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Laser Doppler Flowmetry Evaluation of the Microcirculation in Dentistry

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/64926>

Abstract

This chapter presents the most important features of laser Doppler (LD) techniques: LD flowmetry (LDF) and LD imaging (LDI), together with examples of their clinical applications in dentistry. LDF gives a constant estimation of blood flow at a specified point, whereas LDI gives a 'snapshot' of perfusion at a given point. These methods are non-invasive laser-based techniques for monitoring gingival and pulpal blood flow and could be used as a diagnostic tool. In paediatric dentistry and odontology, LDF proved to be an atraumatic real-time method used for determining the tooth vitality by monitoring the pulp microcirculation in traumatized teeth, fractured teeth and teeth undergoing different conservative treatments (e.g. bleaching, dental preparation for prosthetic restorations, etc.). In periodontology, recent studies showed the ability of LDF to evaluate the health of gingival tissue in different types of periodontal diseases. By using LDF, it is also possible to evaluate the outcome after different periodontal treatments. The laser Doppler line scanning can be used for recording the gingival healing process after a surgical procedure in the anterior area of the oral cavity.

Keywords: microcirculation, dental pulp, gingiva, laser Doppler flowmetry, laser Doppler imaging

1. Introduction

The microcirculation consists of vessels with the diameter less than 100 μm . The structure and topological organization of the microcirculation located within organs differ from the larger conduit vessels that distribute blood flow to the organs. The rheological properties of blood

in the microcirculation differ from those in the large vessels due to the Fahraeus-Lindqvist effect, which lead to diameter-dependent reduction in hematocrit and effective blood viscosity in microcirculatory vessels [1]. The main function of the microcirculation is to deliver nutrients to and remove waste products from the various tissues as well as support the exchange of respiratory gasses. It also plays an essential role in fluid exchange between blood and tissue, delivery of hormones from endocrine glands to target organs, and bulk delivery between organs for storage or synthesis and provides a line of defence against pathogens [2]. An ideal technique for measurement of tissue oxygenation should provide quantitative, accurate and reproducible real-time information about oxygen supply and utilization in specific tissue beds. For clinical applications, such a device should be safe, non-invasive and easy to use.

Laser Doppler flowmetry (LDF) and laser Doppler imaging (LDI) have been widely used to assess tissue micro-vascular function. These techniques have functioned as clinical surrogate markers. However, the lack of standardization in data expression limits the use of these tests in routine practice. Nowadays, LDF is commonly used to assess tissue blood flow; yet, data exhibit great spatial variability. Another way of getting around spatial variability could be to evaluate tissue blood flow over wider areas by using LDI. Successful wound healing following periodontal surgery is strongly influenced by revascularization rate as well as by preservation and reconstruction of the micro-vascularity of the gingival tissues [3, 4]. Regular post-operative assessment of flap perfusion by members of the microsurgery team trained in the use of laser Doppler line scanning might, therefore, represent a practical alternative to more complex and invasive monitoring techniques.

There are numerous applications where LDF was used to non-invasively monitor changes in blood flow in living tissues. LDF has been used to assess blood flow for intact microvascular systems such as the skin, the retina, gut mesentery, renal cortex and mucous membranes [5, 6]. Dental applications include LDF readings (LDFRs) of periodontal ligament [7], pulpal blood vessels [8–12], gingival or sulcular blood flow in health and disease [13–18], evaluation of the degree in healing and revascularization of surgical wounds [19], the effect of orthodontic treatment [20] or the injection of vasoconstrictive anaesthetics on blood flow [21]. Single-point LDF, the technique mentioned above, shows good temporal resolution, poor spatial resolution and poor reproducibility in low capillary density tissue areas [22, 23]. This latter issue can be overcome by using either integrated probes with several transmitting and/or receiving fibres or full field techniques such as LDI. This technique shows excellent spatial resolution but poor temporal resolution for most devices (especially when scanning large areas) [24], but it provides a more valid measure of tissue blood flow [25].

2. Methods and results

The pulpal and gingival blood flows (GBFs) in the clinical situations described in this chapter were monitored using a MoorLab Laser Doppler (LD) equipment (Moor Instruments Ltd., Axminster, UK) with a straight optical probe, MP3b, 10 mm. A double silicone impression fixed perpendicularly on the buccal cervical surface of the tooth was used for stabilizing the

probe. The Moor Instruments MoorLab LD monitor uses laser radiation generated by a semiconductor laser diode operating at a wavelength of 780 ± 10 nm and a maximum accessible power of 1.6 mW. The programmed bandwidth of the recorded LD signal was 20 Hz–20 kHz while sampling frequency was 40 Hz. Calibration was performed according to the manufacturer instructions. LDF was recorded and analysed using MoorSoft MoorLab V2.01 software. The physical parameters assessed were flux, expressed in perfusion units (PU) and perfusion measurement (DC). The term used to estimate blood flow is flux—a quantity proportional to the average speed of the blood cells and their concentration. This is expressed in arbitrary perfusion units (AU) that are linearly related to flux. DC gives an indication of the backscattered laser light intensity. The DC signal indicates a correct positioning of the optical probe, showing the reflected laser radiation level from the level of concerned area. The DC signal is the one that indicates the mechanic stability of the optical probe placed at the level of acquisition area. The data were processed using statistical analysis software SPSS v16.0.1.

2.1. Microcirculation of the dental pulp

The tooth vitality preservation is one of the most important aims in conservative dentistry. This is why the reliable vitality assessment of the dental pulp has always been problematic and therefore, many methods have been suggested to test pulp vitality [26]. Pulp vitality tests should attempt to examine the presence of pulp blood flow, offering a precise, objective and quantitative assessment as opposed to the conventional tests that rely on the patient's subjective sensitivity [27, 28].

It is reported in the literature that the LDF technique is reliable for measuring human pulpal blood flow (PBF) to determine pulp vitality [29, 30]. The technique can measure perfusion quantitatively in real time [31]. However, it has also been claimed that signals from human teeth do not necessarily indicate pulpal blood flow and could be confused with a signal obtained from nearby gingival tissues, suggesting that periodontium and other neighbouring tissues can contribute to the signal [32–34]. Polat et al. [34] examined the scattering and penetration properties of the laser used in LDF by using a camera with slow speed shutters. They demonstrated that the laser can densely penetrate up to 4 mm in depth and less densely for up to 13 mm. This also suggests that even with proper isolation of the tooth, some signal contamination from the periodontium is inevitable. They also demonstrated that without isolation, the laser light could scatter from the source tooth to the whole oral cavity, which can also potentially contribute to signal contamination. Karayilmaz and Kirzioglu [35] indicated that LDF could reliably discriminate the vitality of the teeth with a sensitivity and specificity of 1.0 for studied sample. LDF was found to be a more reliable and effective method than pulse oximetry (PO) and electric pulp tester (EPT) in assessing the pulpal status of human teeth.

The isolation method before LDF measurements is crucial for obtaining an accurate signal. Therefore, many authors have used different isolation techniques, thus the different results. This is why many studies suggest that 45–82% of the blood flow recorded with LDF from human teeth may not be from the pulp [36–39]. Soo-Ampon et al. [33] found that up to 80% of the LDF output signal in human incisors may be non-pulp in origin if attempts at tooth isolation

are not made. Polat et al. [34] compared teeth that had undergone a pulpectomy with contralateral healthy pulps as controls. They also found that approximately 70% of the LDF readings from teeth with the pulps removed were non-pulp in origin. The results obtained by our group [40] show that about 69% of the acquired LD signal is of non-pulp origin, consistent with the existing literature [38, 39, 41, 42].

