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*Published in:*
Journal of Periodontology

*DOI (link to publication from Publisher):*
10.1002/JPER.19-0562

*Publication date:*
2020

*Document Version*
Accepted author manuscript, peer reviewed version

# Adjunctive effects of laser therapy on somatosensory function and vasomotor regulation of periodontal tissues in patients with periodontitis– A randomized controlled clinical trial

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**4 Figures; 2 Tables; 44 references; 3996 Word Count**

**One sentence summary:** The periodontal condition was improved after conventional SRP treatment, however adjunctive laser therapy did not provide any further advantage on fluctuation of periodontal somatosensory function or gingival microcirculation in the present study.

**Running Title:** Periodontal somatosensory function and vasomotor regulation

**AUTHORS CONTRIBUTION:** All authors have made substantial contributions to conception and design of the study. HG, RF, XC, LL, XW, YX have been involved in data collection and

data analysis. HG, RF, YX, PS and KW have been involved in data interpretation, drafting the manuscript and revising it critically and have given final approval of the version to be published.

**Abstract**

**Background:** The purpose of this prospective study was to compare the changes in periodontal somatosensory function and microcirculation in patients with periodontitis following initial treatment with scaling and root planing (SRP) with or without adjuvant laser therapy.

**Methods:** Twenty-four patients suffering from periodontitis were recruited and randomly allocated into a split-mouth design to either SRP combined laser therapy side (test side) or SRP only side (control side). All treatments were performed by the same investigator at a single visit. Laser Doppler Flowmetry (LDF) and Quantitative Sensory Testing (QST) were performed at baseline (W0), 1 week (1W), 2 weeks (2W), and 4 weeks (4W) after treatment on both sides of the attached gingiva of the maxillary lateral incisor. Clinical examination including pocket probing depth (PPD) and bleeding on probing (BOP) was performed at W0, 2W, and 4W on both sides. Data were analyzed with two-way analysis of variance (ANOVA).

**Results:** The PPD and BOP significantly improved after treatment ($P < 0.001$). The LDF values were significantly decreased on both sides at all follow-up time points ($P < 0.001$), temperature was increased only on the test side ($P = 0.017$) whereas there was no significant change on the control side ($P = 0.792$). Significantly less sensitivity was observed for all QST parameters ($P < 0.030$) except for warmth detection after treatment.

**Conclusion:** Adjunctive use of laser therapy did not provide any significant clinically advantage or additional effects on the recovery of periodontal somatosensory function or gingival microcirculation in the present study.

**Keywords:** Quantitative sensory testing, blood flow, Nd:YAG laser, periodontal somatosensory function, inflammation

## Introduction

Periodontitis characterized by progressive inflammation and tissue destruction induced connective tissue attachment loss and alveolar bone absorption[1]. Unlike other painful conditions characterized by persistent inflammation and/or tissue destruction, periodontitis is unique in the sense that there is no obvious pain component. By comparison, painful periodontal diseases do exist under acute symptomatic periodontal conditions such as necrotizing ulcerative gingivitis, acute apical periodontitis, pericoronitis, and the advanced stages of chronic periodontitis[2]. However, it is challenging to make direct comparisons between painful and non-painful stages of periodontitis according to the existing literature, since the inflammatory and nociceptive processes have not been sufficiently elucidated so far[3]. The fundamental principle of periodontal therapy is to remove etiologic factors such as bacterial biofilms, calculus, smear layer and infected cementum[4]. Although mechanical scaling and root planing (SRP) has been regarded as the gold-standard for the management

of periodontitis[5,6], in recent years, lasers have gained increased interest as an adjunct or alternative treatment for chronic periodontitis[7]. A plethora of research data have demonstrated that neodymium-doped: yttrium, aluminum and garnet (Nd: YAG) laser is capable to reach the sites where conventional instruments cannot with advantages of analgesia and hemostasis[8, 9].

A histological review of the periodontal ligament innervations revealed that there are both A-delta fibers, transmitting mainly mechano-sensation, pressure, and proprioception and C-fibers, transmitting mainly nociceptive sensations[10]. However, to date there is a surprising paucity of documentation regarding overall innervation of human periodontal tissues under inflammatory conditions and whether laser procedures can provide further impact on the mechano-nociceptive sensitivity of periodontal tissues after SRP treatment. Quantitative sensory testing (QST) as a reliable, non-invasive psychophysical tool has been developed for assessment of somatosensory function by the German Research Network on Neuropathic Pain (DFNS) with further application to the trigeminal area[11, 12]. The QST parameters are considered to be potentially valuable for evaluating small-fiber function[13]. The application of QST could assist in exploring the underlying neurobiological mechanisms of periodontitis and serve as an objective and valuable evidence to determine the host responses to different treatment modalities of periodontitis dialectically on somatosensory function.

Periodontitis with the initial clinical symptoms of gingival redness, swelling and bleeding has attracted scholars' interests to focus on the relationship between microcirculation and inflammation. The gingival microcirculation presented a dramatic dynamic change in response to the development and progression of inflammation resulting in increased

vascularity with more capillary loops, larger vessel size and slowed blood flow, and a restriction of the afferent blood vessels[14]. The effect of periodontal treatment has been shown to ameliorate the disorganized vascular endothelial architecture associated with periodontitis[15]. Laser Doppler Flowmetry (LDF) has been widely used to determine gingival blood flow (GBF) in animal and human gingiva with inflammation and various periodontal diseases[16, 17].

