

Somatosensory profiling of patients with plaque-induced gingivitis

a case-control study

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Table 1
Mean values ± standard deviation for clinical parameters of tooth 32 and 42in periodontitis patients and healthy controls

	Controls		Periodontitis		<i>p</i> value	
	Tooth 32	Tooth 42	Tooth 32	Tooth 42	Tooth 32	Tooth 42
PPD (mm)	2.0±0.2	2.0±0.2	3.3±0.4	3.4±0.8	<0.001*	<0.001*
CAL (mm)	0	0	2.7±0.6	2.9±0.8	<0.001*	<0.001*
BOP (%)	0	0	79.4%±20.7%	80.8%±16.7%	<0.001*	<0.001*

Table 2

A 2-way mixed model ANOVA for LDF and QST values in different site (tooth 32 and tooth 42) and group (controls and patients) with repeated measures

	Controls		Periodontitis		site		group	
	Tooth 32	Tooth 42	Tooth 32	Tooth 42	F	p	F	p
Flux (PU)	124.3±55.9	117.8±55.3	288.9±136.8	263.6±104.3	0.565	0.457	0.198	0.659
Conc (AU)	206.8±38.4	230.1±66.6	269.8±123.9	209.2±44.5	1.291	0.263	6.522	0.015*
Speed (AU)	31.7±12.0	28.1±13.1	62.8±34.3	67.4±29.0	0.009	0.925	0.554	0.461
Temp (°C)	28.9±2.8	31.0±1.9	30.0±2.0	30.9±1.8	13.921	0.001*	2.322	0.136
CDT (°C)	25.5±5.9	25.7±7.7	24.0±8.5	25.2±7.2	3.389	0.073	0.106	0.747
CPT (°C)	11.5±8.5	14.9±10.7	7.5±9.4	12.2±10.7	8.574	0.006*	0.199	0.658
WDT (°C)	49.4±1.6	49.6±2.2	46.7±2.9	47.4±2.9	0.819	0.371	0.398	0.532
HPT (°C)	48.7±5.1	49.9±1.9	49.6±1.7	48.4±1.9	0.001	0.979	4.675	0.037*
MDT (mN)	3.1±0.5	3.1±0.5	3.0±0.4	2.8±0.5	1.226	0.275	0.809	0.374
MPT (mN)	7.5±6.2	9.0±6.6	8.2±6.2	5.6±3.3	0.960	0.333	10.354	0.003*
PPT-I (KPa)	149.3±68.5	140.7±70.2	198.2±85.6	228.6±112.5	1.006	0.322	3.260	0.079
PPT-v (KPa)	182.0±107.8	189.9±122.8	305.7±151.7	271.3±154.9	0.761	0.389	1.935	0.172
PPT-g (KPa)	69.9±37.5	66.1±29.1	77.1±45.2	66.0±28.0	1.590	0.215	0.371	0.546

