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Research Article

Laser Doppler Flowmeter as a Periodontal Evaluation Method: A Clinical Pilot Study

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Abstract

Background and objectives: Periodontal disease, as an inflammatory pathology, induces hemodynamic changes that can be evaluated by different unbiased methods such as laser Doppler flowmetry. This clinical investigation assesses laser Doppler as a non-invasive procedure to monitor gingival vascularization and its potential relationship with the response to treatment of periodontal disease.

Materials & methods: 45 sites of white Spanish patients with active periodontitis undertake a complete periodontal analysis. This included periodontal pathogens identification along with the monitoring of the gingival margin microvascularization using a Doppler laser at the points exhibiting the most periodontal damage. All assessments were performed before and after periodontal combined treatment PCT (scaling, root planing, and antibiotic therapy prescription) (n = 45 sites).

Results: Parameters of periodontal disease showed a positive correlation with pathogen levels. Blood flow readings decreased significantly after PCT (p < 0,05), although this parameter was not statistically correlated with periodontal nor microbial assessments in a significant range.

Conclusion: Laser Doppler is a complementary method of monitoring periodontal inflammation to traditional techniques of clinical periodontal evaluation. Further studies are necessary to determine its usefulness as a predictive method of periodontal disease evolution.

More Information

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Introduction

Periodontitis is the main cause of dental loss in the adult population. However, diagnosis of periodontal disease is still based upon subjective clinical examination procedures, which are time-consuming and poorly implemented in general dental practice [1]. Research into periodontal disease is one of the leading topics in dental knowledge. Special attention has been focused on new innovative methods in periodontal diagnosis, specifically built on the momentum of the genomic and proteomic era, as well as advances in cell biology and cell signaling upon periodontal diagnosis and therapy. Considering the essential need for translation of basic research into office practice, and the thorny issue of how cost-effective periodontal therapy is, new approaches in parameters for periodontal diagnosis and treatment monitoring are of paramount relevance.

Periodontal health is defined as the absence of inflammation even in a reduced periodontium. Inflammation constitutes a paramount sign of periodontal disease and the first step in the cellular and humoral immune response [2]. Periodontal pathogens and their invasion of gingival epithelium cells, alter periodontum in two ways: A.- causing tissue destruction; and B.- producing harmful substances that, during tissue damage, act as proinflammatory cytokines that stimulate the inflammatory response [3-6]. Early changes of this defensive reaction occur at a microvascular level as a form of angiogenesis, due to dilatation of the capillaries and an increase in the number of them [7,8]. This can be transferred

to both the initial gingivitis and chronic inflammatory periodontal processes.

Several studies employ laser Doppler flowmetry (LDF) as a non-invasive method to assess tissue vascularity. This technique can be used in different microvascular systems such as skin, colonic, muscular, gingival, pulpal, and oral mucosal tissues [9-23]. An animal study measured the influence of irradiation on decreased jaw-bone vascularization, validating LDF to monitor alveolar bone vascularity [24]. The establishment of a normal values range for bone micro-vascularization may reduce the risk of osteoradionecrosis in implant treatment of irradiated patients [25]. Normal blood flow velocity values could be calculated relative to periodontal tissues, as recently reported for eye microvasculature [26]. In Periodontics, LDF appraisals of tissue response to either basic periodontal treatment (scaling and root planning) [27,28] or surgical approach [18,19] have proved laser Doppler readings to be positively correlated to gingival inflammation reduction [28-30]. Other studies that focus on gingival micro-vascularization differences between smokers and non-smokers failed to find a lower gingival blood flow in non-smoking patients with the same degree of periodontitis. Tobacco has not proved to be capable of causing vasoconstriction in oral tissues, contrary to clinical observations in which there is a lower tendency to bleeding on probing in this group of patients [18,21].