For this reason, in our studies investigating dental pulp blood flow, a silicone impression combined with light cure periodontal liquid dam was used in order to reduce the signal contamination. This method offered an excellent isolation certified by the DC values obtained during the measurements. It has been shown that the light from a LDF probe placed at 2 mm above the buccal cement-enamel junction is transmitted apically towards the radicular pulp [41]. There are several studies that have reported the placement of the LDF probe at 1–1.5 [43], 2 [44], 2–3 [45, 46], ~3 [47] and 4–5 mm [48] coronal to the gingival margin. In our studies, the probe was placed on the cervical third of the tooth, at 3 mm away from the gingival margin (Figure 1).

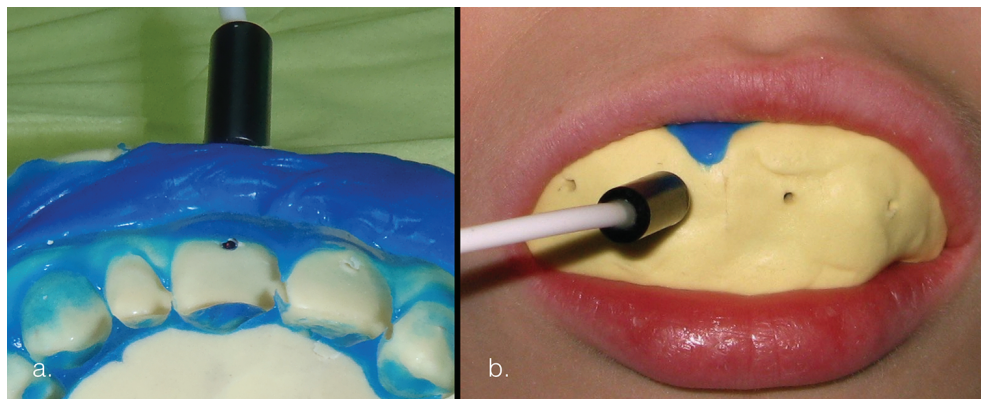


Figure 1. The acquisition technique of laser Doppler signals. (a) Silicone holder with the optical fibre inserted in the canal previously created and (b) intra-oral positioning of the silicone holder together with the stabilized optical fibre.

2.1.1. Bleaching and pulp microcirculation

The treatment of teeth whitening can be performed in the dental office, by the dentist, or at patient's home, and uses whitening agents, such as hydrogen peroxide gel (3–38%), carbamide peroxide (10–30%) or a mixture of hydrogen peroxide and sodium carbonate. Tooth bleaching, as one of the most required dental cosmetic procedures, must imply a consequent tooth vitality assessment. Sensitivity is strongly related to concentration, time and rate of usage of the bleaching gel [39, 42, 49–51]. In general, the activation systems have a role in increasing the temperature of the whitening agent, which penetrates rapidly the dental hard tissues, an aspect that favours the obtaining of an optimal result in a short interval of time but with the risk of increasing the inner pulp temperature. Therefore, this procedure can cause a local irritation to the dental pulp, which affects its micro-vascularization. In one of our studies, we chose the 1064 nm laser instrument for activating the bleaching gel and we compared it with the conventional 'in office' bleaching procedure, using LDF measurements.

In the Nd:YAG (1064 nm) laser-assisted bleaching, the pulp had a much better recovery (**Figure 2**), suggesting that LDF is a suitable method for a continuous monitoring of the dental pulp microcirculation [52].

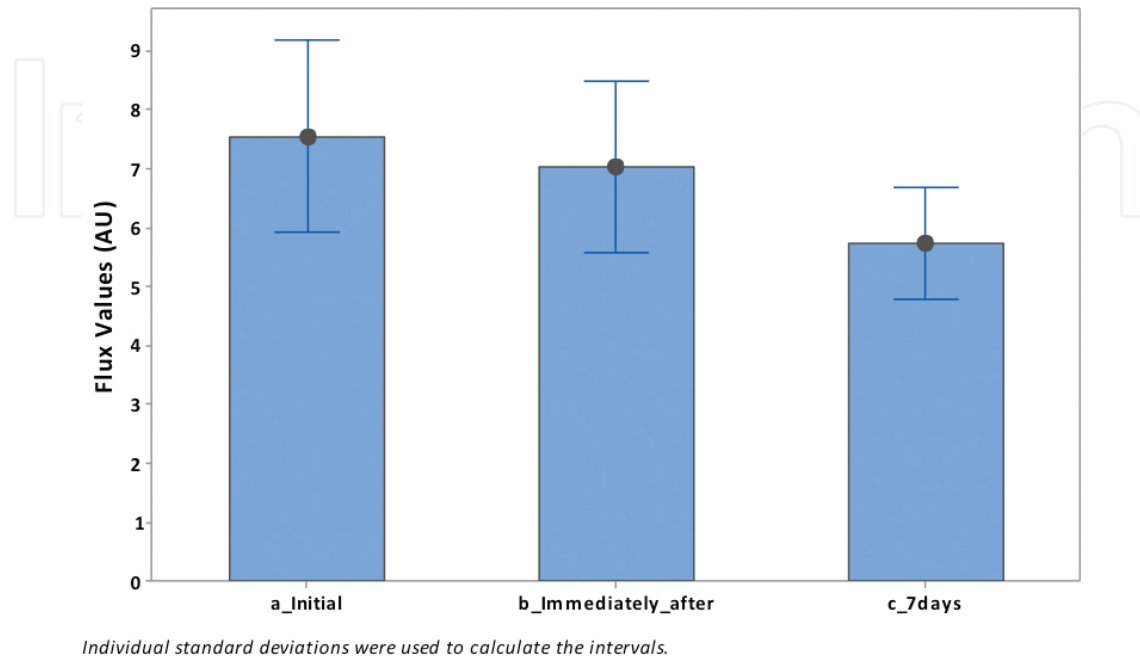


Figure 2. Interval plot of mean recorded before, immediately after and 1 week after treatment, indicating the evolution of the pulp blood flow over time for laser-assisted bleaching procedure.

2.1.2. Prepared teeth and pulp microcirculation

Determination of pulpal health represents an objective of endodontic diagnosis. It is important to assess pulp vitality prior to undertaking extensive tooth preparation in order to improve the prognosis of the restoration. It is also desirable to confirm periodically the pulp vitality in teeth that have undergone pulp preservation procedures or have had extensive restorations [53].

Full crown preparation procedures are probably the greatest restorative injury to which the dental pulp is subjected [54, 55]. The extensive cutting during crown preparation, desiccation, thermal injury and bacterial contamination has been implicated in the injury associated with tooth preparation [56]. Crown preparation without water spray causes about 95% reduction in the pulpal blood flow by 1 h after preparation. In contrast, the use of water spray virtually eradicates any alteration in pulpal blood flow. The reduction in coronal pulp blood flow is the result of an increased blood flow through the apically positioned arteriovenous (AVA) shunts and a redistribution of blood flow from the drilled side to the opposite side of the pulp [28, 57].