Figure 1

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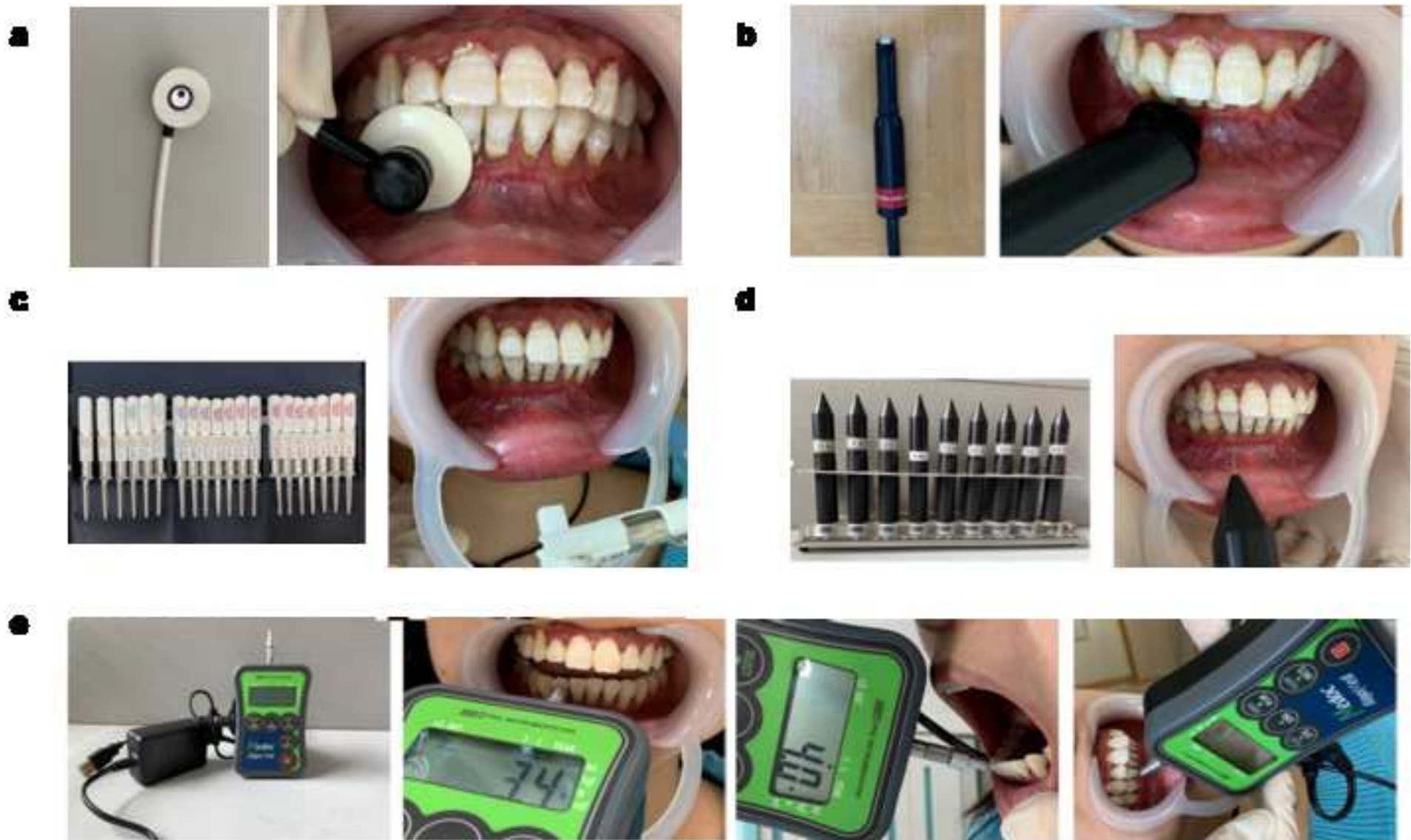
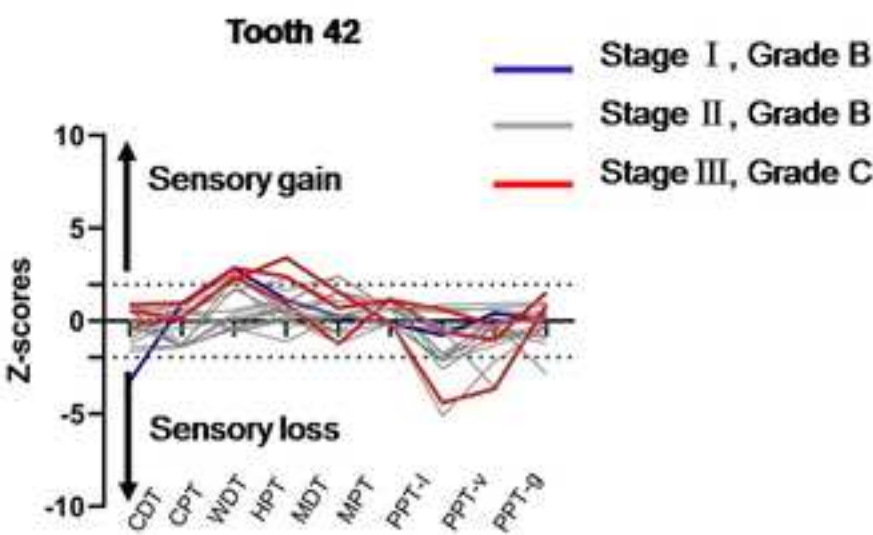
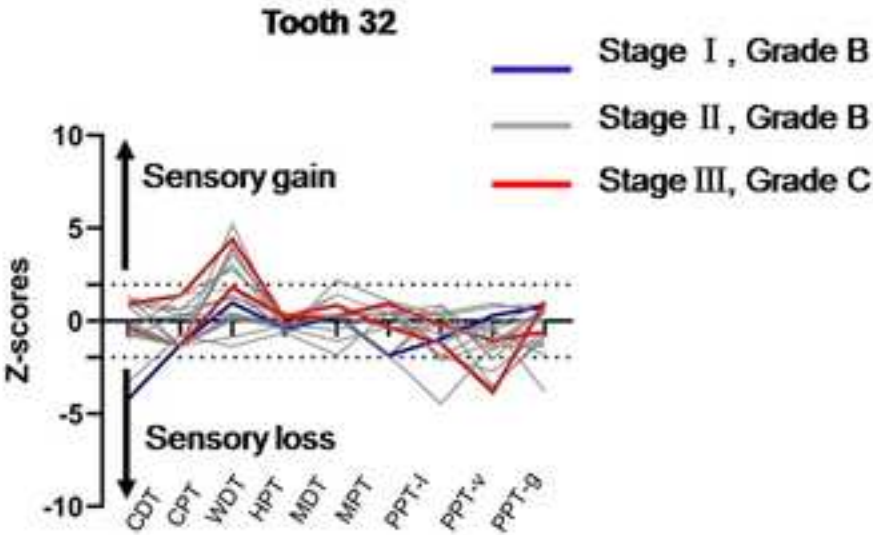