Eventually, it could be possible to establish a threshold in periodontal vascularity value beyond which periodontal bone resorption could be triggered. As a matter of fact, impartial clinical decisions in periodontal diagnosis and treatment outcomes might be influenced by assessing micro-vascularity in periodontal tissues. Hence, clinical implementation of laser Doppler readings may increase the predictability of periodontal treatments. However, to prove this method to be useful in human beings, normal values of human periodontal vascularity measured by LDF constitute a pre-requisite. As far as we know, no study has considered how the presence of periodontal pathogens influences gingival blood flow/gingival inflammation. This study tackles the evolution of the gingival blood flow after combined therapy with basic periodontal treatment and antibiotics.

Materials and methods

Study design and population

This pilot study includes a total of 9 white Spanish patients (4 women and 5 men) between 26 and 71 years old (mean 44.67, standard deviation SD 13.17). All participants sought periodontal treatment in private practice and exhibited periodontal biotype medium [31] and disease stage III and IV, grade C [32]. It was distinguished between smokers and non-smokers.

The following exclusion criteria were considered: patients with a history of excessive consumption of drugs and/or alcohol, pregnant or breastfeeding, infectious diseases, intravenous bisphosphonates treatment, antibiotic treatment for less than 2 months before starting with the first part of the study, diabetics, chemotherapy, head or neck irradiation, hematologic disorders, as well as in psychiatric patients.

The protocol study complies with the ethical precepts formulated in the Declaration of Helsinki of the World Medical Association on the ethical principles for medical research in human beings and in their subsequent revisions, as well as those requirements for the applicable legal regulations. It was approved by the clinical research ethics committee of the San Carlos Clinical Hospital in Madrid (C.I. 17/129C). All patients signed an informed consent of the medical process and its inclusion in the research study.

The timeline of the experiment (Figure 1) had three checkpoints:

1. Periodontal study which included periodontogram, periodontal pathogens identification, and pre-treatment blood flow laser Doppler readings at the 5 sites exhibiting the greatest periodontal disease activity;

2. One-stage scaling and root planning treatment followed by antibiotic prescription (Metronidazole 500 mg / 12h / 7 days [33,34]),

3. Eight weeks later, all parameters were re-evaluated to assess the correlation between clinical intervention, microbial counts, and LDF readings.

Periodontal evaluation

Clinical data from patients included an exhaustive anamnesis form, orthopantomography (OPG), and standardized periapical radiographic series [35]. The periodontal chart was fulfilled using a CP-12 periodontal probe, and included assessments of probing depth (PD), bleeding on probing (BOP) scored according to the Löe and Silness criteria, plaque index (PI) recorded by the O'Leary plaque index, clinical attachment loss (CAL) and recessions (REC). All these measurements involved six tooth aspects (mesiobuccal, buccal, distobuccal, mesio-lingual, lingual, and disto-lingual) [36-40]. Periodontogram also came with tooth mobility, sulcular suppuration, and furcation defects.

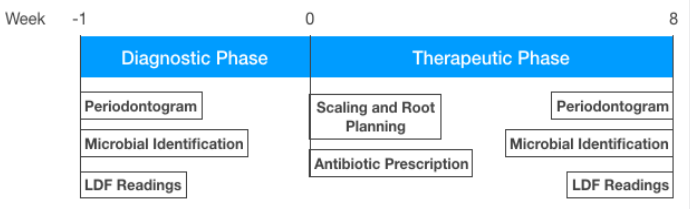

Figure 1: Timeline of the experiment.Week 1: Diagnostic phase with a periodontal chart fulfilled, microbiological testing of periodontal microorganism, and microvascular blood flow readings using laser Doppler flowmetry. Week 0: Periodontal treatment (scaling, root planing, and antibiotic prescription). Week 8: Periodontal reevaluation (periodontal chart, periodontal pathogen identification, and blood flow readings using laser Doppler flowmetry).

Microbiological testing of periodontal microorganisms

The five sites with higher PD values from periodontal evaluation were allocated for bacterial sample collection. Protocol for sulcular crevicular fluid bacterial samples included supragingival extensive drying and plaque removal with sterile cotton pellets.