Nevertheless, so far few randomized clinical trials have specifically investigated whether laser has a positive effect in transforming periodontal somatosensory sensitivity and improving periodontal microcirculation as an adjunctive treatment of periodontitis. Thus, the main purpose of this randomized clinical trial was to explore whether there are different changes of somatosensory sensitivity and blood flow responses in periodontal tissues following SRP with or without adjunctive laser treatment in the short term perspective.

## Materials and methods

### *Study participants*

Twenty-four patients (10 men and 14 women, with age of 19-35 years, mean age ± SD; 26.5 ± 3.6 years) diagnosed with periodontitis from the Periodontal department, Affiliated Hospital of Stomatology, Nanjing Medical University, P.R.C. were recruited and received periodontal treatment. All patients met the basic criterion according to the new classification of periodontitis, i.e. localized or generalized or molar/incisor pattern periodontitis of periodontal stage II, III with grades B or C with probing depths＞5 mm in at least one site in each

maxillary quadrant, and radiologically-detectable alveolar bone loss in all quadrants[18]. Additional criteria were: mobility of maxillary lateral incisor <II degree, without endodontic or orthodontic treatment, crown restoration, or dentin hypersensitivity. There were no histories of smoking, previous treatment of periodontal disease, no systemic antibiotics, pain medication, antidepressants or non-steroidal anti-inflammatory drugs (NSAIDs) intake for at least 6 months before the start of the study. There were no orofacial pain complaints and severe systemic diseases, mental disorders or peripheral neuropathy. Women in the menstrual period were excluded. Declaration and informed consent were obtained from all participants prior to participation. The study was approved by the Nanjing Medical University Research Ethics Committee (approval number: PJ2017-051-001) in accordance with the Helsinki Declaration II.

*Experimental protocol*

After clinical, LDF and QST assessments, patients underwent two different treatment modalities. The left or right sides of the maxillary teeth were randomly assigned as the test side or control side according to a computer-generated randomization list for all patients. The teeth on the test sides received additional laser treatment after SRP, whereas the teeth on the control sides received only SRP treatment without administrating antiseptics. All treatments were carried out by one investigator, whereas the baseline and follow-up examinations were performed by another two investigators. LDF, and QST test were applied again at 1 week (1W), 2 weeks (2W), and 4 weeks (4W) and the follow-up clinical examinations were performed at 2W and 4W after the treatment by the same investigators.

*Examiner calibration*

Periodontal clinical measurement was performed by a calibrated examiner who was not involved in the patients' subsequent treatment and data analysis. The examiner recorded full-mouth pocket probing depth, clinical attachment level and bleeding on probing at 6 sites per tooth (with the exclusion of the third molars) of four patients, using a conventional manual periodontal probe (Hu-Friedy, Chicago, USA) and applying approximately a 0.25 N force. Calibration was deemed satisfactory if ≥90% of the recordings of the two visits that were separated by 48 hours could be reproduced within a 1.0 mm difference.

*Clinical examination*

The following clinical measurements were taken: pocket probing depth (PPD), bleeding on probing (BOP) measurements were carried out at six sites in all teeth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual/palatal, mid-lingual/palatal and disto-lingual/palatal) except for the third molars. PPD was calculated as the distance from the bottom of the pocket to the gingival margin.

*Periodontal treatment*

All patients were examined by a professional dental hygienist for supragingival cleaning of the teeth and instructions in oral hygiene one week before root debridement. The SRP was performed using hand instruments (Gracey Curets, Hu-Friedy, Chicago, IL, USA) and an ultrasonic device (MiniPiezons, EMS Electro Medical Systems S.A., Nyon, Switzerland) under local anesthesia.

*Laser therapy*

A Nd: YAG laser treatment (Fotona, Germany) was performed at an energy level of 100 mJ/pulse, and 20 Hz accompanied by air and water cooling accomplished under a 320 μm fiber optic delivery system according to the manufacturer's instruction following the procedure of SRP. The fiber was inserted into the periodontal pocket base in parallel alignment with the root surface, and the fiber was slowly moved from apical to coronal in a sweeping motion during the laser light emission in a direction from mesially to distally at either buccal aspect or lingual aspect for 10 seconds. All treatments were performed at a single appointment for both the test and control groups.

*LDF recordings*

The LDF recordings presented as tissue microvascular blood perfusion (Flux), concentration of moving blood cells (Conc), the relative velocity of microvascular blood flow (Speed) and the temperature of gingiva (Temp) were assessed by a Laser Doppler Flowmetry (Moor Instruments, England). The LDF technique is based on the Doppler principle. Specifically, a laser beam with a wavelength of 780 nm is emitted by an optical fiber to the tissue to be tested. After the configuration, the probe with a diameter of 1 mm was put on the attached gingival tissues – 1 mm away from the free gingiva of maxillary later incisors for 1 min every run and recorded the average value.

*QST*

Thermal quantitative sensory tests were performed at the attached gingiva of the maxillary lateral incisor using a computerized thermal stimulator (MEDOC TSA-2001 apparatus, Medoc Ltd, Ramat-Yishai, Israel). The contact area of the intra-oral thermode was 6×6 mm. Cold and

warm detection thresholds (CDT, WDT) were measured first, followed by cold and hot pain thresholds (CPT, HPT). The mean thresholds of three consecutive measurements were calculated. The temperature of the thermode started at 37 ℃ for the intra-oral sites and cooled down or heated up at a rate of 1 ℃/s to the lower limit of 0 ℃ or upper limit of 50 ℃. Participants were instructed to press a button on the computer mouse as soon as they perceived the thermal sensation of cold, warm, cold pain, or heat pain following the instructions developed by the DFNS.

