earning a perfect score of 4 on the scale. In contrast, for the 980nm wavelength, half of the samples displayed the poorest incisional quality (Table 1).

Histological Findings in the Epithelium

Examination of the epithelial tissue revealed the absence of carbonization across all three study groups. Notably, more than 50% of the resection margin exhibited preserved cell morphology in all samples obtained with the 450 nm and 940 nm wavelengths. However, in four (40%) of the samples obtained with the 980 nm wavelength, the preservation of cell morphology was not observed. A statistically significant difference was identified between the three groups ($P = 0.008$) (Table 2; Figures 1-3).

Histological Findings in Connective Tissue

Table 3 presents the results of all the evaluated variables. Carbonization was observed in the majority of samples across the three wavelengths. However, it is important to note that this finding was limited to less than 50% of the resection margin in each of the samples examined (Figures 1-3).

In terms of the presence of collagen coagulation, the 940 nm wavelength exhibited the lowest incidence of this finding, with seven samples (63.6%), while the 980nm wavelength had the highest number of samples showing collagen coagulation, observed in more than 50% of the resection margin, accounting for seven samples (70%).

Basophilia was a common observation in most of the samples. However, it is worth noting that among the samples obtained with the 450nm wavelength, three (30%) did not exhibit basophilia, and seven (70%) displayed it in less than 50% of the resection margin. In contrast, the samples obtained with the 940 nm and 980 nm wavelengths consistently showed basophilia in more than 50% of the resection margin. Statistical analysis revealed a significant difference between the groups ($P = 0.006$).

Regarding the preservation of the fibroblast structure,

Table 1. Regularity of the Incision According to the Wavelength

Wavelength	Regularity of the Incision								*P* Value*
	Regular (scale 3)		Irregular (Scale 1 y 2)		Completely Irregular (Scale 0)		Total	Mean According to the Scale (0-4) ± SD	
	No.	%	No.	%	No.	%			
450 nm	0	0	10	100	0	0	10	2.4 ± 0.8	0.034
940 nm	2	20	8	80	0	0	10	2.4 ± 0.8	
980 nm	0	0	5	50	5	50	10	1.1 ± 1.2	

* Kruskal-Wallis test.

Table 2. Histological Findings in the Epithelium

Category	Carbonization of the Epithelium, No. (%)			Cell Structure Preservation, No. (%)		
	450 nm	940 nm	980 nm	450 nm	940 nm	980 nm
Absent	10 (100)	10 (100)	10 (100)	0 (0)	0 (0)	4 (40)
Present (< 50% of the resection margin)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Present (> 50% of the resection margin)	0 (0)	0 (0)	0 (0)	10 (100)	10 (100)	6 (60)
Total	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
P value*		> 0.05			0.008	

* Kruskal-Wallis test.

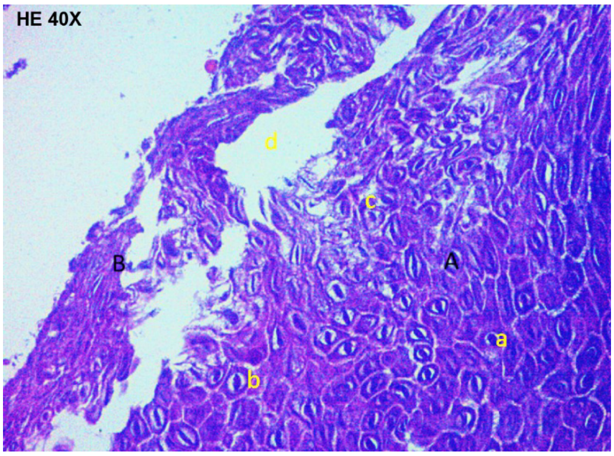

Figure 1. Section Performed With a 450 nm Diode Laser. Keratinized squamous stratified epithelium: the circle shows nuclear changes of pyknosis (a), hyperchromatism with fusiform nuclei (b), loss of intercellular adhesion (c), and loss of connective tissue adhesion (d) of 30%