The processing of the samples was carried out in the Microbiology and Molecular Virology Unit of the Analysis Laboratories Dr. Echevarne (Barcelona, Spain) within 24 hours. For the identification of periodontal pathogens, in samples of crevicular fluid, a conventional polymerase chain reaction (PCR) was performed followed by identification by reverse hybridization on a colorimetric strip. The microIDent® kit (Hain Lifescience, GmbH, Nehren, Germany) was used, based on DNA · STRIP® technology, which allows the semi-quantitative detection of *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td) and *Prevotella intermedia* (Pi).

For this, a multiplex PCR was carried out, using complementary oligonucleotides of fraction 16 of the rDNA corresponding to these microorganisms, followed by simultaneous reverse hybridization on a nylon strip with colorimetric labeling. Multiplex amplification was accomplished with biotin-labeled primers. The reverse hybridization procedure was performed according to the instructions of the manufacturer of the microIDent® kit (Hain Lifescience, Nehren, Germany). Each strip supplied by the manufacturer of the microIDent® test has a total of 7 reaction zones: 2 corresponding to quality control (Conjugate Control (CC) and Amplification Control (AC)), and 5 specific bands for each bacterial species (Aa, Pg, Tf, Td and Pi) (Figure 2A). The results were determined according to the intensity of staining of each line in the specific bands, corresponding to each bacterium (Figure 2B). The degree of staining of each band was represented by crosses (Figure 2C), following a scale from lower to higher: (+), +, ++, +++, which the commercial company makes correspond with a specific concentration for each bacterium (Figure 2D). The absence of staining was considered negative, indicating that the sample contained less than the detectable level of nucleic acid for the target microorganism. The detection limit was determined by the manufacturer and corresponded to 10^3 genomes, in the case of Aa, and 10^4 genomes for Pg, Pi, Tf, and Td.

Micro-vascular flow assessments

Laser Doppler Flowmeter Figure 3 (Moor VMS-PC®, Moor Instruments Limited UK) surveyed blood flow at the periodontal gingival margin. The probe used in this experiment displayed a 1.5 mm diameter and a fiber distance of 0.5 mm (VP3 needle-shaped probe) Figure 4. This flowmeter is a semiconductor diode laser with a wavelength of 780 nm, based on the Doppler effect that provides continuous monitoring of

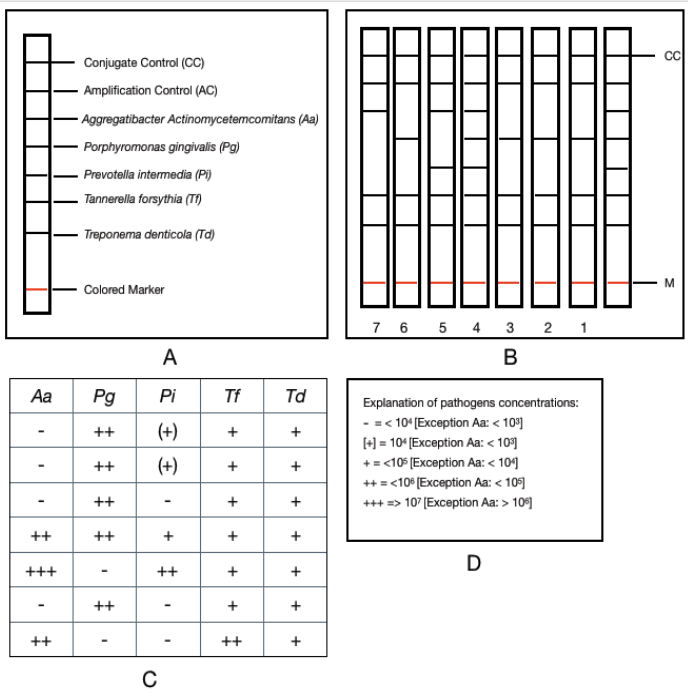

Figure 2: PCR results interpretation accord to the microIDent® kit manufacturer (Hain Lifescience, GmbH, Nehren, Germany) A-Reaction zones (sites 1-7). B-Staining of line in the specific bands, corresponding to each bacterium. C- Degree of staining of each band represented by crosses. D- Explanation of pathogens concentrations.

