Antimicrobial photodynamic therapy mediated by methylene blue in surfactant vehicle as adjuvant to periodontal treatment. Randomized, controlled, double blind clinical trial

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ABSTRACT

Background: Antimicrobial photodymanic therapy mediated by methylene blue has been investigated as an adjunctive to periodontal treatment but the dimerization of photosensitizer molecules reduces the phototoxic effects. Sodium dodecyl sulfate is a surfactant that may control this aggregation. The aim of this study was evaluated the photodynamic effect of methylene blue in sodium dodecyl sulfate in periodontitis.

Methods: 36 participants with periodontitis were selected and allocated randomly in two group for intervention and other two for control – all of them were treated with scaling and root planing before aPDT. Three periodontal evaluations were done: at the selection time, at the day of intervention and thirty-day after this. Pre-irradiation time was 1min and 2min for irradiation. Laser (Therapy XT, DMC, São Carlos, Brazil) with wavelength of 660nm and 100mW of power was used. Two photosensitizer solutions with 100 μM methylene blue was used, one of them was in water and other in 0,25% of sodium dodecyl sulfate. Two sites of each participant were selected for the experimental procedures. Microbiological evaluations were performed to quantify microorganisms before and immediately after intervention. Quantitative microbiological evaluation was the primary outcome; morphological aspects of bacterial colony, and clinical probing depth was the secondary one.

Results: There was no significant difference between the groups in both bacterial reduction and the clinical parameter evaluated.

Conclusion: The effect of methylene blue in surfactant did not cause enough phototoxic effects that could promote reduction of periodontal pocket depth.

Keywords: Antimicrobial photodynamic therapy, sodium dodecyl sulfate, periodontal disease, antimicrobials, laser therapy

1.Introduction

Periodontitis is a disease whose treatment aims to reduce bacterial load of periodontal pockets [1]. It has been shown that conventional mechanical therapy may not reach these goals due to the anatomic roots complexity [2]. The use of antimicrobials may cause adverse effects and lead to the development of bacterial resistance [3]. Antimicrobial photodynamic therapy (aPDT) emerges as an alternative to antimicrobials [4] and a way to increase access to periodontal treatment by reducing costly surgical procedures. aPDT is a noninvasive procedure and has been used as an adjunct to the treatment of periodontitis. It is based on the use of a photosensitizer (PS) which dyes the cells, and in the presence of adequate wavelength light, promotes its death by the formation of reactive oxygen species (ROS) [4–7].

Methylene blue (MB) is a widely used dye, but there are no conclusive clinical trials that show its efficiency when used in aPDT[8]. There is a need to understand how to improve the performance of MB within the periodontal pockets during aPDT treatment. Dimerization is one of the factors that decreases the photochemical reactions of MB. This occurs due to its planar molecular nature that allows the aggregation of the photosensitizer, preventing the arrival of light. Sodium dodecyl sulfate (SDS) acts as a surfactant agent and has been safely used in oral formulations such as mouth rinses and toothpastes [9,10]. SDS controls dimerization [9,11–13] promoting the micelles formation and depending on both concentration, the generation of singlet oxygen is increased. And, it may dissolve the membrane and bacterial wall [14].

The ratio dimer/monomer of MB is used to determine the degree of its aggregation and previous studies showed that MB in a solution of 0,25% w/v SDS produced significant bacterial reduction [9]. Alvarenga, 2019 [15], concluded that the SDS promoted 96% bacterial reduction after 5min of irradiation. Tortamano in 2020 [13], using the same concentration and dilution of the PS, concluded that there was toxicity in *P. gingivalis* cultures and who did not present bacterial death *of A. actinomycetemcomitans.*

Thus, the hypothesis evaluated in this clinical trial was that the use of MB in SDS has a higher antimicrobial photodynamic effect when compared to MB in water.

2. Material and methods

This clinical trial was registered on the Brazilian Registry of Clinical Trials (RBR-2g9cf4). Ethical clearance was obtained from Ethical Committee of UNINOVE (number 4.264.322) and written informed consent was signed by the participants.