However, few reports were found in the literature regarding the use of LDF in assessing the pulpal blood flow in teeth that underwent prosthetic preparations [58, 59]. That is why the aim of our study was to evaluate how teeth preparation for full crown coverage may affect the pulpal blood flow.

The results obtained in our study show a linear increase in pulpal blood flow (PBF) values for all samples after dental prosthetic preparation. The values recorded 7 days after the preparation were higher than those recorded at 24 h after the preparation (**Figure 3**), which suggests that the increase in values does not relate only to optical changes due to the reduction of dental hard tissue but rather to the establishment of proper PBF. As a consequence, it may be assumed that a phenomenon of micro-irritation has appeared in the investigated area.

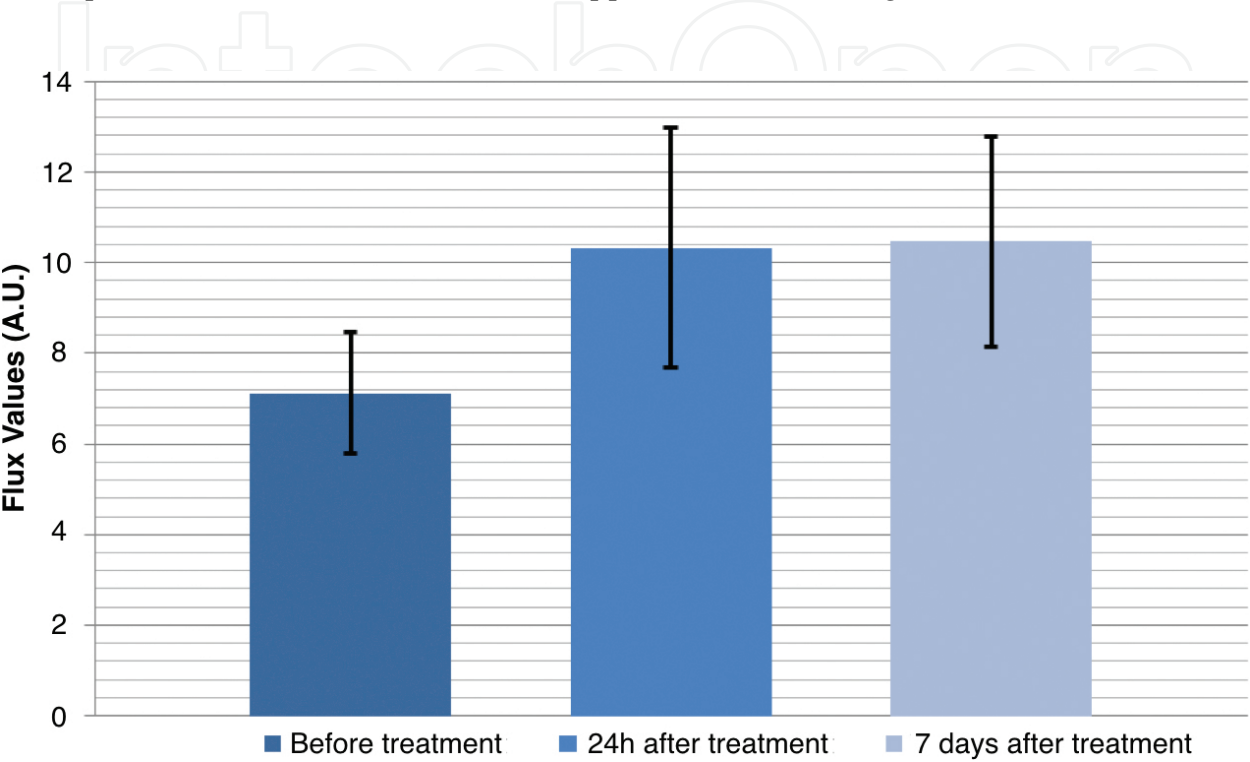


Figure 3. The PBF in time for the prepared teeth.

Only in one sample, the PBF recorded at 24 h was tremendously different from the initial moment and even from the PBF recorded at day 7. The patient did not report clinical symptoms of pulpal inflammation, such as pain and tenderness to percussion.

Yanpiset et al. [43] found LDF measurements to be extremely accurate in differentiating a revascularized (vital) tooth from a necrotic tooth pulp. An exciting finding of their study was that an accurate LDF reading of pulpal revascularization could be established at the fourth week after treatment, which is much earlier than it would be expected from standard sensitivity tests. This finding corresponds to those from the study by Skoglund et al. [60]. The LDF is extremely accurate in non-vital teeth, with almost 100% accuracy, but not as good in vital teeth. The blood vessels, fibroblasts and fibrous connective tissue that occupy the central portion of the pulp chamber can be affected without having a significant inflammatory reaction. While this tissue is vital and would give a radiographic picture of continued root development, the amount of moving blood cells creating a Doppler shift would be minimal. Another reason is that the revascularized teeth containing predominantly osteoid tissue may have a different optical property and the flux value reading from a revascularized tooth may be different from a normal tooth pulp. These teeth might give a false negative result [44]. Clinically, it has to be

assumed that one may not rely solely on the LDF, but an estimation of signs of the pulpal or periapical pathology would still be necessary, before initiating endodontic treatment.

2.1.3. Pulp capping and pulp microcirculation

Injuries to permanent anterior teeth account for the most frequent form of orofacial trauma at a young age. According to various epidemiological studies, the permanent central incisors are mostly involved in traumatic events, sustaining nearly 80% of all registered injuries [61, 62].

Crown fractures may be uncomplicated involving enamel and dentin, without pulp exposure, or complicated, with pulp involvement. Therefore, an efficient clinical evaluation of an injured tooth requires symptomatic, visual and radiographic assessment. This is where LDF steps in, allowing a more accurate assessment of vascularization status in injured teeth whenever required, meaning immediately after the traumatic episode, as well as during and after treatment, justified by the method's safeness and non-invasiveness.

In one of our studies, we aimed to investigate the use of LDF, the pulpal healing process in complicated and uncomplicated crown fractures—with and without pulpal exposure when laser-assisted therapy combined with calcium hydroxide was used. After rubber dam placement, indirect pulp capping and preparation for resin composite restoration were performed for the upper right (#1.1) and left (#2.1) central incisors using Er:YAG laser irradiation (wavelength of 2940 nm; energy 240–80 mJ, SSP). Immediately after the treatment, the LDFRs were analysed and the results showed an increase in PBF on both teeth especially for tooth 2.1. After 7 days, the LDFRs evaluation was performed, and it showed a decrease in PBF in both teeth. The decrease was more notable in tooth 2.1 where the indirect pulp capping was performed. The last LDFRs evaluation was performed after 6 weeks, which revealed the recovery of PBF to a normal value, demonstrating that the pulp reached normal healthy status (**Figures 4 and 5**).