Mechanical detection thresholds (MDT) were measured at attached gingiva of maxillary lateral incisor using standardized Semmes–Weinstein monofilaments with 20 different diameters (North Coast Medical, Canada). The number of each filament (1.65–6.65) corresponds to a logarithmic function of the equivalent forces of 0.008–300 g. The filament was applied vertically to the test sites: attached gingival tissues, and the pressure was applied slowly until the filament bowed with a total contact time of about 1 s. To detect the mechanical pain threshold (MPT), weighted pinprick stimuli delivered with a custom made set of seven pinprick stimulators (Aalborg University, Denmark) were used. Each stimulator had a flat contact surface of 0.2 mm that exerted forces of 8–512 mN[11]. All pinprick tests were made with the stimulator perpendicularly to the examination site and in a vertical position with the contact time of about 1 s. MDT and MPT were measured using the 'method of limits' technique described by Baumgartner[11].

A handheld pressure algometry (MEDOC, Israel) with a probe diameter of 0.8 cm was used to test pressure pain threshold (PPT)[11, 19, 20]. The pressure was applied to the attached gingival tissues perpendicularly and crowns of the maxillary lateral incisors from two

directions: one perpendicular to the center of facial surface of the crown labeled as “lateral”; the other direction was labeled as “vertical” when the pressure was applied parallel to the axis of the tooth. Three trials were made in random order at each tooth for the two sites and two different directions. There was of 1 min interval between each measurement and 10 min between each trial to minimize sensitization and/or habituation to the stimulus. In accordance with previous studies the PPT was determined with a constant application rate of 30 kPa/s. The participant was instructed to press a hold switch as soon as the sensation of non-pain changed to a sensation of pain.

*Statistical analysis*

The sample size was calculated with risk of type I and type II errors of 5% and 20% respectively and an estimate of the inter-individual variation of 25% and a minimal relevant difference to detect as 20% for QST and LDF measures . Thus, a total of 24 participants were needed. However, taking into account an anticipated 20% drop out rate, we aimed to recruit a total of 30 participants. Descriptive statistics were used to summarize all measurements and data were presented as the means ± SD. The necessary logarithmic transformation was performed to secure normal distribution of the data set. All of the LDF and QST data at different time point were normalized to the W0, and the relative changes of each QST and LDF parameter were compared between two different treatment sides. A two-way mixed model analysis of variance (ANOVA) with repeated measures was used to analyze the QST and LDF measurements at the selected sites at different time points. The between group factor in the ANOVA was treatment (test side and control side), while the within-group factor was time point (four levels: W0, 1W, 2W and 4W after treatment). A LSD honestly significant

difference test was employed for post-hoc comparisons. Repeated measure analysis of variance was used for intra-group comparison of the clinical parameters. The inter-group comparisons used the Paired sample t-test. All statistical calculations were performed using the Statistical Package for Social Sciences, version 20 (SPSS, IBM). The significance level was set at 0.05.

## Results

The outline of this study is illustrated in a flowchart (Figure 1). Total of 24 participants completed all treatment procedures successfully and data were available for the analysis. Healing was uneventful after both therapies.

### *Clinical parameters*

Mean PPD (mm), sites with PPD＞5mm and BOP (%) values and changes for PPD, sites with PPD＞5mm and BOP of maxillary lateral incisor and each quadrant side are presented in Table 1 and 2. There were no statistically significant differences in probing depth and bleeding on probing between test and control lateral incisor and quadrant sides at all time points ($P$>0.060). The probing depth reductions were similar on the test and control sides at all time points ($P$>0.060). In addition, both maxillary lateral incisor and sides showed a statistically significant reduction in probing depth and bleeding on probing at all follow-up points (P<0.050) (Table 1).

*LDF recordings*

There were significant effects of time (ANOVA; F>42, df= 3, P <0.001) but no significant effect of treatment (ANOVA; F<1, df = 1, P>0.300) on values of Flux (PU) and Conc (AU) (Figure 2 AB). The interaction between time and treatment was not significant (ANOVA; F <1, df= 3, P> 0.300). There were no significant effects of time (ANOVA; F = 2.485, df = 2.487, P = 0.076) and treatment (ANOVA; F = 0.006, df = 1, P = 0.937) on the values of Speed (Figure 2 C). The interaction between time and treatment was also not significant (ANOVA; F = 1.046, df = 2.487, P = 0.367). There were significant effects of time (ANOVA; F =3.983, df = 1.933, P =0.024) and treatment (ANOVA; F =6.382, df = 1, P = 0.016) on the values of Temp. The interaction between time and treatment was also significant (ANOVA; F =4.521, df = 1.933, P = 0.016). The relative changes of Temp were significantly higher on the laser treatment side at 1W, 2W and 4W (P=0.037, P=0.022, P=0.013 respectively) (Figure 2D).

*QST parameters*

There were significant effects of time (ANOVA; F>1,df >1.5, P=0.031, P=0.005, P<0.0001, P=0.006, P<0.0001, P<0.0001, P<0.0001, P<0.0001 respectively) but no significant effect of treatment (ANOVA; F<3, df = 1, P>0.100) on the values of CDT, CPT, HPT, MDT, MPT, PPT-gingiva, PPT-lateral or PPT-vertical. The interaction between time and treatment was not significant (ANOVA; F <2, df <3, P> 0.100) (Figure 3 and 4).There were no significant effects of time (ANOVA; F = 1.292,df = 2.536, P = 0.279) and treatment (ANOVA; F = 2.181, df = 1, P = 0.144) on the values of WDT. The interaction between time and treatment was also not significant (ANOVA; F = 1.329, df = 2.536, P = 0.249) (Figure 3 C).