Figure 3: Laser Doppler flowmeter monitor Moor VMS-LDF1-HP.

Figure 4: VP3 Needle shape probe.

blood flow with a sampling frequency of 40 Hz and a depth of 1 mm. Doppler effect consists of the apparent frequency change of a wave produced by the relative movement of a source with respect to an observer. In the blood flow, by striking a beam of light on any human tissue, it is dispersed both by the static structures and by the red blood cells. Those beams returned by the red blood cells suffer a deviation in their frequency that is enlarged, however, those that affect the static structures are not modified. Both light fractions are captured by a photodetector and processed to determine blood flow [24].

Flowmeters measure blood flow in perfusion units (PU) as the product of the average speed and concentration of blood cells in a single volume of tissue. "Standard Motility" consisting in a low concentration of polystyrene microspheres in water submitted along thermal movement (brownian movement) calibrates the probe. A memory chip in the probe stores figures from the calibration process. Probe positioning on the gingival margin was stabilized by means of a customized splint in order to sustain measurement reproductivity. Splint is important to minimize scattering from structures other than blood cells that could generate Doppler changes and produce an indistinguishable signal from that caused by blood flow itself. LDF readings were recorded during an interval of 20 seconds (Figure 5). Blood flow assessments were performed at each site independently so that positioning in the split did not scatter readings during probe recording.

Factors such as room temperature and brightness are confounding factors for LDF readings and may cause bias in results. Since low temperatures (< 15 ºC) reduces significantly Doppler laser flowmeter signal, the room was maintained at all times in a 23 ºC - 26 ºC range during procedures. The dental chair was switched off and exposure from outside light was controlled to minimize probe scattering from external light energy sources.

Statistical analysis

Statistics software SPSS® was used to perform data analysis. All measurements were described by means and SD, statistical significance was pointed for a $p < 0.05$. Blood flow was considered a dependent variable and compared with indirect variables such as gender, age, toxic habits (smoke), PD, BOP, PI, CAL, and periodontal pathogens presence. Measurement values at different timeline points were analyzed by the T-Student test for paired samples, using the range with Wilcoxon signs when appropriate. Pearson correlation coefficient was used to determine the correlation between blood flow (PU) and the rest of the parameters. Differences between subjects (smoker or not) were compared by means of the Mann-Whitney U test.

Results

Demographic characteristics and blood flow readings (PU)

LDF readings mean at T1 (week -1) was 86.85 SD of 103.44. After PCT, LDF readings were significantly lower (mean 54.31 and SD 52.82), being statistically significant ($p = 0.048$, mean -32.50, SD 107.32).

Data analysis revealed significantly lower PU in female patients compared with male patients at T1(week -1) and at T2 (week 8). T-Student Test showed also a decrease in PU values statistically significant in female patients ($p < 0.05$) after PCT.

Pearson's correlation test indicates a statistical signification comparing microvascular readings with age, younger patients exhibited bigger difference in LDF readings after PTC than older ones ($p = 0.016$).

No statistical differences were found between smokers or not in microvascular readings previous or after periodontal treatment (Mann-Withney U test $p > 0.05$) (Table 1).

Periodontal parameters and blood flow readings (PU)

Men had worse indicators of periodontal health than women, even this was not statistically significant. Eight weeks after PCT, all patients had improvements in all periodontal parameters, in terms of pocket reduction, gain of clinical attachment level and reduction in full mouth bleeding and

