UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Sabrina Del Bianco Vargas

Análise comparativa da atividade antimicrobiana e dano oxidativo induzido por ablação a laser com indocianina verde versus aPDT com azul de metileno e curcumina em biofilme de E. coli em canais radiculares.

Araçatuba 2024

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Dissertação apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de Mestre em Endodontia.

Orientador: Prof. Ass. Dr. Rogério de Castilho Jacinto

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A Deus

Dedico este trabalho a Deus, pois ele tornou isso possível. Ele me deu forças nos momentos em que estava desacreditada. Ele ouviu meus apelos e orações e me guiou durante todo o caminho.

A minha irmã

A Caroline minha irmã tão querida, me ajudou muito com meus filhos nos momentos da minha ausência. Sempre como minha parceira e amiga não me deixando desistir desse sonho.

Aos meus filhos

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Universidade Estadual Júlio de Mesquita Filho

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A vida não é fácil para nenhum de nós, mas isso não importa. O que importa á preservar e, acima de tudo, ter confiança de si mesmo. É preciso sentir confiança para fazer algo e alcançar os objetivos, custe o que custar.

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Vargas, S.D.B. **Atividade antibiofilme da ablação a laser com indocianina verde comparada à terapia fotodinâmica em canais radiculares infectados com Escherichia coli.** 2024. Nº.Dissertação (Mestrado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2024.

Resumo

Introdução: A ablação a laser e a Terapia Fotodinâmica Antimicrobiana (TFD) servem como tratamentos adjuvantes para aumentar a redução microbiana nos canais endodônticos radiculares. Este estudo in vitro avaliou a ablação a laser com indocianina verde (ICG) em comparação com aPDT usando fotossensibilizadores de azul de metileno (MB) e curcumina (CUR) para reduzir biofilmes de Escherichia coli e induzir danos oxidativos em canais radiculares. Métodos: Raízes padronizadas de incisivos superiores (n=100) foram contaminadas com E. coli por 10 dias para formar biofilmes. Os grupos incluíram ICG 0,05% ativado por laser de diodo infravermelho, CUR 0,05% ativado por LED azul, MB 0,01% ativado por laser vermelho, solução salina estéril (Controle Negativo) e hipoclorito de sódio 2,5% (Controle Positivo). Amostras de canal radicular foram coletadas pré e pós-tratamento, semeadas em ágar

BHI para contagem de unidades formadoras de colonia por mililitro. O dano oxidativo foi avaliado pelos métodos de substâncias reativas ao ácido tiobarbitúrico (TBARS) e proteína carbonilada. Os dados percentuais de redução foram submetidos à ANOVA de dois critérios e aos testes de Student-Newman-Keuls ($p < 0,05$), Kruskal-Wallis e Dunn ($p < 0,05$) para redução de UFC e ANOVA one-way ($p < 0,05$) para dano oxidativo.Resultados: Quando testados individualmente contra bactérias Gram-negativas, a ablação a laser com ICG e aPDT com Cur e MB não foram tão eficazes na redução bacteriana quanto o NaOCl. Entretanto, a ablação a laser com ICG induziu maior dano oxidativo em bactérias Gram-negativas, especialmente na análise do TBARS sugerindo seu potencial como terapia adjuvante em procedimentos de canal radicular.

Palavras-chave: Laser de diodo; Infecções endodônticas; Ablação a laser; aPDT; Azul de Metileno; Curcumina.

Vargas. S.D.B. **Comparative Analysis of Antimicrobial Activity and Oxidative Damage Induced by Laser Ablation with Indocyanine Green versus aPDT with Methylene Blue and Curcumin on Eschirichia. coli Biofilm in Root Canals.**2024 Nº.Dissertação (Mestrado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2024.