After 7 days, the pulp tissue was not restored to a healthy condition, with normal blood flow as shown by LDFRs, but after 6 weeks, the PBF recorded by LDF and the clinical assessment also showed almost a complete restoration of PBF. Vascular changes are essential to the initiation of acute as well as chronic inflammation, and blood flow is essential to its resolution. The inflammation process involves vasodilatation, thus increased circulation and perfusion. Therefore, a successful pulp capping is obtained when the following clinical conditions are met: uninflamed pulp, good antibacterial seal and the use of a capping material tolerated by the pulp tissue; better outcome is mainly registered in young teeth. Consequently, the clinical signs of inflammation correlated with the changes in PBF. LDF may therefore play a key role in clarifying the importance of PBF dynamics in the treatment of young traumatized teeth. Moreover, the recovery of PBF after laser indirect pulp capping was spectacular. This fact has been attributed to laser treatment for preparing the area for a hermetic sealing of the pulp. The practitioner must pay attention to the cavity preparation as well as to optimal placement of the capping material, which is in the benefit of the formation of tertiary dentine. Laser-assisted pulp capping represents a new treatment opportunity that improves the working conditions and the biological quality of the irradiated surface, thus increasing the effectiveness of the interaction between pulp tissue and capping agent.

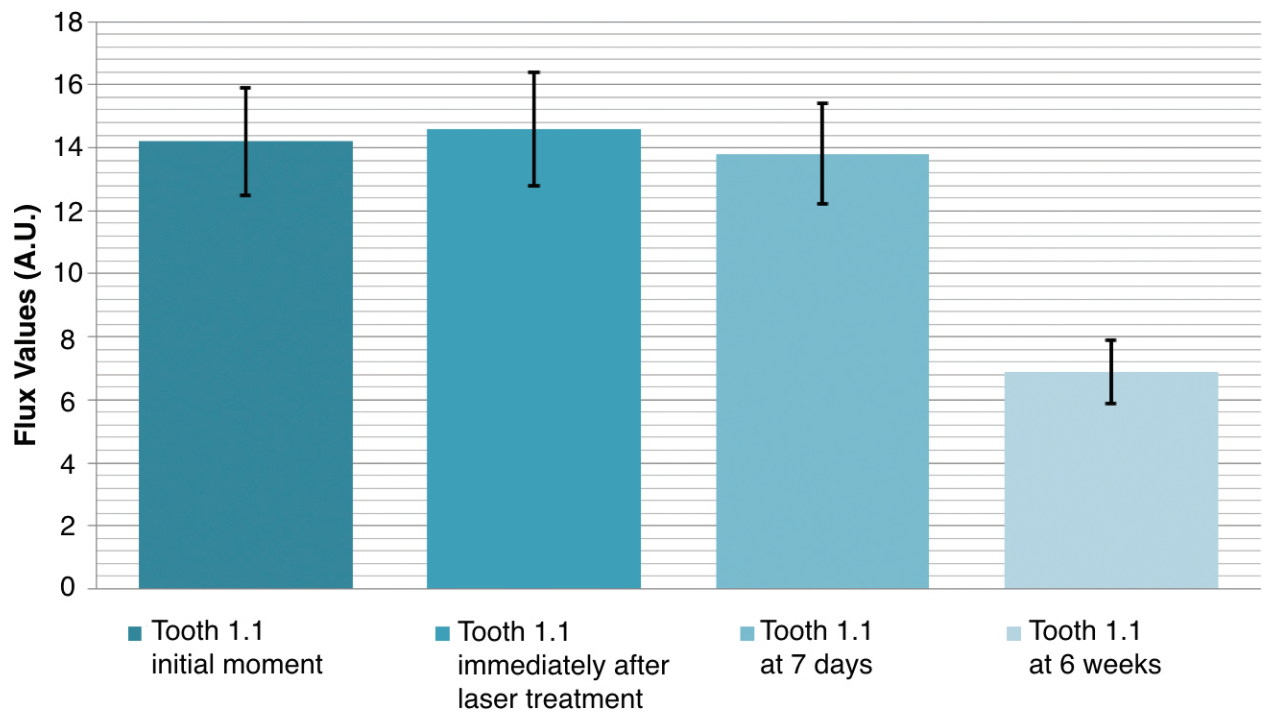


Figure 4. The descriptive graphic for LDFRs in traumatized tooth 1.1.

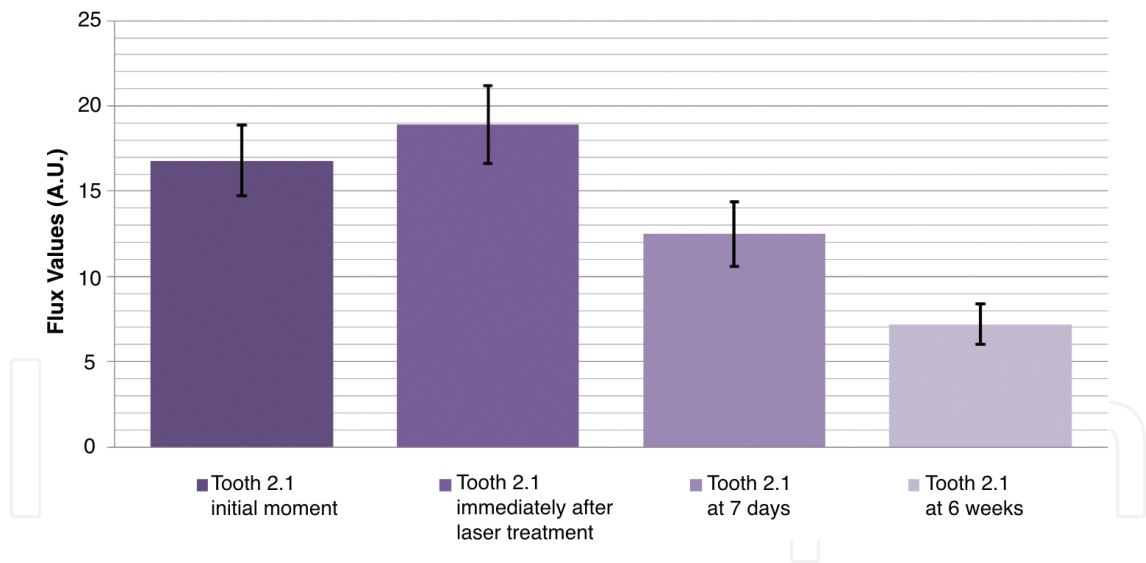


Figure 5. The descriptive graphic for LDFRs in traumatized tooth 2.1.

2.1.4. Traumatology and pulp microcirculation

LDF has been shown to be valuable in monitoring revascularization of teeth following severe dental trauma. During follow-up examinations the traumatized tooth can be unresponsive to traditional vitality testing during the first 6 months; however, LDF indicated that revascularization had occurred much sooner. Until recently, CO₂ ice has been the most effective method

for sensitivity testing in trauma cases but LDF is able to give the assurance that we could defer invasive care during critical time period when the root canal therapy might have been initiated for the patient [63]. The information obtained by LDF is of additional importance for the treatment planning. Since the clinical examination of traumatized teeth is sometimes inconclusive, LDF could be regarded as a further diagnostic tool but it cannot replace the radiological or clinical examination [64].

A prospective, cohort study conducted by Emshoff et al. [65] on patients with dental injuries developed prediction rules for the treatment response related to the management of dental injuries. Treatment response (success or failure) was categorized based on findings of clinical and radiographic evaluation after 9 months. The most important variables were sub-luxation, root fracture, baseline PBF level and a change in PBF level at 3-month follow-up. The results show that the outcome following the management of dental injuries may be predicted from variables collected with LDF and physical examination. Predictive modelling may provide clinicians with the opportunity to identify 'at-risk' patients early and initiate specific treatment approaches.