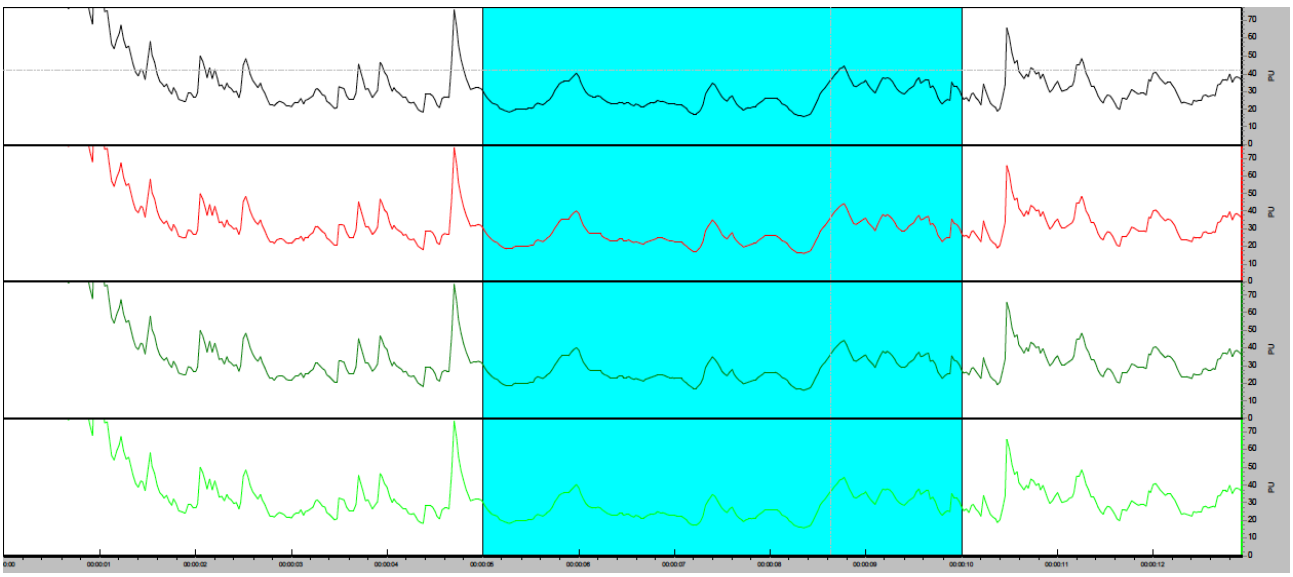

Figure 5: Blood flow measurements in PU with the Moor-VMS software. 20 seconds selected.

Table 1: Demographic characteristics and blood flow readings were measured in perfusion units. Means, standard deviations, and p values.

Perfusion Units	Global population		p value (AGE)	Men			Women			p value	Smokers		Non smokers		p value
	Mean	SD		Mean	SD	p value	Mean	SD	p value		Mean	SD	Mean	SD	
T1.Week -1	86.85	103.44	.023	119.37	142.38		60.83	44.51		.024	67.18	46.82	102.58	131.53	.694
T2.Week 8	54.31	52.82	.679	72.02	70.53		40.20	26.57		.107	47.19	35.96	60.06	63.35	.253
Interventional Change	-32.50	107.32	.016	-47.35	151.93	.179	-20.62	50.13	.05		-19.98	50.24	-42.52	137.42	.607

Table 2: Periodontal parameters correlate with blood flow readings measured in perfusion units. Means, standard deviations, and p values.

Periodontal Parameters	PD			p Value (PU)	CAL			p value (PU)	PI			PU	BOP			p value (PU)
	Mean	SD	p Value		Mean	SD	p value		Mean	SD	P value		Mean	SD	p value	
T1.Week -1	6.27	1.30	-	.935	6.64	1.78	-	.873	79 %	18.52	-	.914	65 %	20.79	-	.123
T2. Week 8	4.2	1.07	-	.170	4.56	1.63	-	.983	48 %	30.57	-	.990	27.44%	10.34	-	.615
Interventional Change	-2.08	1.51	.000	.905	-2.067	1.09	.000	.699	-31 %	12,05	.000	-	-37,56 %	10,45	.000	-

Table 3: Periodontal pathogens correlated with periodontal and microvascular parameters in T1 and T2 (Mann Whitney U test < 0.05 *p* values).