Abstract

Laser ablation and Antimicrobial Photodynamic Therapy (aPDT) serve as adjunctive treatments to enhance microbial reduction in endodontic root canals. This in vitro study assessed laser ablation with Indocyanine Green (ICG) compared to aPDT using Methylene Blue (MB) and Curcumin (CUR) photosensitizers for reducing Escherichia coli biofilms and inducing oxidative damage in root canals. Methods: Standardized upper incisor roots (n=100) were contaminated with E. coli for 10 days to form biofilms. Groups included ICG 0.05% activated by infrared diode laser, CUR 0.05% activated by blue LED, MB 0.01% activated by red laser, sterile saline (Negative Control), and 2.5% sodium hypochlorite

(Positive Control). Root canal samples were collected pre- and post-treatment, plated on BHI agar for CFU/mL counting. Oxidative damage was assessed using Thiobarbituric acid reactive substances (TBARS) and carbonylated protein methods. Percentage reduction data underwent Two-way ANOVA and Student-Newman-Keuls test ($p < 0.05$), Kruskal-Wallis and Dunn's tests ($p < 0.05$) for CFU reduction, and One-way ANOVA ($p < 0.05$) for oxidative damage. Results: No statistical differences were found among groups for E. coli reduction ($p > 0.05$). All groups had higher reduction than NC and lower reduction than PC ($p < 0.05$). ICG and Curcumin showed higher oxidative damage than MB and controls in protein carbonyl analyses. In TBARS analysis, ICG exhibited the greatest oxidative damage, statistically higher than other photosensitizers, negative, and positive controls. Conclusion: There was no difference between Laser ablation with ICG and aPDT with Cur and MB, which were less effective in bacterial reduction than NaOCl. However, ICG-induced higher oxidative damage in

Gram-negative bacteria, suggesting its potential as an adjunctive therapy in root canal procedures.

Keywords: Diode laser; Endodontic infections; Laser ablation; aPDT; Methylene Blue; Curcumin.

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(ICG) Indocyanine Green

(MB) methylene blue

(CUR) curcumin

(FS) photosensitizers

(PDT) photodynamic therapy

(MO) microorganisms

(CFU) colony-forming units

(E. Coli) Escherichia Coli

(LED) Light- Emiting Diodo

Sumário

Artigo

1-Introduction:

Endodontic treatment primarily aims at the effective decontamination of the root canal system (RCS) in teeth with pulp necrosis (1). Various therapeutic strategies, including biomechanical root canal preparation (BCP) (2), the use of sodium hypochlorite irrigating solutions and the application of intracanal calcium hydroxide medication are employed to achieve this goal, reducing the quantity of microorganisms (MO) and their toxic byproducts (3). However, studies indicate that BCP combined with irrigating solutions and intracanal medication alone does not completely eliminate MO from the RCS, especially in cases of persistent infections (1; 4). Consequently, ongoing research investigates novel therapeutic strategies to eradicate endodontic infections.

With the advancement of Laser and LED (Light Emitting Diode) devices, innovations have emerged for various health treatments, such as photodynamic therapy (PDT) (5). PDT involves the action of an exogenous photosensitizer (PS) activated by specific-wavelength light (Laser or LED) to selectively destroy a target site (6; 7). The process of

photosensitization involves the absorption of photons by a photosensitizer (PS), leading to the formation of highly reactive singlet oxygen species that can cause oxidative damage to lipids, amino acids, and proteins (8). This damage can trigger apoptosis, a form of programmed cell death, through a variety of signaling mechanisms, including oxidative stress (9). The oxidation of phosphatidylserine (PS) during apoptosis is a key step in the externalization of PS in the plasma membrane, a process that is essential for the engulfment of apoptotic cells (10). The mechanisms by which reactive oxygen species (ROS) cause or regulate apoptosis include receptor activation, caspase activation, Bcl-2 family proteins, and mitochondrial dysfunction (11). PDT is employed in endodontics after BCP and before intracanal medication (for two-session endodontic treatments) or after BCP and before root canal obturation (for single-session endodontic treatments). Both PDT alternatives aim to enhance RCS decontamination (12). Studies confirm that PDT enhances root canal disinfection, as opposed to antibiotics, which have specific actions. Singlet oxygen derived from a photodynamic reaction, on the other hand, has a nonspecific mechanism of action, preventing the development of microbial resistance (13; 14). Among commercially available PS, methylene blue

(methylthioninium chloride, MB), curcumin, and indocyanine green are noteworthy. MB, possessing photosensitizing characteristics, is utilized in PDT due to its absorption band in the range of 500 nm to 700 nm, activated by red light (15). Curcumin (CUR), a natural compound, has an absorption band between 420 and 480 nm, making it activated by blue light (16).