A total of thirty-six participants diagnosed with periodontitis according to the 2017 World Workshop [16], were selected for the study from Outpatients of UNINOVE School of Dentistry. The whole dentition was assessed to detect more than one non-adjacent tooth interdental clinical attachment level - CAL ≥ 5mm, pocket probing depth - PPD ≥5mm and bleeding on probe - BOP at the day of participants selection, before the first microbial sampling and thirty-days after this sampling [16,17].

2.1. Basic periodontal treatment

All participants received oral hygiene guidance and were motivated to keep these orientations whenever they returned to another sessions, which was included scaling and root planing, as well as polishing with a paste based on pumice and water.

The treatment was performed with manual instruments (Curets Gracey Standard and Curets Gracey mini five, Hu-Friedy) and ultrasonic (Varios, NSK, Japan).

The number of sessions of basic periodontal treatment varied considerably, because the number of sites that were treated and the objective was to reduce the gingival bleeding index around 30% [18]. The variation in the number of interventions of the basic periodontal treatment could not influenced the results because in this study because there was no "conventional treatment" group since the aim of the research was not to compare treatments, but to evaluate the antimicrobial effect of antimicrobial photodynamic therapy.

2.2. Study design

The present study was a randomized, controlled, and double-blind clinical trial.

After the basic periodontal treatment, the participants were randomly assigned into four groups, where two groups received the interventions and the other two were the control groups:

PDT_MB_SDS – treated with aPDT with MB in SDS.

PDT_MB – treated with aPDT and MB in water.

CTR_MB – treated with MB in water without light irradiation.

CTR_MB_SDS – treated with MB in SDS without light irradiation.

A specialist in Periodontics – P1, blinded for analysis, collected all the necessary clinical data at baseline, at the intervention visit, and at the control visit, and the other Periodontist - P2, performed the basic periodontal treatment, the application of photodynamic therapy, and randomization.

Thirty days after the completion of the basic periodontal treatment, the intervention was performed on each of the participants

Block randomization was done with numbers that corresponded to a brown envelope. Inside these envelopes - listed, sealed, and stored in a safe place - there was a sheet printed with the corresponding group. It was done on the www.randomization.com.

The periodontal evaluation was performed with a millimeter periodontal probe (Universal Probe, North Carolina 15, Single Tip, Hu-Friedy PCPUNC156). The clinical parameter was the pocket probing depth -PPD, which is the distance between the gingival margin to the bottom of the pocket. The data were recorded and sent to statistical analysis. These evaluations were done in three moments: at participants selection, before the first microbial sampling at the day of the interventions, and thirty-days after this sampling.

Inclusion criteria were with periodontitis; 10 teeth present; PPD ≥5mm; CAL ≥5mm; BOP <30%; 18 years old. Exclusion criteria were smokers; diabetics; anemia; cancer; pregnant women; antibiotic therapy in the last 6 months; use of

anti-inflammatory drugs in the last 3 months; coagulopathies; orthodontic treatment.

2.3. Antimicrobial photodynamic therapy

The intervention was applied after the basic periodontal treatment. Thirty days were waited to heal periodontal epithelial tissues[20,21] for reducing the risk of dilution of PS by bleeding from the gingival epithelium. The PS (Fórmula e Ação, Vila Mariana, São Paulo-SP) was deposited in periodontal pockets with a syringe and blunt needle, with the application of the bottom to the coronal pocket direction, and a time of 1min of pre-irradiation was adopted. Then, the laser was applied emitting wavelength of 660nm, power of 100mW. The tip of the equipment was placed directly on the mucosa. The irradiation time was 2min per site. Each irradiation point had approximately 0.4cm^2, radiant exposure of 30J/cm^2, irradiance of 0.25W/cm^2. Pockets larger than 5mm did not selected to avoid another irradiation, minimizing the photobiomodulation effects [22] (Table 1). An aluminum foil barrier was placed at the tip of the equipment to block light radiation in control groups. Pre-irradiation and irradiation times were the same. Participants were blinded because they could improve home care if they knew they would be in groups that had received the interventions.