Figure 2



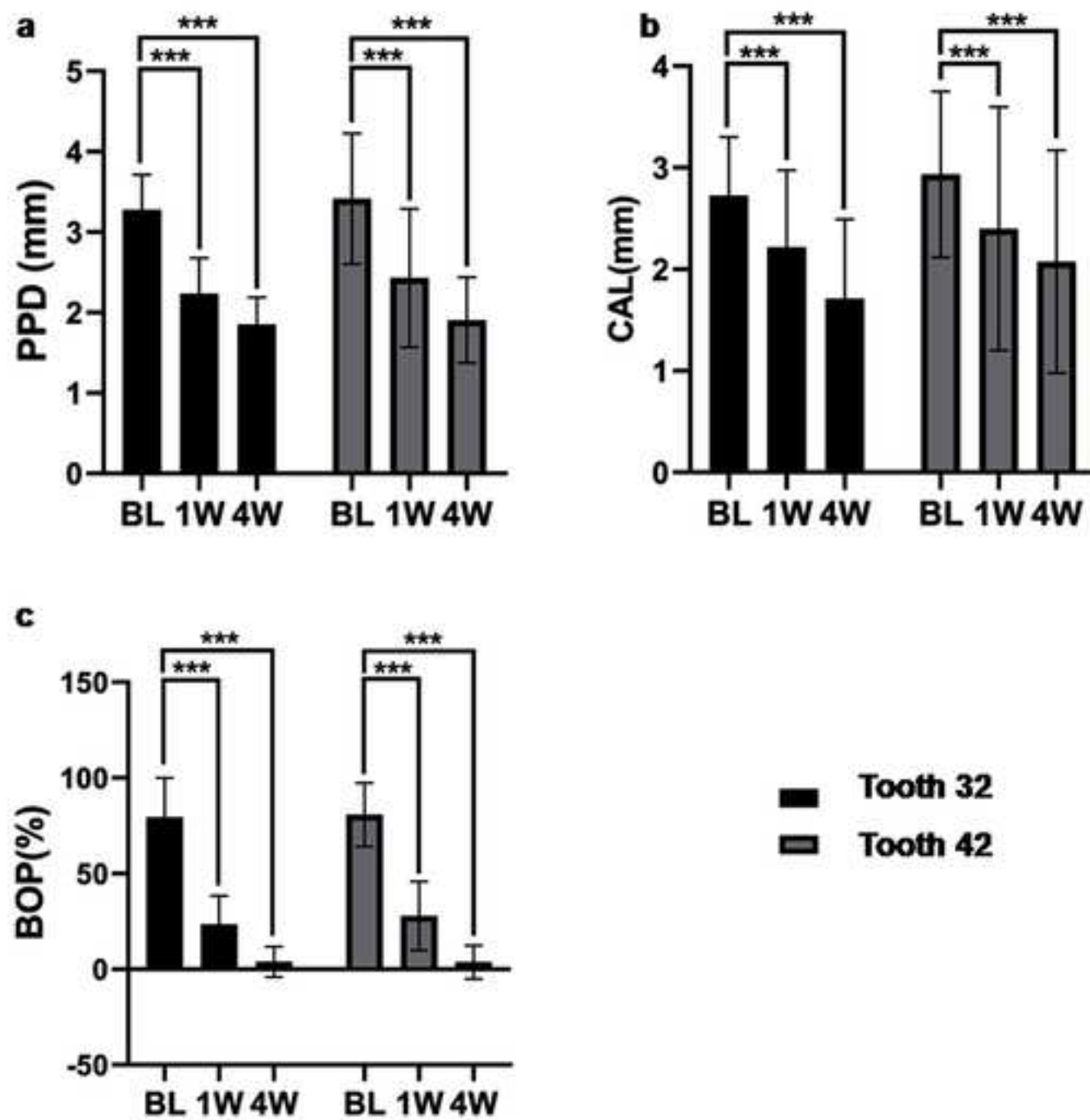


Figure 4

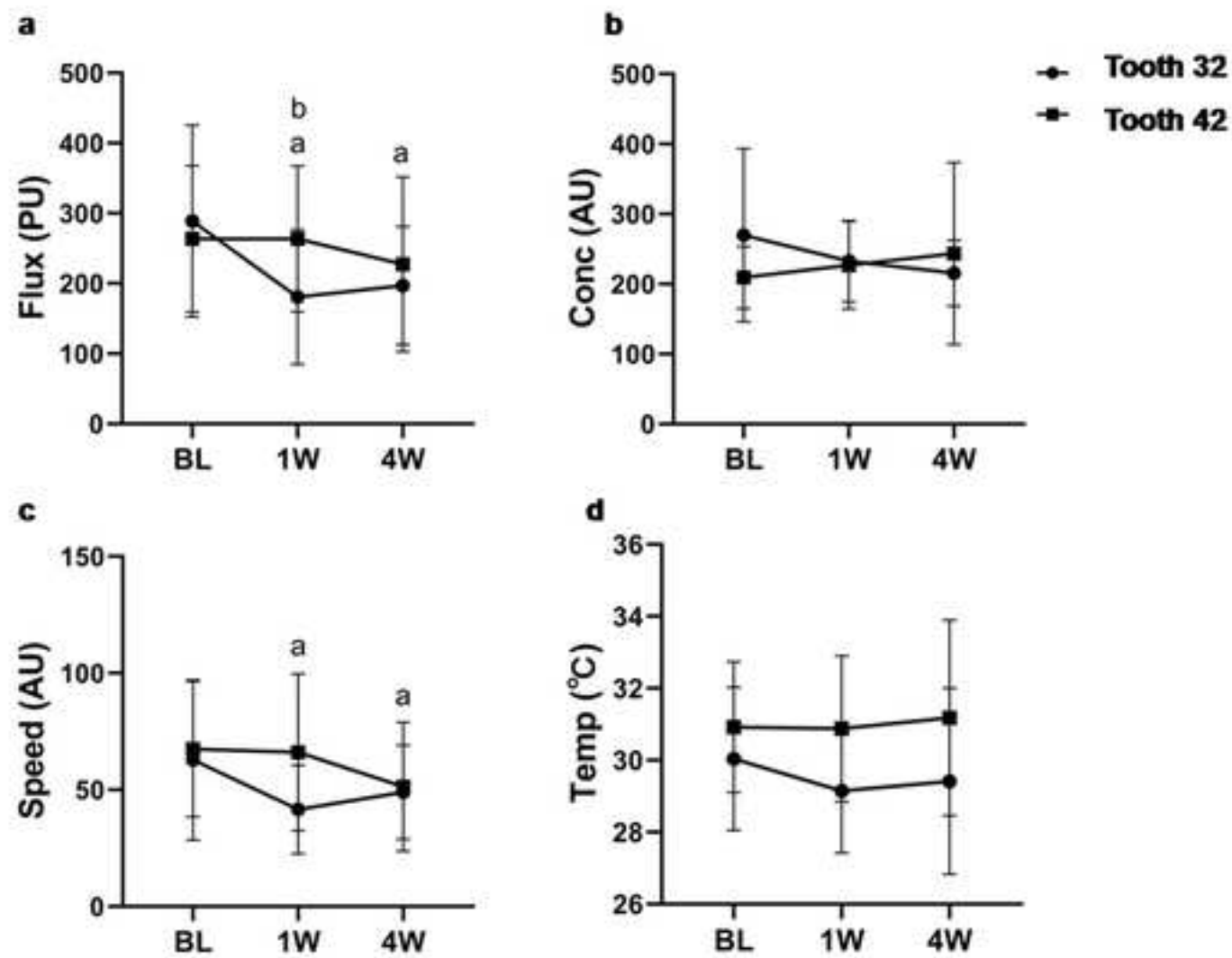
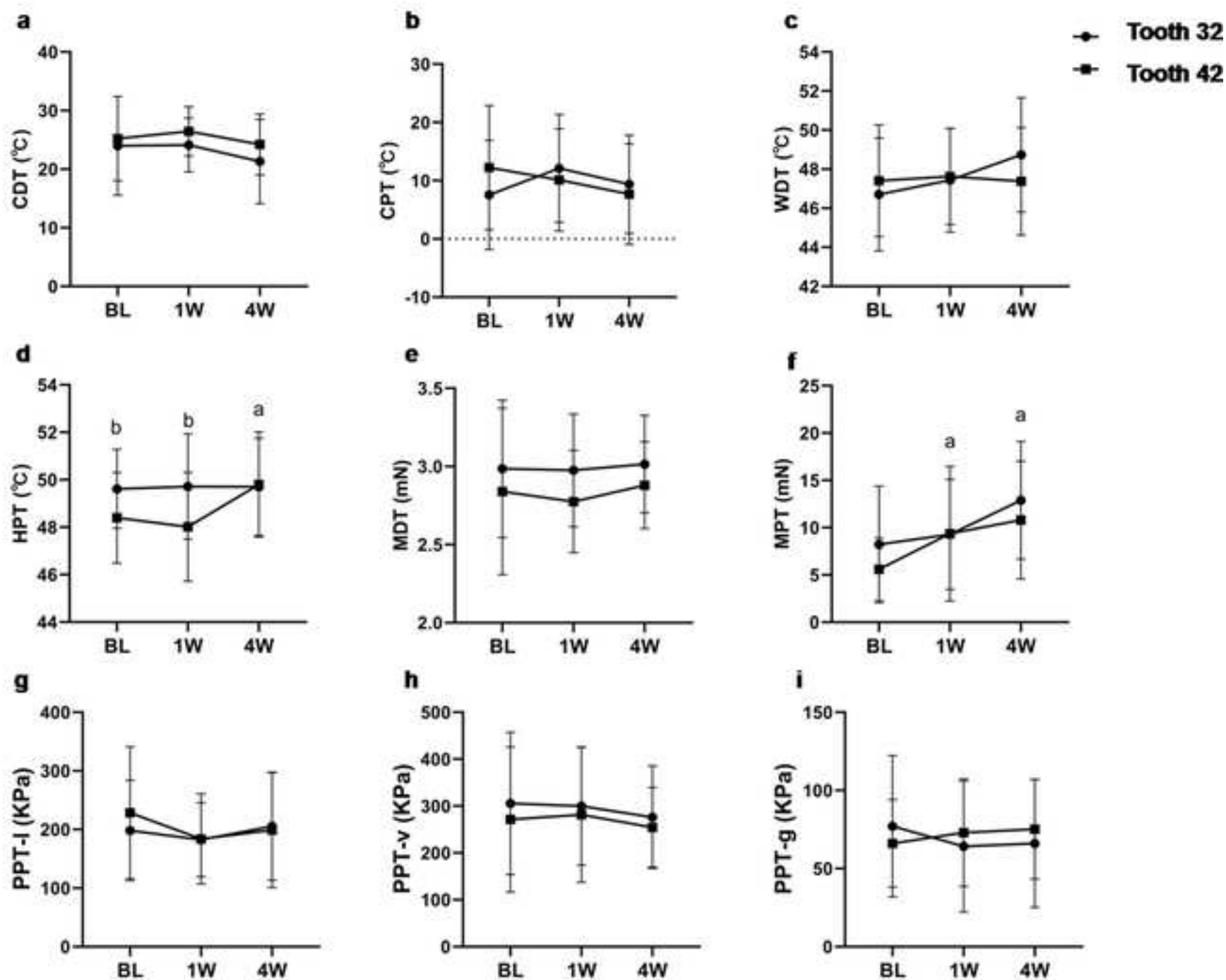