## Discussion

To the best of our knowledge, this is the first study to explore the different changes of periodontal somatosensory sensitivity and microcirculation following disparate non-surgical periodontal treatment. The results of this randomized clinical trial showed that all clinical parameters were improved and periodontal tissues were less sensitive to the thermal and mechanical stimuli after periodontal treatment. Moreover, periodontal therapy resulted in improvement of microcirculation in patients with periodontitis. However, any significant effect of the laser therapy as an adjuvant to scaling and root planing was not detected in the present study.

*Treatment effect on clinical parameters*

In the present study, the Nd: YAG laser was used to remove the epithelium lining the periodontal pocket and to suppress or eradicate periodontal pathogens from periodontal pockets after SRP. The results showed that probing depth and BOP scores were significantly reduced in both groups compared to W0 with no statistically significant differences between the control and test side at any time point. This condition showed no additional clinical improvements and indicates that non-surgical periodontal treatment and optimal personal oral hygiene procedures can provide the resolution of inflammation independently of the treatment method. This finding agrees nicely with a meta-analysis carried out with only three studies and a systematic review included eight publications, which both declared that there

was no sufficient evidence to support the superiority or the efficiency of adjunctive Nd: YAG laser to SRP[8, 21].

*Treatment effect on vasomotor regulation*

In the presence of inflammation, the gingival vascular architecture of animals changes from a regular network into a looped, dilated, and convoluted appearance, which can be reversed by periodontal treatment[15]. The results of this study demonstrated the increased gingival blood flow and concentration of moving blood cells in inflamed gingiva, when compared to mildly inflamed or healthy gingiva after periodontal treatment. This is in accordance with previous studies using LDF showing the increased blood flow in inflamed dog gingiva and also in humans[16, 22] (Figure 2). Interestingly, our issue is consistent with one previous study using laser Doppler periodontal probe developed for monitoring intrasulcular gingival blood flow, which illustrating that after treatment there was a significant decline in the values of laser Doppler recordings[23]. In contrast, Matheny et al., showed a significant decrease in gingival regional blood flow in experimental human gingivitis. This contradiction may be related to the methodological artifacts[14]. In the present study no significant changes were detected in the superficial capillary blood velocity, though the figure showed a tendency of a declined moving speed (Figure 2C). Interestingly, the temperature of the gingiva on the test side showed a significant increase of 2 ℃ on an average with significant difference compared to the control side (Figure 2D). This is in agreement with several previous experimental in-vivo studies showing a thermal increase in the dental and periodontal tissues during Nd: YAG laser application in endodontic, periodontal, and restorative procedures[24-26]. Animal studies have

verified the safety of an oral mucosal temperature increase of overall 2 ℃ on healthy tissues and 6.1 ℃ increase on root surfaces for the periodontal ligament, which were further supported in our clinical investigation in humans[25, 26]. The rising temperature can be explained by two underlying mechanisms. One explanation could be that the heat transportation from tissue to tissue is less dissipated as gingival blood flow declined, and another explanation could be due to the emitting light of Nd: YAG laser which is converted into heat by refraction or diffused reflection at the tip end. These factors together lead to a significantly increasing temperature on the test side of the stimulated gingiva[4]. These findings may help to understand better the influence of periodontal treatment and even the additional effect of Nd: YAG laser on the changes of blood flow in the maxillary region.

*Treatment effect on periodontal thermal and mechanical sensitivity*

Most of the work carried out on the efficacy of conventional periodontal therapy and adjunct Nd: YAG laser periodontal treatment ignores the possibility whether these therapies have impacts on the somatosensory sensitivity of periodontal tissues. Limited by the maneuverability of the experimental equipment and to reduce the experimental error, we choose the lateral incisor as our experimental site.

In most clinical cases with pathology in the periodontium, slight itch, mild to severe intermittent or persistent dull pain from the gingiva or tooth, or occlusal pain, percussion discomfort can be evoked and may imply somatosensory abnormalities in the periodontal tissues. Whilst some research has been carried out on the morphology, distribution and composition of periodontal mechano-nociceptors, very few studies have systematically

evaluated the effects of periodontal treatments on the function of the periodontal ligament receptors. One study aiming to explore the influence of advanced periodontitis pointed out that the periodontium of teeth with advanced periodontitis is less sensitive to pressure detection and also highlighted that the somatosensory qualities of the periodontium depend more on the degree of inflammation than a reduction in the amount of tissue[27]. This pioneering insight provided a valuable theory foundation to carry out this study.