Similar to photodynamic therapy, laser ablation is a two-stage treatment involving the application and retention of a photosensitizer compound in target tissues (first stage), which is then activated by exposure to light at an appropriate wavelength (second stage). Indocyanine green (ICG) has been widely used for successful laser ablation due to its non-toxic and effective properties, with high absorption capacity at a utilized light wavelength. ICG exhibits antimicrobial properties, especially when activated by diode laser with a wavelength (810 nm) matching its absorption peak. Its light spectrum allows for a better water absorption by dental tissues compared to low-power lasers. Increased diode laser power damages bacterial cell viability through irradiation, not solely due to temperature rise (12). Literature demonstrates significant antimicrobial activity using indocyanine green against Enterococcus faecalis or Streptococcus mutans biofilms (12,17), but not as effective against Porphyromonas

gingivalis biofilms (18), which is a Gram negative bacteria. Nevertheless, there is a lack of studies comparing the antimicrobial efficacy of laser ablation using ICG against Gram-negative bacteria.

Based on the above, the objective of this study was to evaluate the efficacy of laser ablation using ICG in comparison to aPDT protocols utilizing MB or curcumin CUR as photosensitizers, specifically in reducing the biofilm formed by a Gram-negative bacterial species (E. coli) within root canals. Additionally, the study aimed to investigate the impact of these treatments on inducing oxidative damage in bacterial cells. The first null hypothesis posits that there are no differences between laser ablation with ICG and aPDT with CUR or MB concerning the reduction of E. coli biofilm. The second null hypothesis asserts that there are no differences between laser ablation with ICG and aPDT with CUR or MB in terms of causing oxidative damage to E. coli cells.

2- Material and Methods

Sample Size Calculation: The sample size calculation was based on data from a previous investigation (19), using the software www.sealedenvelope.com/power, with a Type I error (α) of 0.05 and a power (β) of 0.80. The calculation indicated that 17 teeth were required for each group. Considering the potential loss of teeth during the study, a total of 20 teeth were included per group.

Selection and Preparation of Bovine Tooth Samples:

Endodontic treatment primarily aims at the effective decontamination of the root canal system (RCS) in teeth with pulp necrosis (1). Various therapeutic strategies, including biomechanical root canal preparation (BCP) (2), the use of sodium hypochlorite irrigating solutions and the application of intracanal calcium hydroxide medication are employed to achieve this goal, reducing the quantity of microorganisms (MO) and their toxic byproducts (3). However, studies indicate that BCP combined with irrigating solutions and intracanal medication alone does not completely eliminate MO from the RCS, especially in cases of persistent infections (1; 4). Consequently, ongoing research investigates novel therapeutic strategies to eradicate endodontic infections.

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tissues compared to low-power lasers. Increased diode laser power damages bacterial cell viability through irradiation, not solely due to temperature rise (12). Literature demonstrates significant antimicrobial activity using indocyanine green against Enterococcus faecalis or Streptococcus mutans biofilms (12,17), but not as effective against Porphyromonas gingivalis biofilms (18), which is a Gram negative bacteria. Nevertheless, there is a lack of studies comparing the antimicrobial efficacy of laser ablation using ICG against Gram-negative bacteria.

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Bacterial Culture and Contamination Process: The bacterial strain was transferred to 5.0 ml of Brain Heart Infusion (BHI) broth. The cellular suspension was adjusted in a spectrophotometer to achieve a turbidity of 1.5 x 10^{-8} colony-forming units (CFU/mL), equivalent to 0.5 McFarland standard. Each specimen-containing flask was opened in a laminar flow hood. Sterile pipettes were used to remove 2.0 ml of sterile BHI and replace it with 2.0 ml of bacterial inoculum. This process involved sequential centrifugation at 1,400 *g*, 2,000 *g*, and 3,600 *g* for 2 cycles of 5 minutes each at different speeds. The flasks were sealed and maintained at 37 °C for 10 days, in an aerobic incubator. During this period, 1.0 ml of contaminated BHI was replaced with 1.0 ml of freshly prepared BHI every 1 day to prevent medium saturation. At each medium change, vortexing for 1 minute and centrifugation for 5 minutes at 3,600 *g* at 25 °C were alternately performed. Turbidity of the medium was monitored during the incubation period to observe bacterial growth.