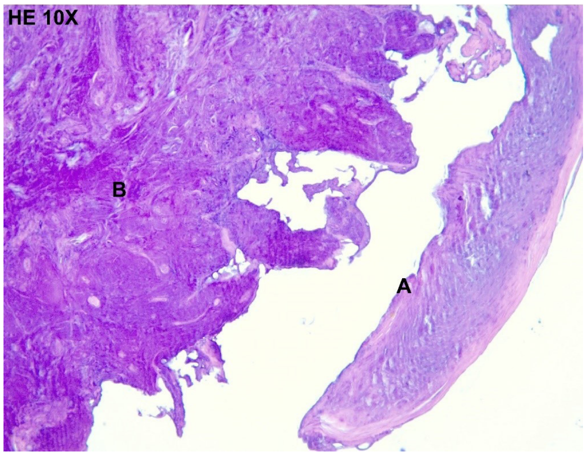

Figure 3. Section Performed With a 980 nm Diode Laser. **(A)** keratinized stratified squamous epithelium with 100% loss of adhesion to the connective tissue. **(B)** Connective tissue with basophilic areas of moderate thermal necrosis

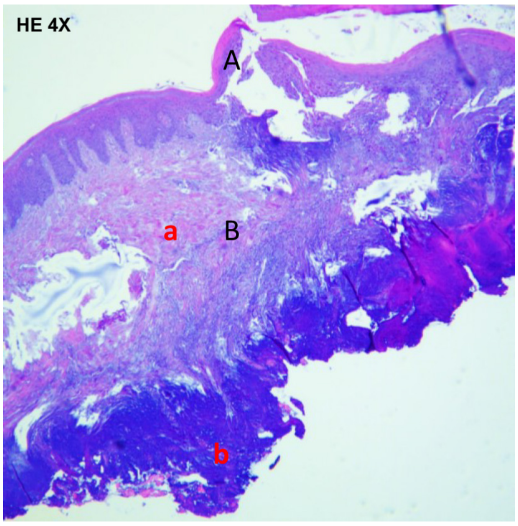

Figure 2. Section performed with a 940nm Diode Laser. **(A)** keratinized stratified squamous epithelium with 30% loss of adhesion to connective tissue. **(B)** Connective tissue showing eosinophilic (a) collagen fibers (pink), with basophilic areas (purple) in the deepest zone of the specimen, with thermal necrosis generating carbonization (b)

most samples displayed this feature in less than 50% of the resection margin. Notably, the highest level of cell preservation was observed in the samples obtained by using the 450 nm wavelength (8; 80%). When comparing the three groups, a statistically significant difference was identified ($P = 0.026$).

Intraoperative Effects

Throughout the surgical procedure, the evaluation of hemostasis and pain demonstrated a consistent pattern across the three wavelengths, with a predominance of cases reporting the absence of bleeding: 450nm 10 (100%); 940nm: 7 (70%) and 980nm: 9 (90%) and absence of pain: 450nm: 10 (100%); 940nm: 8 (80%) and 980nm: 9 (90%). However, it is noteworthy to emphasize that for the 940nm diode laser, there were instances of mild bleeding (3; 30%) and mild pain (2; 20%).

Discussion

Laser devices currently find a wide range of applications in dentistry.[14-18] One of the primary clinical advantages of surgical lasers over conventional scalpel and electrocautery methods is their ability to achieve intra- and postoperative hemostasis. Additionally, they can reduce postoperative pain and stimulate the healing process, leading to faster wound repair.[19-21] These clinical outcomes are closely linked to the effects that occur at the histological level within the tissue resection area. For this reason, it was proposed to conduct a histological study of gingivectomy samples obtained by using diode lasers with different wavelengths, aiming to evaluate whether the effect varies according to the wavelength.

The histological findings reported in this study are intricately tied to the mechanism of action of surgical lasers, which relies on creating a photothermal effect. This effect involves a localized temperature increase induced by the interaction of the laser with the tissue. This rise in temperature is essential for achieving the intended outcomes, including tissue incision/excision, ablation/vaporization, and/or hemostasis/coagulation.