Figure 5



Microcirculation and somatosensory profiling of patients with periodontitis: a preliminary case control report

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Author contributions: All authors have made substantial contributions to conception and design of the study. Ruyi Fan, Huiqing Gou, Xiaoqian Wang, Lu Li, Yan Xu have been involved in data collection and data analysis. Ruyi Fan, Huiqing Gou, Yan Xu, Peter Svensson and Kelun Wang have been involved in data interpretation, drafting the manuscript and revising it critically and have given final approval of the version to be published.

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Abstract

Objectives: The purpose of this preliminary study was to explore blood microcirculation and somatosensory profiles in periodontitis patients before and after non-surgical periodontal therapy.

Materials and methods: Twenty patients (10 men and 10 women, 20 to 30 years old) and twenty age- and gender-matched healthy controls were included. Non-surgical periodontal therapy was performed for all patients. Clinical examination including pocket probing depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BOP) were performed at Baseline (BL), 1 week (1W), and 4 weeks (4W) after non-surgical periodontal therapy on 6 sites of tooth 32 and 42. Laser Doppler Flowmetry (LDF) and Quantitative Sensory Testing (QST) were applied at the attached gingiva of tooth 32 and 42 at BL, 1W and 4W after non-surgical periodontal therapy. Data were analyzed with a 2-way mixed-model of ANOVA.

Results: The PPD, CAL and BOP significantly improved after non-surgical periodontal therapy ($p<0.001$). Periodontitis patients demonstrated a higher tissue microvascular blood cell concentration ($p=0.015$) and a significant gain in thermal ($p=0.037$) and mechanical ($p=0.003$) somatosensory function compared to controls. After non-surgical periodontal therapy, the flux ($p=0.002$) and speed ($p=0.008$) of blood flow decreased significantly and thermal ($p=0.029$) and mechanical ($p<0.001$) somatosensory function were reversed.

Conclusion: Gingival microcirculation and somatosensory function seem impaired in patients with periodontitis and are reversed following non-surgical periodontal therapy.

Clinical Relevance: LDF and QST may be appropriate tools to further characterize gingival inflammation and treatment responses in periodontitis.

Keywords: quantitative sensory testing; microvascular blood flow; periodontitis; non-surgical

periodontal therapy

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Introduction

Periodontitis is a major public health issue with a high prevalence of 45%–50% overall, with the most severe form affecting 11.2% of the world's population, being the sixth most common human disease [1,2]. It usually results from a chronic inflammatory condition of bacterial etiology, which can be associated with gingival inflammation extending into the adjacent attachment apparatuses [3,4]. As periodontitis progresses, it can cause breakdown of the supporting periodontal tissues, including alveolar bone, periodontal ligament and root cementum, results in progressive loss of periodontal attachment and bone loss and eventually leading to tooth loss [4,5].