<enter table 1>

2.4. Microbial sampling

Duplicate subgingival biofilm sample were collected from 5mm depth periodontal pockets before and immediately after intervention [23]. Relative isolation teeth made with cotton rollers was done before sampling, Supragingival biofilm was removed with sterile gauze, and the subgingival biofilm sample was obtained by introducing a sterile endodontic absorbent paper tip number 30 (Tanari Absorbent Paper Tips, Manacapuru-AM, Brazil) inside the pocket for 30s. Then they were removed and stored in a sterile plastic microtube holding one milliliter of BHI (culture broth composed of nutrients of brain and heart of cattle, peptone, and dextrose) and properly named, stored under refrigeration, and processed within

20min after collection. The samples were used to decide the colony-forming units - CFUs.

In a plate of 96 wells were marked the sites corresponding to each sample, and 180µL of PBS was placed in the wells to later perform serial dilution (10^{-1} up to 10^{-5} times of the original concentration). The sample microtube was agitated by vortex and 20µL were removed to perform serial dilution on the 96-well plate. 10µL aliquots in 5 dilutions were sown in duplicate in blood agar Petri dishes (Biplaca-Sangue-Agar-TSA-2x10ml-10PL, Laborclin - Products for Laboratories, Pinhais-PR, Brazil). These plates were incubated in anaerobiosis jars at 37°C for a period of 48h for evaluation of the total recovered bacteria, and anaerobiosis sachets were used following the manufacturer's guidelines (Anaerobac, Probac do Brasil Produtos Bacteriológicos Ltda., São Paulo-SP, Brazil). After this time, the CFUs were counting and converted in survival fraction for statistical analysis. Survival fraction data was calculated as the number of CFU/ml after treatment divided by the number of CFU/ml before treatment at the same periodontal site [11]. Visual characteristics of the bacterial colony were evaluated as colony visual size, colony borders and color.

2.5 Sample size calculation

The sample size was calculated using the standard deviation of the bacterial survival fraction randomly extracted from 3 participants of Alvarenga, 2019 [11] was applied in this equation below:

$$n = \frac{\delta^2 Z\gamma^2}{\varepsilon^2}$$

Where *n* is the sample size, □ is the maximum error adopted of 5%, δ is the standard deviation and $Z\gamma$ is the 95% confidence interval, with *z* of 1.96. Resulting in a sample of 5 participants per group to achieve the probability of 95% certainty [24]. And to overcome losses throughout the study, 5 participants were added in each group.

2.6 Statistical analysis

All data were analyzed regarding its distribution using the Shapiro-Wilks test. Parametric data was analyzed using Anova test followed by Tukey test as post-hoc. Nonparametric data was analyzed using Kruskal-Wallis ANOVA followed by Wilcoxon test as post-hoc. The post-hoc p-values were adjusted using the Ryan-Holm stepdown Bonferroni procedure when necessary. The significance level is $\alpha = 0.05$.

3. Results

Thirty-six participants were treated in this clinical study, and they were distributed in the 4 groups. Samples of subgingival biofilm were collected from two sites in each participant, before and immediately after intervention, totaling 144. All were plated in blood agar in duplicate, finishing 288 plaques. Thirteen were lost by failure in laboratory manipulation, but all groups respected the sample size calculation of this assay.

When the growth pattern of bacterial colonies in petri dishes was verified, it can be observed that there was no bacterial reduction, and the growth pattern of the colonies remained the same before and after irradiation in the CTR_MB and PDT_MB groups.

In the PDT_MB_SDS group, there was a reduction in the number of CFU/ml and the colonies presented lower volume when compared to those before irradiation, demonstrating delay in their growth (fig 1).

<enter figure 1>

To analyze the CFU, the survival fractions of each participant were calculated. From the counting of the number of colonies present in each dilution, the CFU/ml was calculated in each of the 5 dilutions. The mean CFU/ml of all dilutions was calculated. The survival fraction was obtained by dividing the mean of the final CFU/ml by the average of the initial CFU/ml, and they were submitted to statistical analysis.