A diversity of factors originating from both invading bacteria and the host cells have been identified to strongly link to pain and hyperalgesia in non-oral tissues[28]. Specifically, *Porphyromonas gingivalis* regarded as a "keystone" periodontal pathogen has been shown to be associated with other oral conditions characterized by symptoms that include spontaneous pain or pain on palpation[29-32]. Moreover, the component lipopolysaccharides (LPS) of this critical pathogen has been identified to have a positive relationship with not only animal model of inflammatory pain but also inflammatory pain in humans[29]. Its hyperalgesic effects may contribute to the up-regulation of pro-inflammatory mediators, such as the cytokines interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), neuropeptide calcitonin gene-related peptide (CGRP) and substance P (SP)[29-34]. Evidence-based up-regulation of pro-inflammatory, pro-algesic or hyperalgesic bioactive molecules such as IL-6, IL-8, MMPs, CRP, PGE2 under the condition of periodontitis have all been illustrated to potentially contribute to hyperalgesia[35-39]. The underlying mechanisms pertaining to the apparent anti-hyperalgesia of periodontal treatment which cause periodontal tissues hyposensitive to cold, mechanical detection and cold, heat, mechanical, and pressure pain still remain an enigma (Figure 3 and 4). The CPT, HPT, MPT and PPT can be detected in the periodontal

ligament and gingiva to test deep pain sensitivity regarded as conduction by both Aδ and C nerve fibers[40, 41]. Periodontal conventional treatment or even adjuvant Nd: YAG laser therapy may have caused suppression or marked decline of pro-inflammatory/pro-algesic host mediators and bacterial abundance which sensitized the somatosensory receptors. This induced the phenomenon observed in this study that there is a significant higher threshold of CDT, CPT, HPT, MDT, MPT and PPTs after active periodontal interventions, which was in accordance with van Steenberghe's declaration that periodontal somatosensory alterations rested more on the inflammatory level[27]. Interestingly, from Figure 3 and 4 there is a trend towards better effects on the test side in most parameters, especially for HPT and PPTs, even with no significant difference between the two sides due to the small sample size. Curiously, participants could not discriminate warm detection from heat pain sensation at the first examination, but after periodontal treatment, they were slightly better to distinguish the differences. A hypothesis suitable to interpret this observation may related to the fact that WDT is considered to represent unmyelinated C nerve fiber conduction whereas HPT represents conduction by both Aδ and C nerve fibers[41, 44]. Some studies have revealed that C-fibers are kind of slow adaption afferents while fast pain is transmitted by Aδ afferents[42, 43] which are predominant in the periodontal ligaments[10, 40]. In this way when periodontal nociceptors are sensitized due to the periodontal inflammation, the imbalance of afferent nerve conduction velocity could contribute to this abnormality[40]. However there was no alteration in the thresholds of warm detection through pre- and post-treatment (Figure 3C). This intriguing phenomenon reflects the complexity of nerve conduction and merits further studies. Thought-provoking, the QST device we used in this study unveiled the delicate

changes of periodontal psychophysical threshold of thermal, mechanical and pressure detection or pain sensation after active periodontal interventions supported by subjective quantitative evidence.

*Study limitations*

The participants of the present study were young and middle-aged patients who may represent a more aggressive form of periodontitis. The results may therefore not be applicable to a more typical type of periodontitis. Most parameters were significantly improved after periodontal therapy, therefore any further effect from adjunct laser therapy could have been difficult to be detected in a short–term study because of a “ceiling effect”. Further, the relatively small sample size might have been insufficient for assessing possible treatment differences.

## Conclusions

The clinical periodontal condition was improved, the somatosensory sensitivity decreased and the gingival blood flow declined after conventional SRP treatment. The adjunctive use of Nd: YAG laser therapy did not provide any significant clinically advantage or additional effects on the fluctuation of periodontal somatosensory functions and gingival microcirculation in the present study.

## Acknowledgements

This investigation was founded by the National Natural Science Foundation of China (81771074) and from A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD, 2018-87). We would like to thank the Orofacial Pain and TMD Research Unit, Institute of Stomatology, Affiliated Hospital of Stomatology, Nanjing Medical University, for their support.

**Conflict of interest**

The authors have stated explicitly that there are no conflicts of interests in connection with this article.

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**Figure and table legends**