The abundant nerve fibers in the periodontal ligament originate from the trigeminal nerve, and are always accompanied by blood vessels [6,7]. The fine unmyelinated nerve fibers are C-fibers, and the free nerve endings are associated with a painful sensation. The thick myelinated nerve fibers, including A-delta-fiber and A-beta-fiber, are primarily involved as mechanoreceptors mediating mechanosensations such as touch and pressure. We hypothesized that the free nerve endings in periodontal ligament may be sensitized as well as the blood microcirculation may be altered by the inflammatory response in patients with periodontitis.

Laser Doppler Flowmetry (LDF) as a noninvasive and easy-operated measurement of microcirculation has been applied in many research projects in different body regions, including in the orofacial area, such as the oral mucosa, masseter muscle, as well as periodontal tissue [8-11]. It has now become a gold standard for dynamic microvascular blood flow assessment, however, although many studies have applied it to evaluate microcirculation of inflammatory mucosa, they yielded conflicting results [8].

Quantitative Sensory Testing (QST) is a standard procedure operationalized by the German

1 Research Network on Neuropathic Pain (DFNS), which has been used to characterize the
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3 somatosensory function in different body regions [12,13]. Recently, an increasing number of studies
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5 have applied QST to assess the possible somatosensory changes in varies orofacial disorders including
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7 temporomandibular disorders, atypical odontalgia, and trigeminal neuralgia and shown that QST is
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9 reliable application [14,15]. Nonetheless, QST has not been applied to the gingiva to test
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11 somatosensory changes in different types of periodontitis. However, a recent study from our group first
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13 applied QST to assess the possible somatosensory changes in patients with plaque-induced gingivitis,
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15 and found that gingivitis patients had changed mechanical and thermal somatosensory sensitivity when
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17 compared to a normal population. The results indicated that the inflammatory reaction in the gingiva
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19 seem to be associated with hypo- and hyperesthesia in somatosensory function [16].
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28 The primary aim of this study was to test the blood microcirculation changes and somatosensory
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30 function in periodontitis patients compared to healthy controls to better investigate the effect of
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32 inflammatory changes in periodontitis, and second to investigate the effect of non-surgical periodontal
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34 therapy on blood flow and somatosensory function. The hypothesis was that there would be significant
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36 differences both in the gingiva and periodontal ligament due to the inflammatory response in patients
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38 with periodontitis, besides, there would also be significant differences in patients after non-surgical
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40 periodontal therapy.
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50 **Materials and methods**

51 **Study participants**

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53 Twenty patients (10 men, 25.8 ± 3.4 years) diagnosed with periodontitis were recruited in the study at
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55 the Department of Periodontology, Hospital of Stomatology, Nanjing Medical University, P.R.C.
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Twenty gender- and age-matched healthy volunteers (10 men 25.6 ± 3.4 years old) were recruited as a control group.

The inclusion criteria of periodontitis patients included: 1. more than 20 functional teeth including two mandibular lateral incisors (tooth 32 and 42); 2. diagnosed with periodontitis in the whole dentition according to the new classification scheme of periodontal diseases determined by the joint European Federation of Periodontology (EFP) and American Academy of Periodontology in 2017 [17]: probing pocket depth (PPD) of the most serious site ≥ 4 mm; clinical attachment loss (CAL) of the most serious site ≥ 1 mm; positive percentage of bleeding on probing (BOP) $\geq 10\%$; with horizontal alveolar bone loss and/or vertical bone defects on x-ray imaging. 3. Tooth 32 and 42 have the clinical features that PPD of the most serious site ≥ 4 mm, CAL of the most serious site ≥ 1 mm, BOP (%) of 6 sites $\geq 10\%$, with horizontal alveolar bone loss and/or vertical bone defects, and tooth movement $< II^\circ$.

The exclusion criteria for all participants were: 1. ongoing dental treatment or other active oral diseases; 2. history of systemic diseases or mental disorders; 3. gingivitis or periodontitis related to systemic, medical or malnourishment; 4. taking antibiotics or pain control medication in the last three months; 5. being pregnant or in the menstrual period; 6. smoking.

Study design

All the participants were treated and tested clinic by the same investigator, who had been trained in the clinical examination and in the use of LDF and QST measurements. All participants were informed of the test procedures and pre-experimental assessment was performed for being familiar with the procedure before the tests.

The clinical examination, LDF and QST were performed in the same sequence, in order to prevent

LDF parameters from being influenced by QST. All tests were determined from the attached gingiva and surface of tooth 32 and 42 in both groups of patients and controls before treatment (BL). The recorded parameters were compared between two groups. Then the non-surgical periodontal therapy was performed in the patient group and all tests were recorded again at 1 week (1W), and 4 weeks (4W) after the therapy.

Clinical examination

PPD, BOP and gingival recession (GR) were measured by the same investigator with a sterilized periodontal probe. All present teeth were examined at 6 sites: mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual of the tooth, and the average value of the six sites was presented [18]. CAL was calculated by PPD and GR, and the positive percentage of BOP sites was calculated.

Non-surgical periodontal therapy

Supragingival scaling, subgingival scaling and root planning was performed using an ultrasonic device (MiniPiezons, EMS Electro Medical Systems S.A., Nyon, Switzerland) and hand instruments (GraceyCurets, Hu-Friedy, Chicago, IL, USA) under local anesthesia. And all patients were given professional tooth cleaning and oral hygiene instructions.

Laser Doppler monitoring

The gingival microvascular blood perfusion (Flux), tissue microvascular blood cell concentration (Conc), the relative velocity of microvascular blood flow (Speed), and temperature were assessed by

the LDF (moorVMS-LDF2, Moor Instruments Ltd, Millwey, Axminster, Devon, England) connected to a computer which recorded the data in real time. The probe touched the attached gingiva of tooth 32 and 42, 1mm away from the free gingival groove (Fig.1a). LDF readings were monitored for a 2-3 min until they had reached to a stable state [19].