Experimental Group Design: The samples were randomly distributed into 5 experimental groups (n=20) based on the decontamination protocol:

- **Group A:** MB+RL (Methylene Blue + Red Laser) - Root canals were filled with 0.01% MB (OS ChimioLux, DMC Importação e Exportação de Equipamentos LTDA, São Carlos – SP, Brazil) with a pre-irradiation time of 180 s. Activation was done with a RL (λ 660 nm - Laser DUO, MMOptics, São Carlos, SP, Brazil) for 60 s, using a flexible optical fiber with a diameter of 300 μm (MMOptics, São Carlos, SP, Brazil), with a final energy of 72 J/cm^2.

- **Group B:** CUR+BL (Curcumin + Blue LED) - Root canals were filled with 0.05% CURC(Apothicário – Manipulação Pharmacy, Aracatuba-SP) with a pre-irradiation time of 300 s. Activation was done with a blue LED (λ 480 nm - Poly Wireless – Kavo Kerr, Joinville-SC) for 240 s, using a flexible optical fiber with a diameter of 300 μm (MMOptics, São Carlos, SP, Brazil), with a final energy of 72 J/cm^2.

- **Group C:** ICG+DL 2.5/300/100 (Indocyanine Green + Infrared Diode Laser 2.5 W power, 300 ms interval, and 100 ms duration) - Root canals were filled with 0.05% ICG (MP Biomedicals - Thermo Fisher Scientific) with a pre-irradiation time of 30 s. Activation was done with an infrared diode laser (λ 810 nm - Donatello, CAO Group,

West Jordan-UT) for 30 s, performed twice, with a power of 2.5 W, interval of 300 ms, and duration of 100 ms.

- **Group D:** NC (Negative Control) n=20 - Root canals were irrigated with 2 ml of sterile saline and did not receive laser treatment.
- **Group E:** PC (Positive Control) n=20 - Root canals were irrigated with 2 ml of 2.5% NaOCl, neutralized with 2 ml of 5% sodium thiosulfate.

Sample Collection and Microbiological Analysis:

The root canal content was collected at two time points: 10 days after bacterial contamination (S1) and immediately after the different treatment protocols (S2). Each canal was filled with Ringer's solution (1 ml) (Sigma-Aldrich, St. Louis, MI, USA)., and three sterile absorbent paper points #60 (Dentsply Maillefer, Switzerland) were inserted into the canal for 1 minute each. Subsequently, the paper points were transferred to 2 ml Eppendorf tubes containing 1 ml of sterile Ringer's solution.

Each sample was homogenized and diluted at 10^{-3} before and 10^{-1} after different treatment protocols. The dilutions were cultured on Brain Heart Infusion (BHI) agar plates and

incubated for 24 hours at 37 °C, in an aerobic incubator. Microbial growth was determined by counting the number of colony-forming units (CFU/ml) of *E. coli*.

Assessment of Oxidative Damage Products:

The *E. coli* strain was reactivated on BHI agar and transferred to BHI broth. The bacterial broth was incubated at 37 °C for 7 hours. Following this period, the cellular suspension was adjusted in the spectrophotometer to achieve a turbidity of 1.5 x 10^{-8} CFU/mL, equivalent to 0.5 McFarland standard. Subsequently, 2 ml of the inoculum was placed in a 2 ml Eppendorf tube and centrifuged at 10,000 rpm at 4 °C for 10 minutes to form a pellet. The supernatant was discarded, and the process was repeated 12 times. After the final centrifugation, the supernatant was discarded, and the treatment protocols were applied to the pellet for each photosensitizer and controls.

Each group consisted of n=10 and underwent treatment as described in the Experimental Group Design. Pellets were washed with 2 ml of 0.9% NaCl to remove excess photosensitizer. The samples were frozen at -20 °C until oxidative damage analysis.

Homogenates were prepared in a 50 mmol/L sodium phosphate buffer (pH 7.4) containing 0.2% (v/v) Triton X-100

and 2 mmol/L phenylmethylsulfonyl fluoride (PMSF). The samples were sonicated for 10 seconds at 100% amplitude. Homogenates were centrifuged at 10,000 rpm for 10 minutes at 4 °C. The supernatant was collected for the analysis of substances reactive to thiobarbituric acid (TBARS) and carbonyl proteins.