Figure 1 Growth pattern of bacterial colonies before and after intervention in the group treated with 100 μM methylene blue in SDS. CTR_MB_SDS group treated without

Click here to access/download;Figure;FIGURE1_KASSA2022.jpg

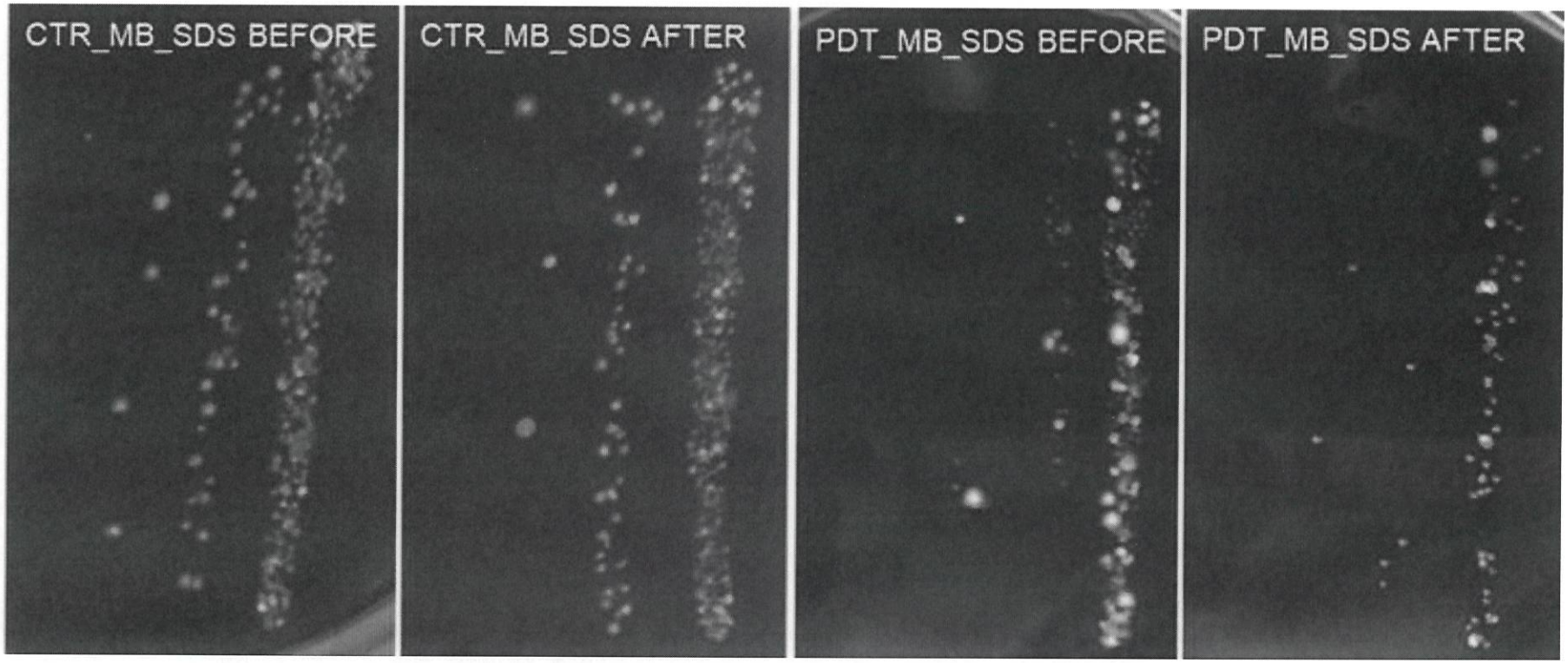

Figure 2