2.2. Microcirculation of the gingiva

There is quite little information in the literature about the vascular dynamics of the gingival circulation in healthy and diseased sites. LDF emerged more than 30 years ago as a non-invasive and real-time method for perfusion measurements [66]. The LD technique made it possible to demonstrate that blood flow wave patterns differ consistently among gingival tissue types [67, 68] and that there are no within-subject differences over time in LDFRs [16].

One of the earliest signs of any inflammatory process is the change in the vascular architecture and microvasculature. This is also true for gingivitis [69]. The healthy gingiva is characterized by a sub-epithelial vascular plexus consisting of a capillary network with loops arching towards the epithelium [70]. Gingival inflammation presents an increased vascularity with larger vessel size, more capillary loops, [71] slowed blood flow [72] and a restriction of the afferent blood vessels [73]. The capillary units are among the first vessels affected by inflammation in the crestal gingiva [74]. If changes of the vascular morphology in inflammation are related to blood flow changes, they may be the first sign to predict the onset of pathological events in the gingiva [75]. Thus, gingival blood flow (GBF) may serve as a prognostic marker. Gingival microcirculation (GM) has lacked exact evaluation for a long time. This was mainly due to methodological difficulties. Different methods, such as impedance plethysmography or the implantation of microspheres, have been employed to study GBF [76–82]. Unfortunately, most of them were invasive or inapplicable to humans. Other studies on dogs have shown that predictable morphologic changes occur in the blood vessels at the gingival margin with the onset of inflammation. These vascular changes precede recognizable histopathological alterations, starting as early as 2 days after the induction of gingivitis [36, 37, 83].

In our studies, in order to obtain a correct LDF measurement of the gingival blood flow, the probe was positioned 4 mm above the cervical line of the upper incisors and was also distanced using a gingival dam (LC Block-Out Resin, Ultradent Products, Inc.) before creating the silicone holder. This distance was necessary in order to avoid pressure on the gingival tissue when

applying and removing the silicone holder during measurements phases. A silicone rubber holder was used in order to secure the gingival LDF probe in position at the studied site. A small hole for the laser probe was placed in the holder at 4 mm away from the gingival margin, using a high-speed handpiece and a 1.5 mm diameter fissure bur. After calibration and disinfection, the laser probe was inserted into a rigid opaque plastic tube with a 1.5 mm diameter and 0.1–0.2 mm longer than the fibre. The plastic tube was used to reduce the movement artefacts of the fibre inside the impression, by increasing adherence and protection of the active optic surface. The plastic tube was forcefully inserted in the canal carved in the impression and positioned afterwards according to study protocol. With the purpose of insuring the reproducibility of LD signal acquisition, a guiding mark was set on the fibre in order to allow its placement in the same position for each testing.

2.2.1. Healthy and inflamed gingiva

Previous researchers have shown that an interaction between GBF and gingival health exists [84]. One of our studies [18] aimed at evaluating the microcirculation in subjects with gingivitis compared to healthy gingiva by using LDF. The subjects of the present study were young adults in whom oral hygiene and dietary habits were well established. Ramsay et al. [85] indicated that the reliability of blood flow measurements required accurate repositioning of the measurement probe; that is why the technique used in the study aimed at achieving a correct reproducibility of the LDF measurements.

The results showed that LDF could be a useful non-invasive, sensitive, reproducible and harmless method for measuring GM in humans. LDF may therefore be an important element in clarifying the role of GBF dynamics in clinical gingivitis as well as in understanding the blood flow dynamics in the gingiva. At the seventh day, the gingiva was not restored to a healthy condition, with normal blood flow as shown by LDFRs but after 14 days, the GM recorded by LDF and the clinical assessment also showed almost a complete restoration of the gingivitis group. Consequently, the clinical signs of inflammation correlated with the changes in GBF (**Figure 6**).

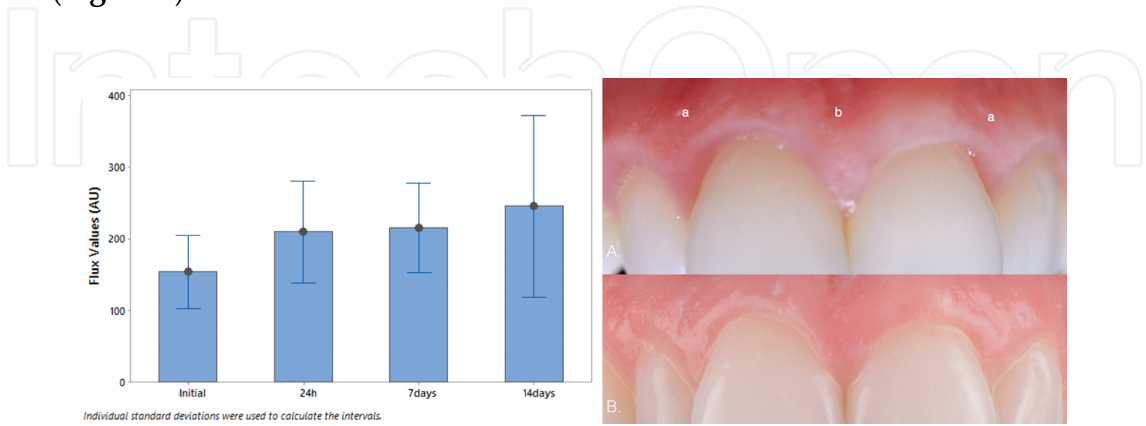
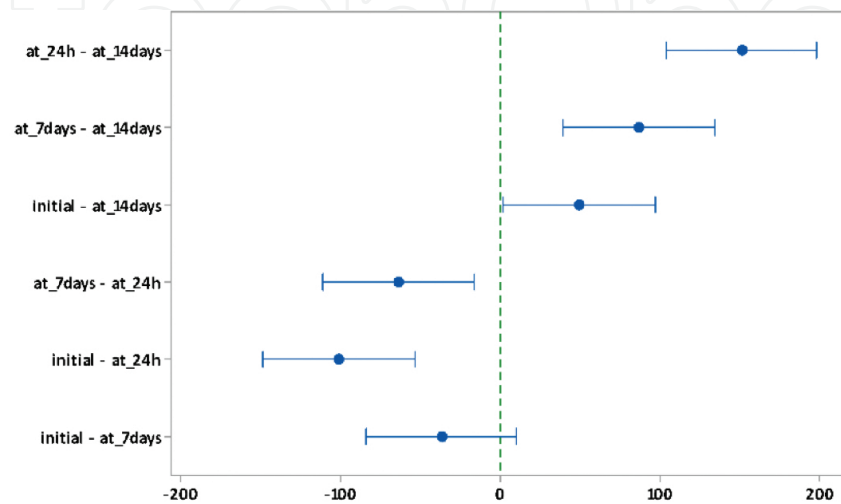


Figure 6. The mean values of the gingival blood flow (GBF) recorded at various moments of time; interval plot of the four moments of time in which the LDF measurements were carried out ($SD = 74.9411$); A. (a) sites with gingivitis; (b) healthy gingival site; B. restored gingival health after 14 days.

The results showed significant statistical differences between the four recordings in time. At 24 h after the initiation of therapy, the GBF was significantly increased compared to the baseline values suggesting local inflammation of the tissues after the initial therapy. No significant differences were noticed between initial moment and 7 days after the treatment and also between initial moment and 14 days after. The GBF values at 14 days were not significantly different compared to the control group (Figure 7).