Statistical Analysis:

Collected data (UFC/ml) were statistically analyzed using Sigma Plot 12.0 software for Windows (Systat Software Inc, San Jose, CA). A comparison before and after decontamination was conducted using Two-way ANOVA and Student-Newman-Keuls test ($p < 0.05$) and Kruskal Wallis and Dunn's tests, $p<0.05$ for percentage of CFU reduction. and One-way ANOVA ($p < 0.05$) for oxidative damage

Results

Table 1 displays *E. coli* CFU (log_{10}) counts before and after decontamination procedures, along with the percentage of *E. coli* CFU reduction (n=20 per group). All groups exhibited statistical differences before and after decontamination protocols ($p < 0.05$), except for the negative control (physiological saline).

Following the decontamination protocols, all groups showed statistically significant cfu reduction compared to the negative control (saline) ($p < 0.05$). There were no statistical differences between the aPDT protocols with MB or CUR and the laser ablation protocol with ICG ($p > 0.05$).

Figure 1 shows Mean and Standard Deviation of TBARS concentrations in *E. coli* cells after treatment with each

photosensitizer (PS) in comparison to NaOCl and Saline Solution.

Results from Figure 1 reveal that ICG induced the highest oxidative damage in *E. coli* cells, exhibiting statistical differences compared to other photosensitizers and both the negative control (saline) and positive control (sodium hypochlorite).

Results from Figure 2 illustrate the Mean and Standard Deviation of carbonyl protein concentrations in *E. coli* cells following treatment with each photosensitizer (PS) compared to NaOCl and Saline Solution. In protein carbonyl analyses, ICG and Curcumin exhibited statistically higher oxidative damage than MB and the controls. In TBARS analysis, ICG demonstrated the most substantial oxidative damage in *E. coli* cells, showing statistical significance compared to other photosensitizer s and both the negative (serum) and positive (sodium hypochlorite) controls.

Tabela 1 - E. coli CFU (log_{10}) counts before and after decontamination procedures, and percentage of E. coli CFU reduction

Groups	Treatments				
	MB	CUR	ICG	NC	PC
Before decontamination	6.13*(0.76)* ^{A,a}	5.99*(0.68)* ^{A,a}	6.27*(0.66)* ^{A,a}	6.40 *(0.43)* ^{A,a}	5.82 *(0.83)* ^{A,a}
After decontaminat	4.38 *(0.49)* ^{B,a}	3.91*(0.48)* ^{B,a}	3.86 *(0.23)* ^{B,a}	6.03*(0.65)* ^{B,a}	2.76(2.86) ^{B,c}
% CFU reduction*	28*(6)* ^{a}	34*(12)* ^{a}	38 *(9)* ^{a}	6 *(7)* ^{b}	55*(46)* ^{a}

Results expressed as mean (SD). Superscript letters indicate pairwise comparisons between before and after decontamination within each group (upper-case), and among treatments within before or after decontamination (lower-case) (2-way, ANOVA and Student-Newman-Keuls test, $p<0.05$, $n=20$). For % CFU reduction, superscript letters indicate significant differences among groups (Kruskal Wallis and Dunn's tests, $p<0.05$, n=20/group). (MB – Metilene Blue; CUR – Curcumin; ICG – Indocyanin Green; NC - Negative Control; PC – Positive Control).