Periodontal and Microvascular Parameters	Aa		Pg		Tf		Td		Pi	
	T1 Week -1	T2 Week 8	T1 Week -1	T2 Week 8	T1 Week -1	T2 Week 8	T1 Week -1	T2 Week 8	T1 Week -1	T2 Week 8
PU	0.05	-	-	-	-	.028	.045	-	.049	-
PD	-	-	-	-	-	-	-	-	.006	-
BOP	-	-	.015	-	.000	.000	-	.05	.045	.006
IP	-	-	-	-	-	.001	.000	.000	.000	-
CAL	-	-	-	-	-	-	-	.000	.004	-
DIFF PU	.05	-	-	-	.047	-	-	-	-	-

plaque scores. Probing depth decreased mean -2.08, SD 1.51 $p < 0.05$. The full mouth bleeding on probing percentage was 65% in week -1 and after PCT (week 8) was 27.44%. In addition, the plaque index was significantly reduced and the mean percentage of plaque in the whole mouth went from 79% to 48% after PCT. Regarding CAL, it experienced an average improvement of -2.08 SD 1.51. Pearson's correlation tests did not show a significant correlation with the decrease in LDF ($p > 0.05$) (Table 2).

Periodontal pathogens count and periodontal and microvascular parameters

Pearson's correlation test showed a significant reduction in all periodontal pathogens ($p = 0.000$) after PCT, except for the reduction in Td, which was not statistically significant.

A positive relationship could be established between certain pathogen reductions and PU levels, as well as plaque index and bleeding on the probing index (Table 3).

Discussion

Until today, the most used methods for evaluation and monitoring periodontal disease are indirect procedures based on clinical evaluations and determinations of the subgingival microflora. The direct methods used previously required invasive techniques such as biopsies, which cause an irreversible change in the tissues studied [41].

Several studies in animals have shown that blood flow is greater in an inflamed gingiva than in a healthy one due to blood stasis [42,43]. Likewise, when gingival inflammation occurs in humans, changes are detected in the marginal gingiva, consisting of an increase in the number of visible vessels, among others [44]. These changes never reverted back to the original blood flow healthy pattern even when inflammation had resolved [45]. Therefore, the use of blood flow as a predictive indicator of future healing and prognosis of periodontal disease is a concept worth pursuing [46].

In the present experiment, the blood flow in the marginal gingiva was notably greater before PCT than 8 weeks after it, having produced both a clinical and statistical reduction in the evaluation parameters of gingival inflammation and periodontal status, considering in some cases the site studied without gingival inflammation (no BOP, PD£3mm). The classic diagnostic parameters of PD, BOP, and CAL, as well as their effectiveness in the monitoring of periodontal disease, have been widely described. It was possible to establish a positive correlation between these parameters and gingival blood flow in the marginal and intracellular gingiva at the disease sites compared to healthy ones. This relationship is also directly proportional to the severity of periodontitis, increasing with greater severity [27,47]. In this study, we also have found a positive relationship, although not statistically significant, between the classic parameters to evaluate periodontal disease and blood flow, the analysis of the data shows a significant decrease after treatment in all parameters, both periodontal and flow of blood.

A possible limitation in the data collection process was the size of the probe. The gingival LDF signal is dominated by the flow mainly from the superficial vessels, so with the current wavelength of the laser light and the construction of the probe, it did not allow us to obtain records of the blood flow within the periodontal pocket, where the first inflammatory changes occur and the largest number of pathogens are present.

All patients included had established stage III and IV and grade C periodontitis, but it would be interesting to focus

future research on the evolution of blood flow in relation to inflammation development since greater readings have been described in chronic periodontitis than in the initial gingivitis [28].

Probe splinting or manual blood flow measurement was a controversial issue in previous studies [28,48]. It has been reported higher measure values when performed by hand than splinting, which may be due to the pressure of the splinting material that compresses the gingiva. For this reason, in this study, the measurements were always carried out by the same operator with a customized splint, seeking not to interfere with the natural vascularization conditions by not contacting the gingival tissue and avoiding previously performed injections of anesthesia with a vasoconstrictor Figure 6.