Click here to access/download;Figure;FIGURE2_KASSA2022.jpg

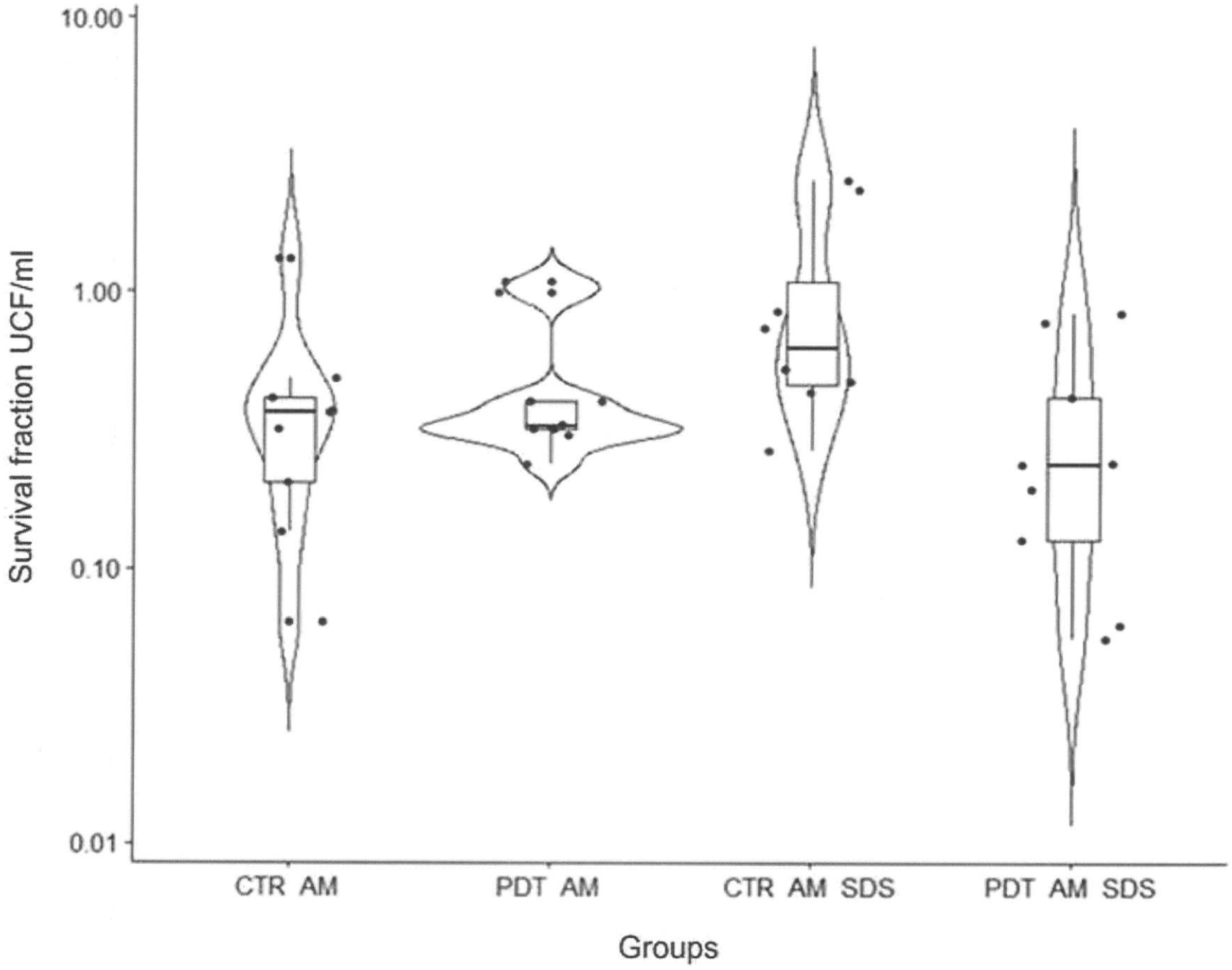

Figure 3

Click here to access/download;Figure;FIGURE3_KASSA2022.jpg

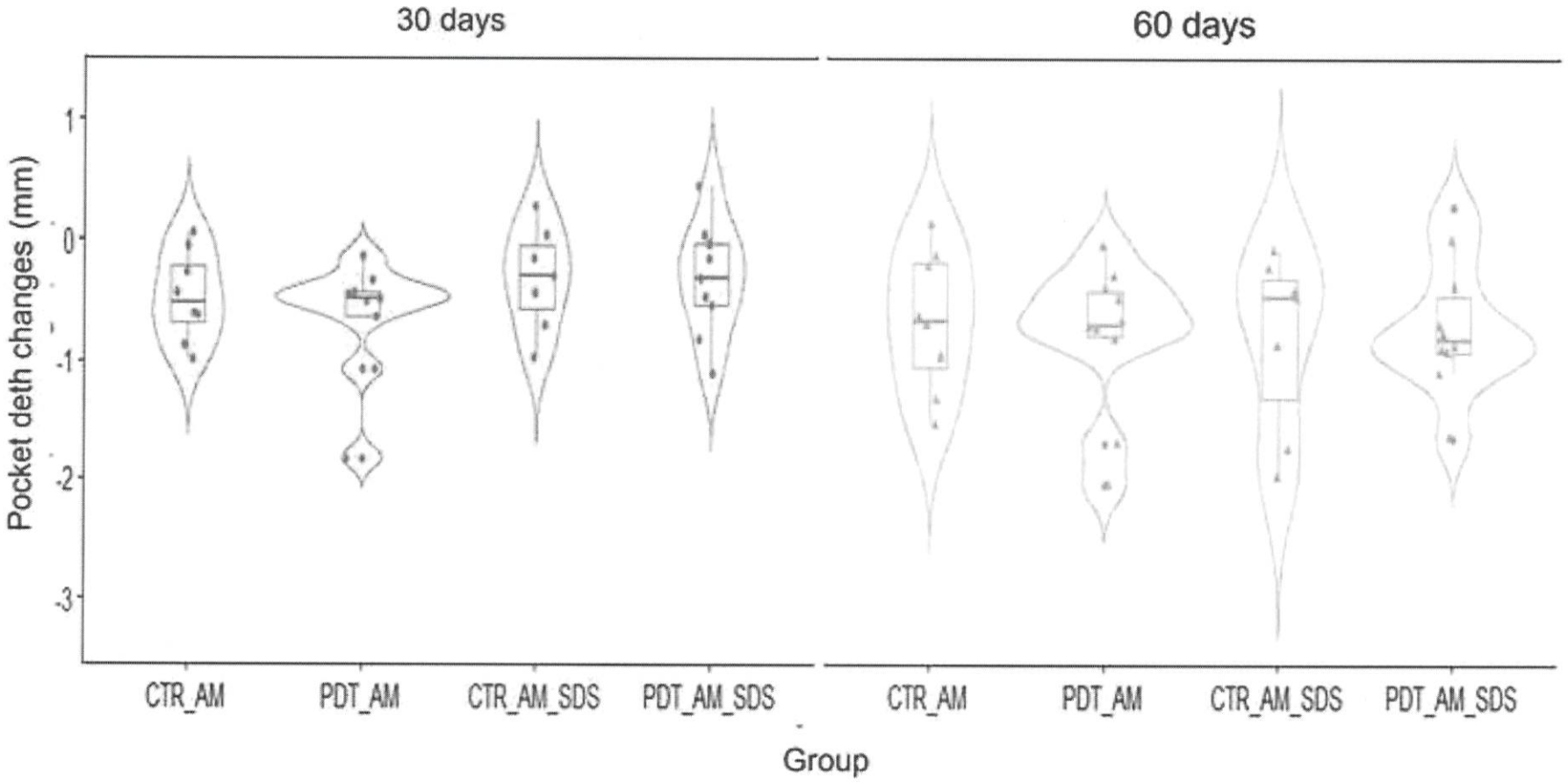

Table 1 – Dosimetric parameters

Parameter	
Wavelength (nm)	660 ±10
Operation mode	Continuous
Radiant average power (mW)	100
Polarization	Randomized
Device irradiated area (cm^2)	0,002827
Target irradiated area (cm^2)	0,4
Irradiance (W/cm^2)	0,25
Exposure time (s)	120
Radiant exposure (J/cm^2)	30
Radiant energy (J)	12
Application technique	contact
Session	Single
Number of dots	1 dot every 3mm