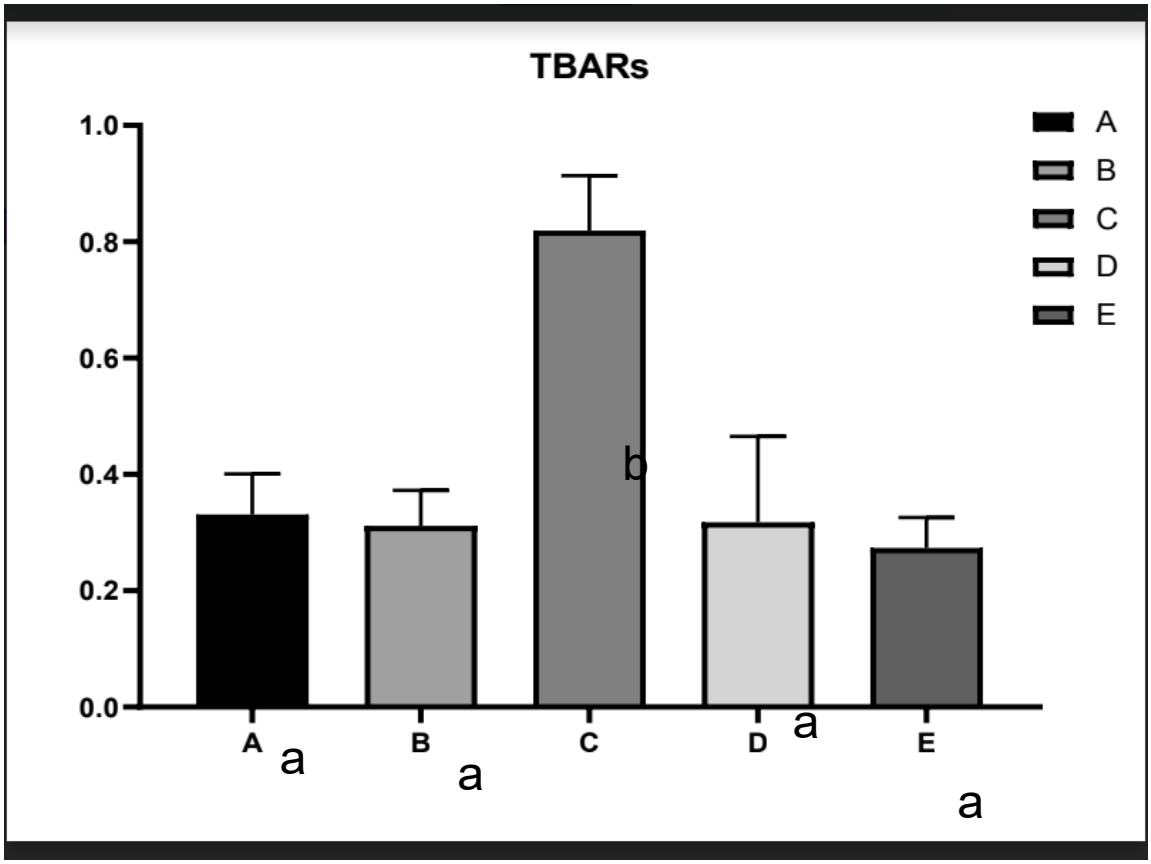

Figura 1: Mean and Standard Deviation of TBARS concentrations (mmol/g of total protein) in *E. coli* cells after treatment with each photosensitizer (PS) compared to NaOCl and Saline Solution. A = MB; B = CUR; C = ICG; D = Saline; E = NaOCl. Different lowercase letters indicate statistically significant differences among the groups (One Way Anova $p < 0.05$,