España-Tost et al have outlined a range of effects that can occur based on the temperature achieved within the target tissue.[22] These effects vary, starting with transient hyperthermia (42–45 °C), progressing to protein denaturation (>65 °C), collagen coagulation and tissue fusion (70–90 °C), and vaporization (>100 °C), and ultimately, ending with carbonization (>200 °C). The most efficiently absorbed lasers induce a rapid temperature increase in the application area. However, because diode lasers are well absorbed by soft tissues, there is a risk of thermal accumulation in the tissue, which is contingent on the duration of application. If temperatures rise significantly, it can lead to tissue

Table 3. Histological Findings in the Connective Tissue

Category	Carbonization, No. (%)			Collagen Coagulation, No. (%)			Basophilia, No. (%)			Preservation of Fibroblast Structure, No. (%)		
	450 nm	940 nm	980 nm	450 nm	940 nm	980 nm	450 nm	940 nm	980 nm	450 nm	940 nm	980 nm
Absent	0	1 (9.1)	0	2 (20)	7 (63.6)	2 (20)	3 (30)	1 (9.1)	0	1 (10)	4 (36.4)	7 (70)
Present in < 50% of the resection margin	9 (90)	9 (81.8)	8 (80)	5 (50)	1 (9.1)	1 (10)	7 (70)	7 (63.6)	4 (40)	8 (80)	6 (54.5)	3 (30)
Present in > 50% of the resection margin	1 (10)	1 (9.1)	2 (20)	3 (30)	3 (27.3)	7 (70)	0	3 (27.3)	6 (60)	1 (10)	1 (9.1)	0
Total	10(100)	11 (100)	10 (100)	10 (100)	11 (100)	10 (100)	10 (100)	11(100)	10 (100)	10 (100)	11 (100)	10 (100)
P value*		0.529			0.086			0.006			0.026	

* Kruskal-Wallis test.

necrosis.

The aforementioned background information aligns with the observed findings of carbonization, collagen coagulation, and basophilia in the gingival samples acquired in this study, all of which can be attributed to the cumulative thermal effect. However, achieving the most desirable outcome, which is to minimize this thermal effect, hinges on various factors, including the operator's technique and the specific characteristics of the periodontal tissue. In cases where a thick gingival biotype is present, the operator may need to pass through the cutting area multiple times to achieve tissue separation, potentially leading to prolonged exposure and increased thermal accumulation

The diode laser not only induces carbonization and coagulation in the resection area but also possesses a distinctive advantage that sets it apart from other instruments. According to Kurtzman and Mahesh,[23] the laser creates a biostimulation zone adjacent to the coagulation area when it comes into contact with soft tissue. This irradiation stimulates tissues and cells, expediting the wound healing process compared to tissues treated with a scalpel or electrocautery. This stimulation can directly affect fibroblasts, promote wound repair, or even induce the proliferation of mesenchymal stem cells, all without altering the DNA of the affected cells, as mentioned in Barboza and colleagues' study.[24]

When comparing our findings with those of other researchers, such as Kazakova et al,[25] it is notable that histological sections of samples obtained with a diode laser in their study exhibited a wider coagulation layer compared to other cutting instruments. They highlighted the excellent deep hemostasis achieved with the diode laser, which is advantageous for surgical procedures from a clinical perspective due to the absence of bleeding.

As for the present study, it was revealed that the samples taken with all wavelengths exhibited some degree of coagulation. Notably, the 980 nm wavelength was the most prominent in generating coagulation, with 70% of the samples showing it in more than 50% of the resection margin, while in 63% of the samples taken with a 940 nm laser, coagulation was not observed. This suggests that the 940 nm wavelength may induce less thermal effect and result in reduced tissue damage. Furthermore, the 450 nm wavelength exhibited even lower thermal effects, as evidenced by the basophilia report. Statistically significant differences were observed when it was compared to the other wavelengths, as 70% of the samples presented basophilia in less than 50% of the resection margin, and 30% did not exhibit it, indicating the preservation of connective tissue and collagen fibers.

On the other hand, Monteiro et al[13] examined the incision regularity in the surgical margins of human oral fibroepithelial hyperplasias removed with lasers of different wavelengths. Similar to our findings, they reported that the 980 nm diode laser exhibited the most irregular incisions, with an average score of 1.86 ± 0.73 on a scale of 0-4. Likewise, in our study, irregularities were observed in most of the samples obtained with the three evaluated wavelengths, with an average score of 1.1 ± 1.2 for the 980 nm wavelength, which displayed the poorest incisional quality at the histological level.