Fig. 1

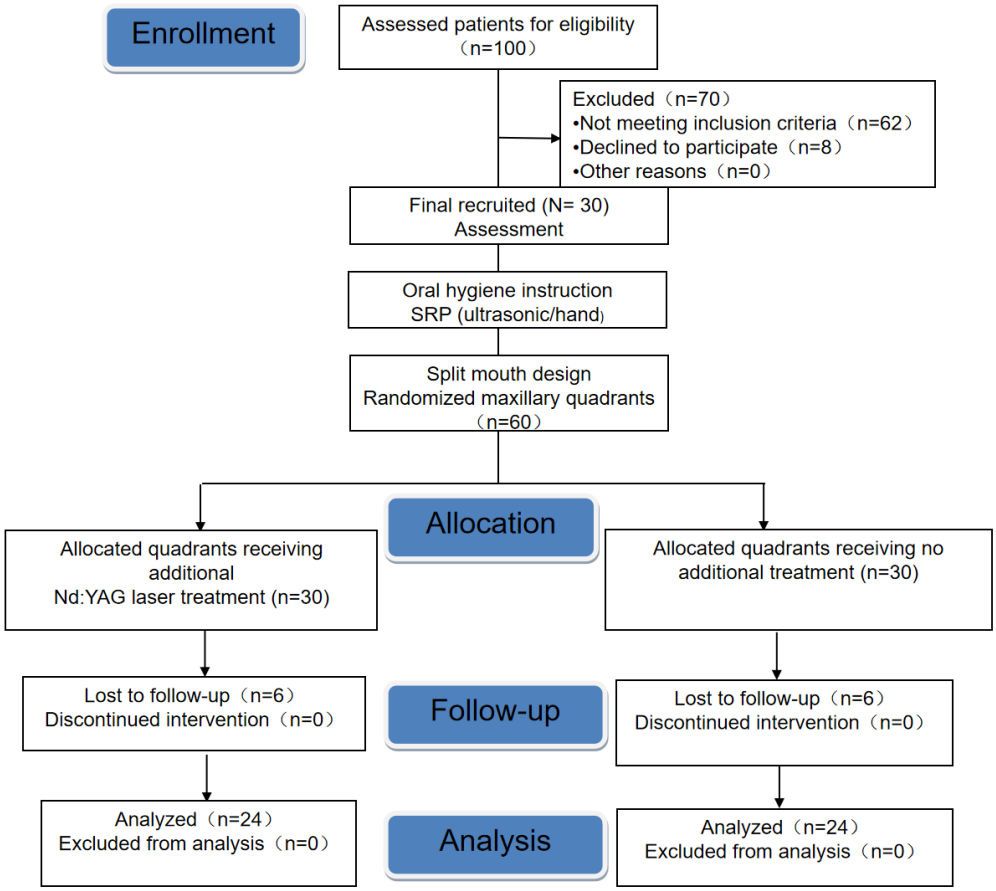


**Figure 1:** Study flowchart

Fig. 2

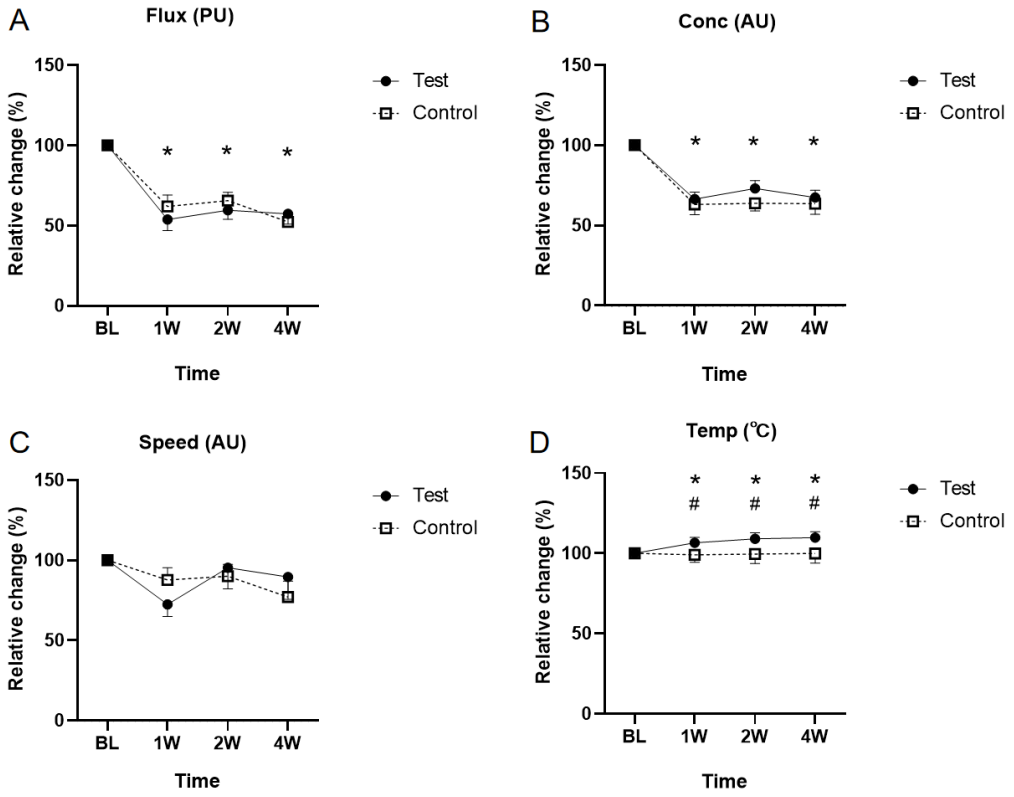


**Figure 2:** Relative changes for tissue microvascular blood perfusion (Flux) (A), concentration of moving blood cells (Conc) (B), the relative velocity of microvascular blood flow (Speed) (C) and the temperature of gingiva (Temp) (D) of the participants at baseline (0), 1, 2 and 4 weeks after treatment of 12 and 22. The data are presented as the mean ± standard error of the mean. Black circles represent Laser-treatment side; white squares represent laser-non-treatment side; *indicates a significant difference ($P < 0.05$) between baseline; a indicates a significant difference on laser-treatment side between baseline ($P < 0.05$); #indicates a significant difference between the laser-treatment side and laser-non-treatment side ($P < 0.05$).