Thermal threshold testing

A MEDOC TSA 2001-II apparatus (Medoc, Ramat-Yishai, Israel) and its intra-oral probe (6mm x 6mm) was used to test thermal threshold. Cold detection threshold (CDT) and warm detection threshold (WDT) were measured at the attached gingiva of tooth 32 and 42 followed by cold pain threshold (CPT) and heat pain threshold (HPT) (Fig.1b). The mean thresholds of three sequential measurements were calculated [20]. The initial temperature of the thermode was 37°C, cooled-down or heated-up at a rate of 1°C/s, and the limitations were set at 0°C and 50°C, respectively. The participants were asked to press the button as soon as they felt the respective thermal sensation of cold, warm, cold pain, or heat pain following the instructions according to the DFNS [20].

Mechanical threshold testing

Mechanical detection thresholds (MDT) was tested by modified von Frey monofilaments (North Coast Medical, Gilroy, CA, USA), which had 20 different diameters, numbered 1.65-6.65 and equivalent forces of 0.008-300g. The filament was applied perpendicularly to the attached gingiva of tooth 32 and 42, and the pressure was applied slowly until it bowed. Each monofilament was tested five times and each touch lasted 1-2 seconds (Fig.1c).

The mechanical pain threshold (MPT) was measured using seven pinprick stimuli corresponding

to 8, 16, 32, 64, 128, 256, and 512 mN (Aalborg University, Aalborg, Denmark) (Fig.1d). Threshold measurements were made by application of a series of ascending and descending stimulus intensities and the values were determined by calculating the geometric mean of these five series of tests [21].

Pressure pain threshold testing

A hand-held pressure algometry (Medoc Ltd, Israel) and a probe diameter of 0.8 cm was used to test pressure pain threshold (PPT), with a constant application rate of 30kPa/s. The pressure was first applied perpendicular to the center of facial surface of the tooth crown labeled as “lateral”, then applied parallel to the axis of the tooth labeled as “vertical” and finally to the attached gingival tissue, about 1mm away from the free gingival groove (Fig.1e). Three measurements were made at each target side, and there was of 1-min interval between each measurement. Participants were asked to press the hold switch as soon as they felt the pressure as a painful sensation [22].

Statistical analysis

The sample size was calculated a priori based on the detection of a minimum clinically relevant difference of 25% at an α level of 0.05 and 80% power (i.e., the risk of a type I and type II error was 5% and 20%, respectively). However, taking into account an anticipated 20% dropout rate, a total of 20 participants were recruited in each group.

Clinical parameters, LDF and QST values were all considered discrete variables, expressed as means \pm SD in text and tables. The necessary logarithmic transformation was performed to secure normal distribution of all data. A 2-way mixed-model ANOVA with repeated measures was performed for each LDF and QST value, with site (tooth 32 and tooth 42) as a within-subject factor and group

(controls and patients) as a between subjects factor. When it came to comparisons of pre- and post-non-surgical periodontal therapy in patients, the 2-way mixed-model ANOVA with repeated measures was conducted again, with time (BL, 1W, and 4W) as a within-subject factor and site (tooth 32 and tooth 42) as a between subjects factor. The significance level was set at 0.05.

Z-score were adopted to demonstrate the degree of differences between QST data from periodontitis patients and healthy controls, using a $Z - socre = (value\ patient - value\ controls) / SD\ controls$. A Z-score above +1.96 or below -1.96 indicates gain or loss of somatosensory function [23]. All statistical calculations were performed using the Statistical Package for Social Sciences, version 17 (SPSS, IBM).

Results

Clinical features in periodontitis patients

Most of the patients (80%) included in the experimental group were in stage II, Grade B of generalized periodontitis, except for one patient who was in stage I and Grade B, and three patients were in stage III and Grade C. As for the status of tooth 32 and tooth 42, most of them were in stage II, Grade B. One single tooth 32 was in stage I and Grade B, and two patients had tooth 32 in stage III and Grade C. One patient had one tooth 42 in stage I and Grade B, and three patients had one tooth 42 in stage III and Grade C.

Clinical parameters in periodontitis and controls

For both tooth 32 and 42, PPD ($p < 0.001$), CAL ($p < 0.001$), and BOP (%) ($p < 0.001$) in patients were all significantly higher than in the control group (Table 1).

LDF data in periodontitis and controls

No significant site differences were detected for Flux ($p=0.457$), Conc ($p=0.263$) and Speed ($p=0.925$), while a significant site difference was found for Temp ($p=0.001$). Conc ($p=0.015$) was significantly higher in patients with periodontitis compared to controls, whereas there were no significant group differences for Flux ($p=0.659$), Speed ($p=0.461$) and Temp ($p=0.136$) (Table 2).

QST data in periodontitis and controls

Significant site difference was found for CPT ($p=0.006$), whereas no significant site differences were detected for any other QST parameters ($p>0.05$).

HPT ($p=0.037$) and MPT ($p=0.003$) were significantly lower (more sensitive) in periodontitis patients compared to controls. There were no significant group differences for any other QST parameters ($p>0.05$) (Table 2).

Figure 2 shows the Z-scores profiles of 20 patients for tooth 32 and 42 for the QST variables. Significant gain of function can be identified for WDT and HPT, and significant loss of function can be identified for PPT-I and PPT-V. The figures as such may indicate differences in somatosensory function related to the status of periodontitis for individual patients.

Clinical parameters after non-surgical periodontal therapy

For both tooth 32 and 42, the values of PPD, CAL and BOP (%) were significantly lower after non-surgical periodontal therapy at 1W compared to BL ($p<0.001$), and significantly lower at 4W compared to BL ($p<0.001$) (Fig.3abc).

LDF values after non-surgical periodontal therapy

No significant site differences were detected for Conc ($p=0.053$), Speed ($p=0.05$) and Temp ($p=0.437$), while significant site differences were found for Flux at 1W ($p=0.013$).