In the context of microscopic tissue carbonization, Cercadillo-Ibarguren et al[26] conducted a histological evaluation of the thermal effects induced on the oral mucosa of pigs when exposed to CO2, Er,Cr:YSGG and diode lasers. They discovered that all samples irradiated with a diode laser exhibited a wide area of noticeable carbonization across almost the entire irradiation perimeter. A parallel finding was observed in the gingival tissue samples in our study, where carbonization was present but limited to less than 50% of the resection margin for all wavelengths.

This suggests that the emission parameters of each laser system play a crucial role in influencing the extent of thermal damage inflicted on soft tissue. However, the wavelength of each laser is a key determinant of the absorption characteristics of the tissue and the resulting thermal effect. To mitigate the thermal effect, the authors recommend configuring the laser parameters appropriately, including power density, continuous or pulsed mode, pulse duration, and pause intervals, to achieve the therapeutic effect with the shortest possible irradiation time.

Upon comparing the histological findings across each study group, it was observed that the effects were similar with the 450 nm and 940 nm wavelengths. However, the 980 nm laser exhibited higher indicators of thermal damage, resulting in some statistically significant differences. Nevertheless, it is essential to note that a larger sample size would be necessary to establish a definitive conclusion regarding the ideal wavelength or the one that generates the least thermal damage.

In a recent in vitro study conducted by Gutiérrez-Corrales et al,[27] it was determined that using an 810nm diode laser with power settings ranging from 0.5 W to 2 W for oral soft tissue biopsies is the optimal choice for minimizing thermal damage in peri-incisional margins.

The findings of this study, along with the existing literature reports, underscore the importance of further research in this field. Expanding this line of inquiry will yield valuable data that can inform decision-making when implementing laser technology in dental practices. Such research endeavors will contribute to maximizing the potential benefits of laser technology for the benefit of patients and the dental community.

Conclusion

The 450 nm, 940 nm, and 980 nm diode lasers demonstrated the ability to induce histological changes in both epithelium and connective tissue, including the carbonization of the connective tissue, basophilia, and collagen coagulation. Upon comprehensive analysis of the study results, it becomes evident that the 450 nm and 940 nm wavelengths tend to result in less thermal damage and better preservation of cells when compared to the 980 nm wavelength. Nevertheless, it is prudent to validate these findings with a larger sample size to strengthen their reliability and significance.

Authors' Contribution

Conceptualization: Jennifer Orozco Páez, Pilar Blanco Flórez.
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Investigation: Pilar Blanco Flórez, Jennifer Orozco Páez, Helida Helena Avendaño Maz.
Methodology: Jennifer Orozco Páez, Helida Helena Avendaño Maz, Josep Arnabat Domínguez.
Project administration: Josep Arnabat Domínguez and Antonio Jesús España Tost.
Resources: Pilar Blanco Flórez, Helida Helena Avendaño Maz.
Supervision: Josep Arnabat Domínguez, Antonio Jesús España Tost.
Validation: Pilar Blanco Flórez, Jennifer Orozco Páez, Helida Helena Avendaño Maz.
Visualization: Pilar Blanco Flórez, Jennifer Orozco Páez, Antonio Jesús España Tost.
Writing–original draft: Pilar Blanco Flórez, Jennifer Orozco Páez.
Writing–review & editing: Josep Arnabat Domínguez, Antonio Jesús España Tost.

Competing Interests

None declared.

Ethical Approval

The researchers in this study adhered to both national and international guidelines, including the Nuremberg Code, the Declaration of Helsinki, the Belmont Report, and relevant Colombian regulations, notably Resolution 008430 of 1993, which outlines the scientific, technical, and administrative standards for health research. Given that the samples used in the study were donated by patients from a private dental office, the researchers obtained informed consent from each participant. This informed consent detailed the objective and justification of the study, information regarding sample processing, and potential benefits of the research. Furthermore, the research underwent a thorough review and received approval from the Institutional Ethics Committee of UNICOC University in Bogotá, Colombia, with approval number 02-02-2023-04, ensuring that the study complied with ethical and regulatory standards.

Funding

Research funding for this project was provided by Doctor Pilar Blanco Flórez.

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