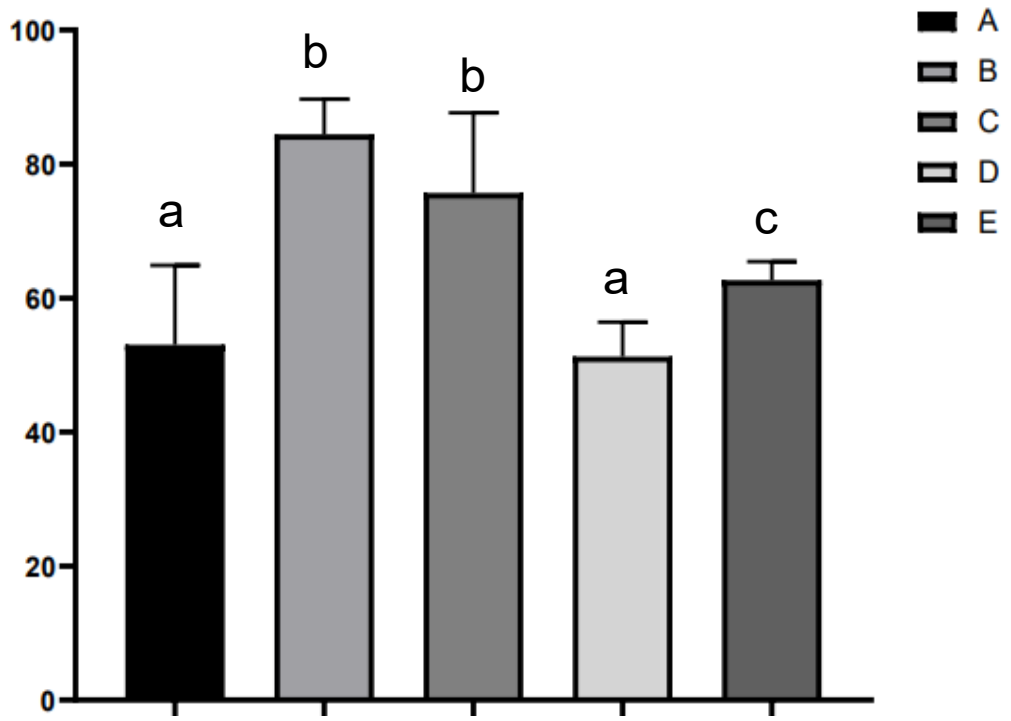

Figura 1 - Mean and Standard Deviation of carbonyl protein concentration

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Figure 2: Mean and Standard Deviation of carbonyl protein concentrations (µmol/g of total protein) in E. coli cells post-treatment with each photosensitizer (PS) compared to NaOCl and Saline Solution. A = MB; B = CUR; C = ICG; D = Saline; E = NaOCl. Different lowercase letters indicate statistically significant differences among the groups (One Way Anova, $p < 0.05$, n=10/group).

Discussion

The primary goal of endodontic therapy is to reduce bacteria and their byproducts, crucial for preventing apical diseases (1). Pathogenic bacteria, particularly Gram-negative rods, can contribute to osteoclastogenesis, invade root canals, and induce osteoblast apoptosis (20-22). Gram-negative bacteria are also associated with symptomatic endodontic infections, manifesting as pain, purulent exudate, and tenderness to percussion (23). This study assessed the efficacy of laser ablation with indocyanine Green (ICG) in reducing bacterial cells and causing oxidative damage in biofilms from Gram-negative bacteria within root canals, in comparison with aPDT using Curcumin (CUR) or Methylene Blue (MB). Although no statistical differences were found between the photosensitizers, confirming the first null hypothesis, ICG induced higher oxidative damage to *E. coli* cells compared to MB and CUR, thus rejecting the second null hypothesis.

Antimicrobial photodynamic therapy has been utilized to enhance root canal disinfection, involving a photosensitizer (PS) activated by specific light, leading to microbial destruction (24). Laser ablation, akin to aPDT, involves applying and retaining a PS compound in target tissues, subsequently activated by light exposure (24). In this study,

there was no difference between laser ablation with ICG and aPDT with CUR or MB, differing from Yamamoto et al. (12) who reported superior results for ICG against Gram-positive bacteria (*E. faecalis*). On the other hand, Rabelo et al (25) showed that aPDT was not effective in reducing Gram-negative bacteria products.

Sodium hypochlorite (NaOCl) is a widely used irrigant during biomechanical preparation due to its antimicrobial and tissue-dissolving properties (26). In this study, all laser protocols were less effective in reducing *E. coli* compared to NaOCl. Conversely, against *E. faecalis*, aPDT and laser ablation were as effective as NaOCl (12). The Gram-negative structure of *E. coli*, with an outer layer of lipopolysaccharide (LPS), may explain the reduced efficacy of laser therapy, as LPSs decrease its antimicrobial efficacy Shrestha et al. (27).

Although no statistical difference in bacterial reduction was observed among the photosensitizers, ICG demonstrated the highest reduction. Additionally, ICG caused significantly higher oxidative damage, as observed in protein carbonyl and TBARS analyses. Photosensitizers, when activated, produce reactive oxygen species (ROS) attacking bacterial targets, leading to oxidative damage to lipids and proteins (28). Further analysis with a detailed understanding of

intracellular effects through transcriptomics, proteomics, and metabolomics are crucial for comprehending the mechanisms of laser therapy.