Comparison with previous studies was hampered by the variety of Doppler lasers used with different calibration constants and units of measurement, as well as by the different study protocols. Even so, the minimum time required to measure blood flow was established, in line with these studies, at 20 consecutive seconds per monitored site [21,49-51]. Other studies based on microvascular flow assessments, employed different methods such as Optical Coherence Technology Angiography (OCTA) [52]. This technique, although non-invasive, is also limited by its expensiveness, slow acquisition time, and small field of imaging among others [53]. Fluorescein angiography (FA) has considerable shortcomings, such as being invasive, having a long image acquisition time, and not providing quantitative data. Also, near-infrared spectroscopy provides deeper tissue information on blood flow but it's not vessel-specific [54].

LDF is a proven monitoring tool of vascularization in other human tissues as alveolar bone [25]. The main application will be evaluating the osseointegration of dental implants, making microvascularization a crucial factor in implant stability. Human besides animal studies showed that LDF is an adequate method for bone microvascularity evaluation and might determine future implant success [55]. Therefore, laser Doppler flowmetry seemed to be a valid option in the search for new direct and non-invasive methods of microvascular flow measurements.

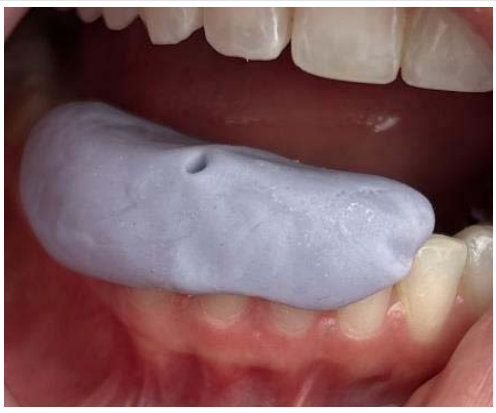

Figure 6: Customized splint.

One of the factors that influence the progression of periodontal disease is the combined action of related pathogens, capable of causing changes in the total oral microbiota, altering the homeostatic balance of the tissue [55-58]. Above all, the presence of the Socransky red complex bacteria (dominant in the late stages of plaque development and mainly found in cases of periodontitis in adults) is strongly associated with parameters of periodontal inflammation, including PD and BOP [56,59]. The presence of these pathogenic species has also been detected in healthy individuals, so for periodontal disease to manifest itself clinically, in addition to a subgingival microbiota shift (favoring excessive growth of pathogenic species), a suitable environment and susceptible host are essentiall [57,60].

Recent studies have focused on the role of innate immunity in the pathogenesis of periodontitis [61]. The final immune response occurs at the local level, being essential for microbiota regulation, by stimulating microenvironmental changes and limiting the increase in periodontal pathogens that help the most protective bacteria to predominate [62]. Despite the fact that, in this study, the relationship between the decrease in periodontopathogenic species in the studied sites and the evolution of blood flow and inflammation parameters appears to be positive, it lacks sufficient statistical significance. This supports the theory that, although the role of periodontopathogenic bacteria in the development of periodontal disease is undeniable, it is not exclusively an infectious but an inflammatory pathology, with an essential role in the host's immune system.

Since the exact mechanisms of the pathogenesis of periodontitis remain unclear, and multiple factors can destabilize this balance contributing to the pathogenesis of periodontitis, the counterbalance of different diagnostic parameters results in challenging for such a multifactorial disease.

Conclusion

In light of the findings of this study, LDF is a feasible method for gingival vascularization assessments, which in combination with traditional periodontal parameters, provides useful information for periodontal disease diagnosis and treatment monitoring.

Especially in the most clinically affected sites, a reduction in the means of blood flow readings was detected, as well as the correlation between the parameters of the evaluation of periodontal disease and the number of pathogens found.

Further studies are necessary to focus on its usefulness as a predictive method of the evolution of periodontal disease with a larger sample size and an increased follow-up time.

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