If an interval does not contain zero, the corresponding means are significantly different.

Figure 7. Fisher individual 95% CIs. Comparison of GBF values of the gingivitis group among the four moments of time recorded in the study. Showing that there are no statistical significant differences between the initial and the 7-day groups as well as between the initial and the 14-day groups.

2.2.2. Laser periodontal surgery and gingival recovery

When performing gingivoplasty by conventional methods, there are limitations regarding healing by secondary intention, post-operative bleeding, loss of keratinized gingiva and inability to treat the underlying osseous deformities, which leads to the inability to complete the treatment [87]. Performing surgical procedure using laser technology can solve most of these limitations.

LDF found an excellent utility in the evaluation of the gingival recovery after surgery performed with the high-end methods available today.

When using lasers, the depth and amount of soft tissue ablation are more precisely established than with mechanical instruments [88, 89]. In particular, Er:YAG laser is very adequate and useful for aesthetic periodontal soft tissue management because this laser is capable of accurately ablating soft tissues using various handpiece tips, and therefore, the healing process is faster and favourable due to the minimal thermal alteration of the treated surface [90].

Diode lasers act as a useful tool for cutting gingival tissue, producing good haemostasis and reducing bacterial growth in periodontal surgery. There is evidence that this wavelength can

reduce gingival inflammation and also the need for local anaesthesia during surgical procedures.

In order to establish the efficiency of one laser in comparison with other, we decided to perform a study where LDF was used to compare GBF after Er:YAG (Fotona Fidelis Plus II) and 980 nm diode laser (Diode Laser Smile Pro 980 Biolitec) gingivectomy.

The evaluation was carried out on 20 anterior teeth that underwent reshaping of gingiva in five female patients (four anterior teeth/patient), aged between 20 and 35, capable of adequate compliance. The Er:YAG laser was used in Long Pulse: 600 μ sec (LP) and Very Long Pulse: 1000 μ sec (VLP) modes, 140–250 mJ, 10–20 Hz frequency, contact mode and using cylindrical sapphire tips. The parameters were established according to previous research [26] and were found suitable for soft tissue without causing visible major thermal damage to root dentin or bone. The 980 nm diode laser was used in continuous wave mode, 4 W, contact mode and cooling with saline solution using a 360 μ m diameter quartz fibre as delivery system (**Figure 8**).



Figure 8. (a). Initial intra-oral status, (b) immediately after laser surgery, (c) 24 h after the laser surgery with indirect provisional restorations, and (d) clinical intra-oral aspect 2 months after treatment with the final ceramic restorations.

At first appointment, the initial measurements were carried out. Post-operative controls and LDF measurements were accomplished after 24 h, 7 and 14 days to evaluate healing and wound evolution on a total of eight points/patient (two points on each tooth) for each patient.

As for the gingival surgery with Er:YAG laser, significant differences in LDF recordings over time were established between different times ($p < 0.001$ with a significant level $\alpha = 0.001$, Friedman test). The results showed that after 24 h the differences are significant compared to the initial moment; 7 days after the treatment, with the Er:YAG, LDF was slightly raised compared to the initial moment ($p = 0.256$), and after 14 days, LDF the values were insignificantly lower compared to pre-treatment ($p = 0.431$) (**Figure 9**).

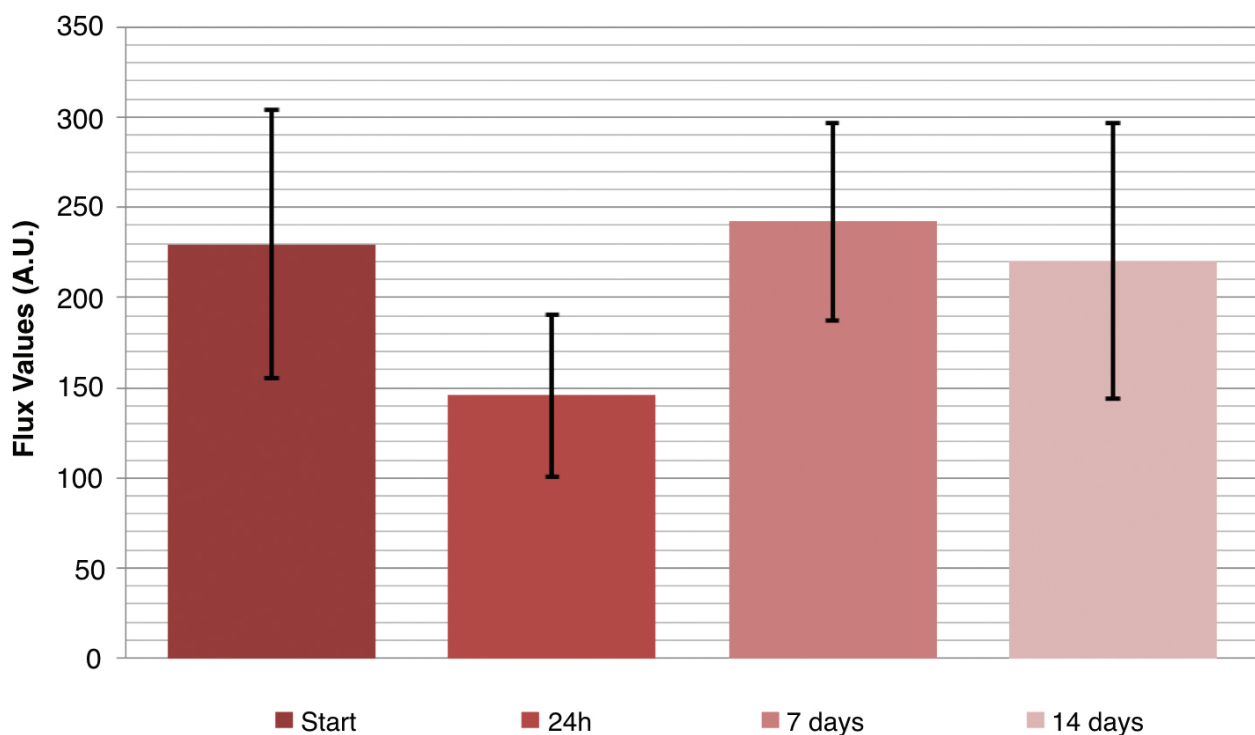


Figure 9. The descriptive graphic for 'Laser 1' method applied at the four moments of time.

Regarding gingival surgery with the diode laser, significant differences between the four tracings over different times were found ($p < 0.001$ with a significant level $\alpha = 0.001$, Friedman test). After 24 h, the differences were significantly lower compared to the initial moment; whereas after 7 and 14 days, the recorded LDF values were significantly raised compared to the initial moment ($p < 0.001$) (**Figure 10**).

The Levene's test for equality of variances was used in order to establish the equal variances assumed at the initial moment as well as after 14 days, and afterwards, the independent sample test was used for comparing the values obtained for the Er:YAG area and for the diode area at the initial moment (insignificant differences $p = 0.897$) and after 14 days (significant difference $p < 0.001$). We established that after 14 days, the recorded fluxes for the diode area were significantly higher compared to the values obtained for the Er:YAG area ($p < 0.001$).