Considering the in vitro nature of this study, caution is necessary in interpreting the results due to inherent limitations. The use of a single strain and the absence of a polymicrobial nature may limit the generalizability of findings. Future investigations with multi-species biofilm models could provide valuable pre-clinical insights. While the present study evaluated the antimicrobial action of aPDT and laser ablation as standalone protocols, their clinical use is recommended as adjuncts to biomechanical preparation, irrigating solutions, and intracanal medication. Despite its limitations, the study suggests that ICG, with its distinctive mechanism of action causing damage to bacterial walls, could be a valuable adjuvant to root canal therapy.

Conclusion

In conclusion, when tested individually against Gram-negative bacteria, laser ablation with ICG and aPDT with Cur and MB were not as effective in bacterial reduction as NaOCl. However, laser ablation with ICG induced higher oxidative damage in Gram-negative bacteria, especially in TBARS

analysis, suggesting its potential as an adjunctive therapy in root canal procedures.

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Anexo 1

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Projeto de Pesquisa intitulado **"Atividade antibiofilme de ablação a laser com indocianina verde comparada à terapia fotodinâmica em canais radiculares infectados com Escherichia coli"**, Processo FOA nº 191-2023, sob responsabilidade de Rogério Castilho Jacinto apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 21 de Março de 2023.

VALIDADE DESTE CERTIFICADO: 06 de Novembro de 2023.
DATA DA SUBMISSÃO DO RELATÓRIO FINAL: até 06 de Dezembro de 2023.

CERTIFICATE

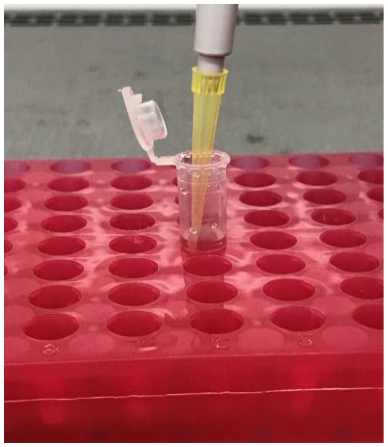
We certify that the study entitled **"Antibiofilm activity green laser ablation compared to photodynamic therapy in root canais infected with Escherichia coli"**, Protocol FOA nº 191-2023, under the supervision of Rogério Castilho Jacinto presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on March 21, 2023.

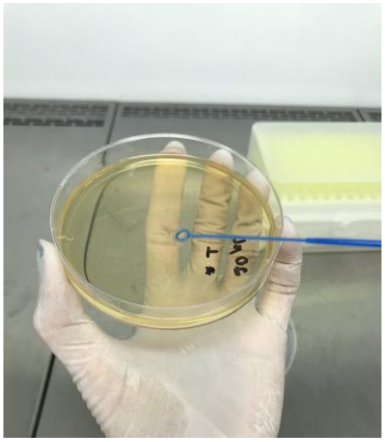
VALIDITY OF THIS CERTIFICATE: November 06, 2023.
DATE OF SUBMISSION OF THE FINAL REPORT: December 06, 2023.

Prof. Dr. Fellippo Ramos Verri
Coordenador da CEUA
CEUA Coordinator

Anexo 2

Cultivo de *Escherichia coli*





Anexo 2

Preparo do inóculo e contaminação das amostras

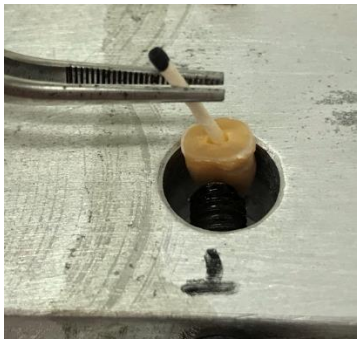

Substituição do BHI estéril pelo inóculo bacteriano


Centrifugação das amostras

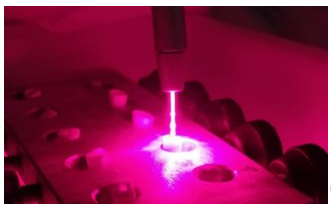
Agitação em vórtex


Amostras em aparelho de alumínio estéril

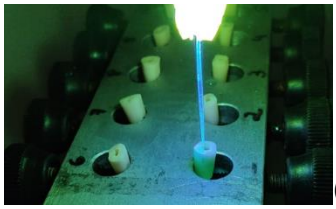
Coleta das amostras



Protocolo de ativação da luz



Grupo MB+RL



Grupo CUR+BL

Grupo ICG+DL