Flux values were significantly lower after non-surgical periodontal therapy at 1W ($p=0.006$) and 4W ($p=0.002$) compared to BL (Fig.4a). Speed values were significantly lower after non-surgical periodontal therapy at 1W ($p=0.031$) and 4W ($p=0.003$) compared to BL (Fig.4c). No significant time differences after non-surgical periodontal therapy compared to BL were detected for Conc ($p=0.751$) and Temp ($p=0.472$) (Fig.4bd).

QST values after non-surgical periodontal therapy

No significant site differences were detected for CDT ($p=0.714$), CPT ($p=0.11$), WDT ($p=0.051$), MDT ($p=0.773$), MPT ($p=0.368$), PPT-l ($p=0.296$) and PPT-v ($p=0.848$), while significant site differences were found for HPT at BL ($p=0.034$) and 1W ($p=0.022$), and for PPT-g ($p=0.026$).

HPT was significantly higher (less sensitive) after non-surgical periodontal therapy at 4W compared to BL ($p=0.043$) (Fig.5d). MPT was significantly higher (less sensitive) after non-surgical periodontal therapy at 1W compared to BL ($p=0.024$), significantly higher at 4W compared to BL ($p<0.001$) (Fig.5f). No significant differences were detected after non-surgical periodontal therapy for any other QST parameters ($p>0.05$) (Fig.5abceghi).

Discussion

In the present study, the blood microcirculation, thermal and mechanical sensitivity of the gingiva were

evaluated in patients with periodontitis compared to a normal population. The results revealed that gingiva in periodontitis patients appeared more sensitive to thermal stimulation and mechanical stimulation.

Clinical features in patients and effect of non-surgical periodontal therapy on clinical parameters

In most clinical situations, the worst prognosis of periodontitis and the greatest degree of inflammation can be seen at the mandibular incisors [24]. In addition, the test sites in the present study were limited by the size of the probe and measuring direction. In order to test the tooth in expected directions and avoid the influence of the mandibular labial frenum, the mandibular lateral incisors were selected as the test sites in this study.

Most of the patients had teeth that were in Stage II and Grade B of periodontitis, which is equivalent to moderate periodontitis with moderate rate of progression, and the periodontal tissue lesion as assessed during the clinical examinations, was limited to 1/3 of the crown. There were few site differences among patients or controls, which may due to normal variation between the two sites. However, there were some sites differences before and after non-surgical periodontal therapy, and it is reasonable that different healing process may exist at the two sites.

Non-surgical periodontal therapy is effective for this stage and grade of periodontitis thorough a disruption and careful removal of the biofilm [25]. This effect is reflected in Figure 3, which showed remarkable improvements after treatment, with decreases in PPD, CAL and BOP (%). All periodontitis patients in this study obtained a state free from inflammatory periodontal diseases, in the whole dentition with $PPD \leq 4mm$, $BOP (\%) < 10\%$, and usually with a reduced periodontium [17].

Gingival blood microcirculation abnormalities in periodontitis patients and after non-surgical periodontal therapy

In the present study, it was shown that periodontitis patients have higher values of Conc, consistent with previous research suggesting capillary dilatation and increased blood flow in inflamed gingiva and periodontal tissues [26,27]. Previous studies have demonstrated the production of vascular endothelial growth factor (VEGF) can be up-regulated in periodontitis sites while compared to the healthy sides, which can accelerate angiogenesis, and inflammation can cause vasodilation and increase the circulation. These factors might contribute to increased capillary blood flow [28,29]. However, controversies have existed over the past few decades, because other studies showed a significant decrease in blood flow in the patients with inflamed gingiva [30,31]. Nevertheless, this protocol reveals a microcirculation change in gingiva in periodontitis patients, and further investigations with larger sample and possibly connected to the histopathology are needed to clarify these issues.

The significant decrease in Flux and Speed after non-surgical periodontal therapy in the present study may be due to the decrease in VEGF, and decreased levels of pro-inflammatory cytokines in the gingival crevicular fluid after treatment [29]. Most of the healing occurs in 6 weeks after treatment, but the periodontal repair may continue for a longer time. Previous research reported that deep periodontal pockets showed the most marked decrease in VEGF 3 months after treatment and it takes approximately 3 months for inflamed gingiva to revert to completely healthy gingiva [32,29]. Therefore, future studies with longer follow-up should be initiated.

These preliminary finding may help to understand better the influence of inflammation on vasodilation in periodontal tissues and the effect of non-surgical periodontal therapy on the blood flow changes. LDF may be a way to estimate the degree of periodontitis and the effect of non-surgical

periodontal therapy by evaluating microcirculation in gingival tissues.

Somatosensory abnormalities in periodontitis patients and after non-surgical periodontal therapy

As a reliable, quantitative and painless technique, the QST battery has been used to assess somatosensory loss and gain in a large number of conditions in the last couple of decades, however, there were very few studies had applied a QST battery in periodontitis patients comparing to healthy controls to our knowledge.

WDT and HPT are considered to represent C-fiber function. MDT is considered to assess A-beta-fiber function. CDT and MPT are considered to represent A-delta -fiber function [23]. Our results showed lower HPT and MPT in periodontitis patients compared to controls, indicating a significant gain in somatosensory function. The abnormalities of these small fiber functions maybe caused by the pathological processes of inflammation in periodontitis. The Z-scores profiles make it clearer to understand “loss and gain” of somatosensory function in individual patients with different status of periodontitis. It seems that an abnormal WDT, HPT and PPT more frequently can be observed around the tooth with a more severe status of periodontitis, indicating that the abnormality of somatosensory may be related to the state of inflammation in periodontal tissues. However, the sample size was relatively small and there was a great individual variance in sensory testing, so further analysis with a larger sample size is required.

Though periodontitis is generally considered to be a painless condition, for example when compared to pulpitis, it has been reported that pain associated with chronic periodontal diseases may be mild, persistent, or episodic dull and periodontitis can results in some degree of trigeminal neural sensitization [33]. Previous literature has indicated that periodontal pathogens in dental plaque can not

only directly produce a range of bioactive molecules which may sensitize receptors and ion channels on neuronal endings, it can also induce the production of host response mediators related to inflammation and pain [34]. Furthermore, tissue-damaging stimuli and inflammatory mediators can cause “peripheral sensitization”, by increasing excitability of nociceptive endings [35]. Such as interleukin 1β (IL- 1β) and tumor necrosis factor α (TNF- α), which can promotes release of prostaglandins, then sensitize nociceptors on C-fiber and decrease the nociceptor threshold [36]. Therefore, it is possible that these locally produced cytokines at the site of tissue injury lead to the changes in thermal threshold and mechanical threshold in periodontitis patients.