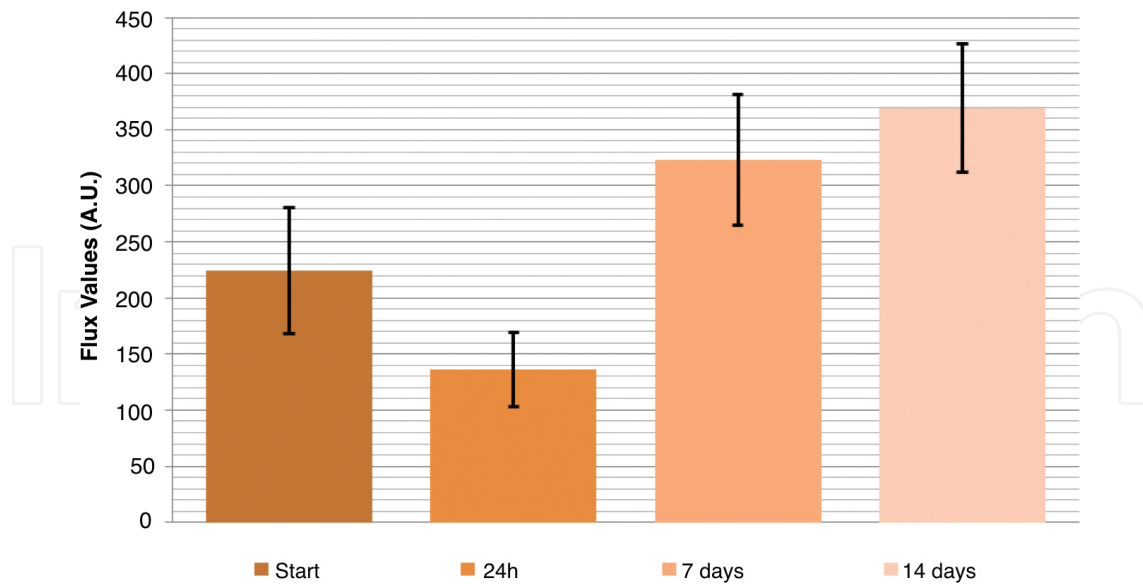


Figure 10. The descriptive graphic for ‘Laser 2’ method applied at the four moments of time.

The results obtained after the laser treatment on the free gingival area indicate a modification in the micro-vascular blood flow response. Furthermore, our measurements, which are in accordance with other studies [91], indicate that LDF technique can offer information regarding the micro-vascular changes during healing period. These results showed an evident decrease in perfusion for both areas in comparison with the baseline values 24 h after surgical procedure. The micro-vascular blood flow increased significantly after 7 days in both areas but mostly in the diode area. After 14 days, the blood perfusion returned to the initial value in the Er:YAG-treated area. The results in the diode-treated area remained at a higher level, showing that after 14 days, the healing in this area was not complete. The response after laser treatment in both areas was an obviously hyperaemic one. The difference in haemodynamic changes that occurred after 14 days can be explained by the differences in tissue interaction of the different laser procedures applied in our study.

2.2.3. *Mucositis and gingival blood flow*

In a study [92] conducted by our group, we evaluated the immediate effects of radiotherapy, more precisely, the oral and perioral soft tissue changes that appear after the radiotherapy treatment period. Additionally, we measured the gingival blood flow using LDF, in order to objectively determine any changes of the microvascular system of the gingiva.

Even after the first radiotherapy exposure, the blood flow values increased towards the irradiated area and remained increased throughout the entire treatment. This suggests that the periodontal tissue responds immediately to radiotherapy (as expected), and an inflammatory state is established even after the first exposure and it persists during treatment. What we found interesting was that this increase in vascularity preceded the clinical modifications, which means that with the help of LDF, we can diagnose an inflammation and we can predict the setting of the clinical side effects of radiotherapy. On the other hand, we did not find any

numerical correlation between blood flow values and the severity of the clinical manifestation of the radiation-induced side effects.

LD was a useful instrument in establishing the kind of dental procedures we can perform during treatment. Based on our results, we recommend to perform, during this time frame, mostly conservative measures, surgical measures should be performed keeping in mind that the tissues are inflamed and that the bleeding would be greater than normal and wound healing difficult. Prosthetic treatments, if performed, should be done with consideration towards the periodontal tissue that should not be additionally irritated. The clinician should carefully wage the advantages of the treatment against the possible complications that it could bring. The goal of any dental treatment should be increasing the patients' quality of life and decreasing the risks of interrupting radiotherapy, due to the onset of the side effects that it causes.

2.2.4. Smokers and gingival microcirculation

One of our studies [93] aimed at investigating microcirculatory alterations of the gingiva occurring after smoking tobacco compared the periodontal status of both smoker and non-smoker patients and also the registered values between the sexes (**Figure 11**).

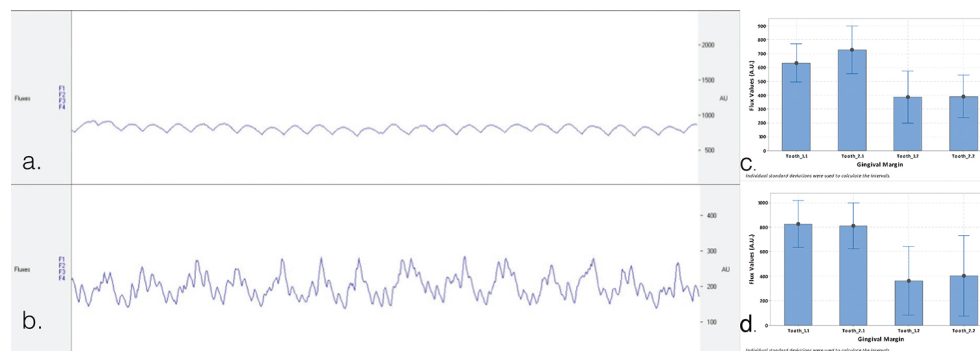


Figure 11. (a) Example of LDF recording from a non-smoker patient; (b) example of LDF recording from a smoker patient; (c) interval plot of flux values (AU) in smoker male group; and (d) interval plot of flux values (A.U.) in smoker female group.

We found no significant differences (*t*-test) between non-smoker male group (I-M) and non-smoker female group (I-F). On the other hand, LDF in the smoker female group (Group II-F) was significantly elevated compared to the smoker male group (Group II-M). The Group II-M LDF values were slightly increased compared to the Group I-M. The LDF values in the Group II-F were significantly higher than the LDF values in Group I-F.

2.2.5. Laser Doppler imaging and gingival microcirculation

Essentially, LDI works by scanning a monochromatic laser across the surface of the tissue. Light, which is backscattered from moving erythrocytes, undergoes a shift in frequency proportional to its velocity, according to the Doppler principle. Most laser Doppler set-ups use a helium-neon laser (RED, 632.8 nm), providing an estimate of perfusion up to a depth of 1–

1.5 mm into the dermis of white skin and thus mainly measure the perfusion in arterioles, venules and capillaries. LDI gives a 'snapshot' of perfusion at a given point.

The objective of one of our studies [19] was to evaluate the applicability of LD line scanning in recording the gingival healing process after a surgical procedure followed by two types of plastic provisional restoration. As a secondary objective, we also aimed at testing two different techniques and materials for performing the plastic temporaries. The results were also validated by clinical examination.