Fig. 3

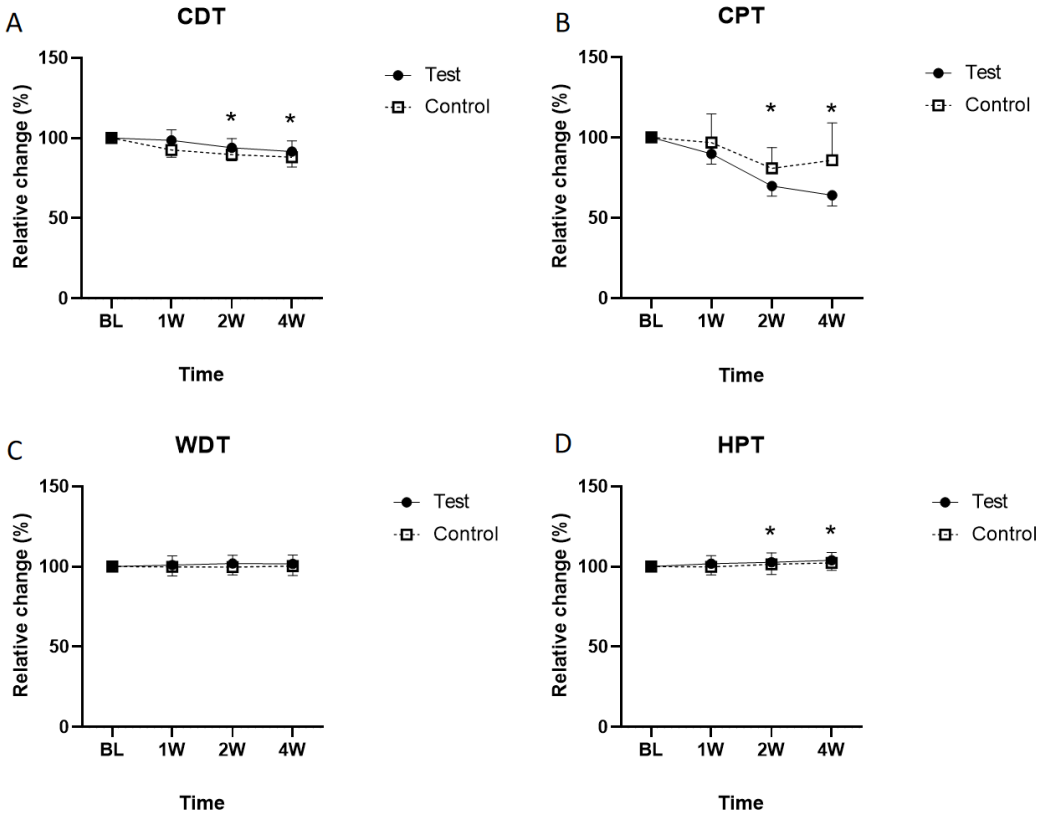


**Figure 3:** Relative changes for CDT (A), CPT (B), WDT (C), HPT (D) of the participants at baseline (0), 1, 2 and 4 weeks after treatment of 12 and 22. The data are presented as the mean ± standard error of the mean. Black circles represent laser-treatment side; white squares represent Laser-non-treatment side; *indicates a significant difference ($P < 0.05$) between baseline; # indicates a significant difference between the laser-treatment side and laser-non-treatment side ($P < 0.05$).

Fig. 4

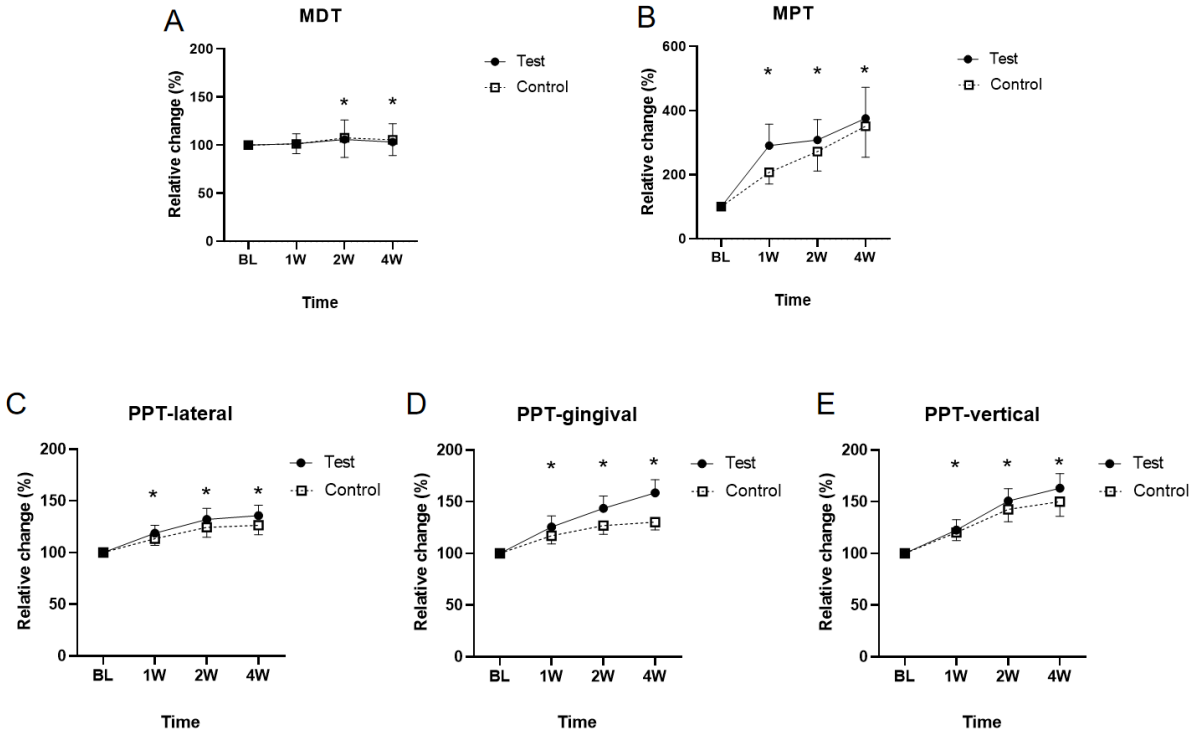


**Figure 4:** Relative changes for MDT (A), MPT (B), PPT-Gingival (C), PPT-Lateral (D) and PPT-Vertical (E) of the participants at baseline (0), 1, 2 and 4 weeks after treatment of 12 and 22. The data are presented as the mean ± standard error of the mean. Black circles represent laser-treatment side; white squares represent laser-non-treatment side; *indicates a significant difference ($P < 0.05$) between baseline; #indicates a significant difference between the laser-treatment side and Laser-non-treatment side ($P < 0.05$).