Previous study has detected that patients after treatment would be accompanied with significantly decreased levels of pro-inflammatory cytokines in the gingival crevicular fluid, like IL- 1β and TNF- α [29]. That may explain the decreased sensitivities of CPT, HPT and MPT after non-surgical periodontal therapy compared to baseline. The somatosensory function gradually recovered and would eventually return to the normal range when these inflammatory mediators were further decreased. Meanwhile, even though nonsurgical therapy did not cause injuries like those caused by surgical operations, it may result in some degree of damage to the periodontal tissues due to the deep mechanical scaling and planning. Perhaps nerve fibers and receptors in the periodontal ligament were further damaged, which can result in dysesthesia or numbness of the test area [37].

The inter- and intra-examiner reproducibility was not investigated in present study, however previous studies have shown a good inter- and intra-examiner reproducibility in terms of the thermal and mechanical QST in the orofacial regions [38, 39].

Since the QST device can detect even minor changes in thermal and mechanical thresholds in the periodontal tissues with inflammation, such a mean of somatosensory measurement may also be used

1 to evaluate the stage and prognosis of periodontitis. Further studies are needed to test the
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3 maneuverability and reliability of these techniques in evaluating periodontitis.
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8 **Study limitations**

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11 Most of the periodontitis patients were in stage II , Grade B of generalized periodontitis, and therefore
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13 the results only apply to this cohort and further studies in other stages of periodontitis will be needed to
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15 be better understand the somatosensory changes in the gingiva. The patients recruited in the present
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17 study were also fairly young and might not represent the profile of the normal clinical periodontitis
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19 patients, as the risk of periodontitis increases with advancing age and usually steadily until individuals
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21 reached approximately 40 years of age [1]. The relatively small sample size in the present study could
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23 be insufficient for assessing possible treatment effect, furthermore, the follow-up time was only 4
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25 weeks, therefore longer follow-up with bigger sample size need to be performed in future study.
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36 **Conclusions**

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38 The present study demonstrated significant disturbances in blood microcirculation and somatosensory
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40 function in periodontitis patients, and significant recovery of somatosensory function was observed
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42 after the non-surgical periodontal therapy. These results might help understand pathological changes of
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44 periodontitis, and LDF and QST might be used as complementary tools to evaluate the status
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46 of periodontitis and treatment outcomes.
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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interests.

Ethical approval: All procedures performed in studies involving human participants were approved by Ethics Committee of Nanjing Medical University (No: PJ2018-013-04)and conducted in accordance with the Declaration of Helsinki.

Informed consent: Informed consent was obtained from all participants included in the study.

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Figure and table legends

Figure1. Clinical photos demonstrating the tests methods employed in this study.

a: Clinical assessment of laser Doppler monitoring (LDF)

b; Clinical assessment of thermal threshold testing

c: Clinical assessment of mechanical detection thresholds (MDT)

d: Clinical assessment of mechanical pain thresholds (MPT)

e: Clinical assessment of pressure pain threshold testing (PPT)

Figure2. Individual Z-score profiles of the 20 patients with periodontitis

CDT=cold detection threshold, CPT=cold pain threshold, WDT=warm detection threshold, HPT=heat pain threshold, MDT=mechanical detection threshold, MPT=mechanical pain threshold, PPT-l=pressure pain threshold, lateral of teeth, PPT-v=pressure pain threshold, vertical of teeth, PPT-g=pressure pain threshold of attached gingiva.

Figure3. Clinical parameter changes after non-surgical periodontal therapy

PPD=probing pocket depth, CAL=clinical attachment loss, BOP=bleeding on probing. Black bars show data of the tooth 32 and grey bars present data of tooth 42 tested at baseline (BL), 1 week (1W) and 4 weeks (4W). (*: $p < 0.05$).

Figure4. LDF value changes after non-surgical periodontal therapy

Flux=microvascular blood perfusion, Conc=blood cell concentration, Speed=the relative velocity of microvascular blood flow, Temp=temperature. ^a Difference between baseline (BL) and 1 week (1W) or

4 weeks ($p<0.05$). ^b Difference between tooth 32 and tooth 42 ($p<0.05$).

Figure5. QST value changes after non-surgical periodontal therapy

CDT=cold detection threshold, CPT=cold pain threshold, WDT=warm detection threshold, HPT=heat
pain threshold, MDT=mechanical detection threshold, MPT=mechanical pain threshold,
PPT-l=pressure pain threshold, lateral direction, PPT-v=pressure pain threshold, vertical direction,
PPT-g=pressure pain threshold of attached gingiva. ^a Difference between baseline (BL) and 1 week
(1W) or 4 weeks ($p<0.05$). ^b Difference between tooth 32 and tooth 42 ($p<0.05$).

Table1. Mean values \pm standard deviation for clinical parameter of tooth 32 and 42 in periodontitis patients and healthy controls

PPD=probing pocket depth, CAL=clinical attachment loss, BOP=bleeding on probing. (*: $p<0.05$)

Table2. A 2-way mixed model ANOVA for LDF and QST values in different site (tooth 32 and tooth 42) and group (controls and patients) with repeated measures

Flux=microvascular blood perfusion, Conc=blood cell concentration, Speed=the relative velocity of microvascular blood flow, Temp=temperature. CDT=cold detection threshold, CPT=cold pain threshold, WDT=warm detection threshold, HPT=heat pain threshold, MDT=mechanical detection threshold, MPT=mechanical pain threshold, PPT-l=pressure pain threshold, lateral of teeth, PPT-v=pressure pain threshold, vertical of teeth, PPT-g= pressure pain threshold of attached gingiva. (*: $p<0.05$)