**Table 1:** Mean (±standard deviation) PPD，sites with PPD＞5mm , and BOP outcomes at baseline, 2 weeks (2W) and 4 weeks (4W) of test and control maxillary lateral incisor PPD, probing depth, BOP, bleeding on probing, Test Laser + SRP treatment quadrant, Control SRP treatment quadrant *P value refers to the statistically significant difference for each group between different time points and #P value refers to statistically significant difference between groups in the same period groups (P < 0.05).

| | **Baseline** | **2W** | **Δ(0-2W)** | **P*-value** | **4W** | **Δ (0-4W)** | **P*-value** | **Δ (2-4W)** | **P*-value** |
|---|---|---|---|---|---|---|---|---|---|
| **PPD (mm)** | | | | | | | | | |
| Test tooth | 3.9±1.2 | 2.4±0.9 | 1.5±0.7 | ＜0.001 | 2.3±0.9 | 1.6±0.6 | ＜0.001 | 0.1±0.3 | 0.038 |
| Control tooth | 3.7±1.1 | 2.4±0.9 | 1.3±0.5 | ＜0.001 | 2.3±0.8 | 1.5±0.6 | ＜0.001 | 0.1±0.3 | 0.020 |
| P#-value | 0.066 | 0.125 | 0.186 | | 0.457 | 0.089 | | 0.624 | |
| **sites with** | | | | | | | | | |
| Test tooth | 1.5±1.0 | 0.5±0.9 | 1.0±0.2 | ＜0.001 | 0.4±0.7 | 1.1±0.3 | ＜0.001 | 0.1±0.3 | 0.024 |
| Control tooth | 1.4±0.9 | 0.5±0.9 | 1.0±0.2 | ＜0.001 | 0.4±0.7 | 1.0±0.3 | ＜0.001 | 0.1±0.3 | 0.04 |
| P#-value | 0.210 | 0.710 | 0.083 | | 1.000 | 0.103 | | 0.570 | |
| **BOP (%)** | | | | | | | | | |
| Test | 70.6±21. | 20.7±16 | 50.0±23. | ＜0.001 | 16.3±18 | 54.5±22. | ＜0.001 | 4.4±17. | 0.133 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| tooth | 0 | .6 | 8 | | .7 | 8 | | 6 | |
| Control tooth | 69.3±21.0 | 21.2±18.8 | 48.2±25.1 | ＜0.001 | 19.8±17.6 | 49.6±23.4 | ＜0.001 | 1.3±18.8 | 0.655 |
| P#-value | 0.729 | 0.878 | 0.729 | | 0.174 | 0.345 | | 0.356 | |

**Table 2:** Mean (±standard deviation) PPD，sites with PPD＞5mm, and BOP outcomes at baseline, 2 weeks (2W) and 4 weeks (4W) of test and control maxillary quadrant side PPD, probing depth, BOP, bleeding on probing, Test Laser + SRP treatment quadrant, Control SRP treatment quadrant *P value refers to the significant statistically difference for each group between different time points and #P value refers to statistically significant difference between groups in the same period groups (P < 0.05).

| | **Baseline** | **2weeks** | **Δ(0-2W)** | **P*-valu** | **4W** | **Δ** | **P*-valu** | **Δ** | **P*-val** |
|---|---|---|---|---|---|---|---|---|---|
| **PPD** | | | | | | | | | |
| Test | 4.4±0.8 | 3.3±0.7 | 1.1±0.6 | ＜0.001 | 3.0±0.6 | 1.4±0.7 | ＜0.001 | 0.08±0. | ＜ |
| Control | 4.3±0.8 | 3.2±0.6 | 1.1±0.5 | ＜0.001 | 3.1±0.6 | 1.2±0.5 | ＜0.001 | 0.1±0.2 | 0.001 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| P#-value | 0.227 | 0.172 | 0.993 | | 0.541 | 0.078 | | 0.585 | |
| **sites** | | | | | | | | | |
| Test | 5.0±1.8 | 3.1±1.8 | 1.9±0.5 | ＜0.001 | 3.0±1.8 | 2.0±0.4 | ＜0.001 | 0.1±0.3 | 0.013 |
| Control | 4.9±1.7 | 3.1±1.9 | 1.8±0.6 | ＜0.001 | 3.0±1.8 | 1.9±0.7 | ＜0.001 | 0.2±0.5 | 0.033 |
| P#-value | 0.627 | 0.736 | 0.256 | | 0.855 | 0.377 | | 0.812 | |
| **BOP** | | | | | | | | | |
| Test | 72.5±18. | 25.0±18 | 47.5±21. | ＜0.001 | 17.9±15 | 54.6±20. | ＜0.001 | 7.1±18. | 0.018 |
| Control | 67.1±21. | 27.5±18 | 39.6±20. | ＜0.001 | 18.3±16 | 48.7±22. | ＜0.001 | 9.2±17. | 0.002 |
| P#-value | 0.176 | 0.514 | 0.095 | | 0.873 | 0.163 | | 0.543 | |