The moorLDI2-IR instrument, infrared diode laser 785 nm nominal, maximum power 2.5 mW with a visible diode laser (target beam for infrared systems) 660 nm nominal, maximum power 0.25 mW, was used in our study. The microcirculation in the investigated areas suffered changes in the analysed period (14 days) and was monitored with the Moor laser Doppler line scanner (**Figure 12**).

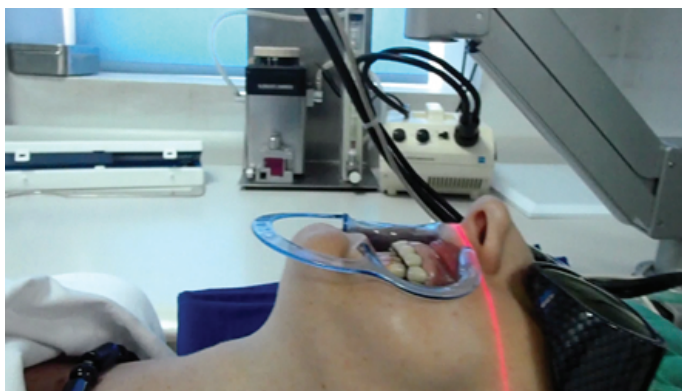


Figure 12. Laser Doppler line scanning procedure.

LDI recordings were performed in the labial regions of the operated areas at the day of the surgery, prior to local anaesthesia, after 24 h, after 7 days and 14 days following the intervention. The scanner used in this study was placed so that it was directed to record the vessels within the selected area. The differences between the four recordings clearly demonstrated adjustments in the micro-vascularity of the region in the healing period. The initial images of the area (**Figure 13(a)**) showed a certain perfusion map that differed completely from the LDI images at 24 h after the surgical procedure and the cementation of the plastic temporaries. The image at 24 h showed increased microcirculation as a reaction to the surgical procedure (**Figure 13(b)**). This situation is represented by an increase in the red colour of the affected areas in the perfusion map. The LDI images, 7 days after the surgical procedure, showed an improvement in the microcirculation healing in the interested area while the LDI images, 14 days after the surgical procedure, confirmed healing by offering a perfusion map similar to the initial one. The clinical examination asserted the changes observed on the perfusion maps in both cases.

With the aid of LDI, it was possible to obtain information regarding the impact of different materials for aesthetic prosthetics temporary restoration after surgical treatment on GM. The

two types of plastic materials had no negative influence on the healing process of investigated area.

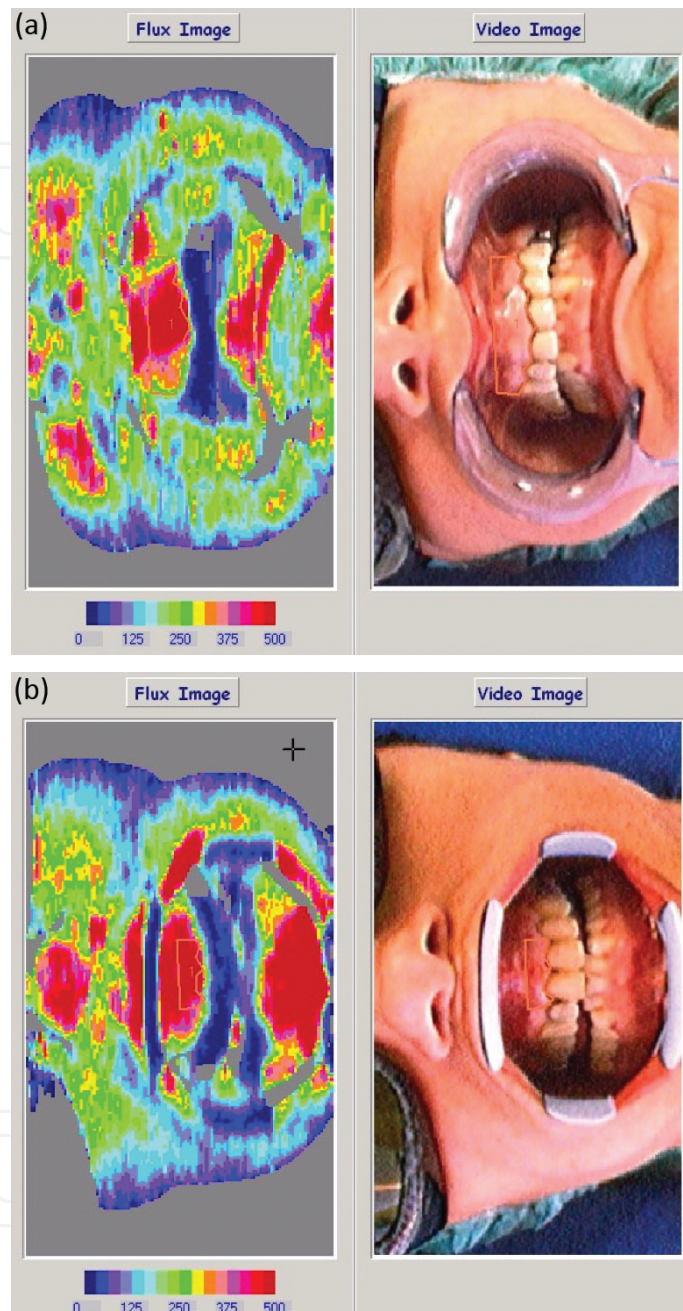


Figure 13. (a) Initial LDI recording and (b) LDI recording at 24 h with an increase in the red colour of the affected areas in the perfusion map.

The major advantages of LDI over LDF are the fact that there is no need for direct contact with the tissue (max. distance 19 cm), the possibility to accomplish multiple measurements allowing to obtaining many images in the area of interest (120 pixel/cm) and most importantly, it allows a global analysis of blood flow in the area of interest.

This technique has been shown to be easy to learn by surgeons. Regular post-operative assessment of flap perfusion by members of the microsurgery team trained in the use of LD line scanning might, therefore, represent a practical alternative to more complex and invasive monitoring techniques. Issues of inter- and intra-examiner reliability have yet to be examined, and in an area where only a low percentage of flaps undergo vascular compromise, this may prove impractical.

One advantage that LDF has over LDI is that it gives a constant measure of blood flow at the specified point, whereas LDI gives a 'snapshot' of perfusion at a given point.

2.3. Limitations

Although LDF has proved valuable for a variety of clinical applications, there are some limitations to its use in oral medicine. A major drawback is that LDF can only detect red blood cell movement in a small volume of tissue (1 mm^3); thus, variables such as the number of vessels with active flow, changes in vessel diameter and flow in individual micro-vessels cannot be analysed. The small measuring area may also influence the reproducibility of the results due to the fact that a minimal displacement of the optical probe would lead to a change in the investigated area [20]. Another source of error in LDF measurements are the artefacts caused by tissue motion in relation to the probe. Additionally, oral LDFRs have demonstrated considerable intra- and inter-individual variability [94, 95]. A part of the limitations is being solved by the fact that the velocity of PBF in humans is very low and that LDF modified for the measurement of slow blood flow is appropriate for PBF measurement in humans [96]. One of the most important limitations of the LDF is that each patient presents variation of blood flow because the measurement is influenced by the thickness of the connective tissue and local distribution of the vessels and also the recording site (free gingivae, inter-dental gingivae, attached gingivae or alveolar mucosae) [35–37, 40]. Other limitation of LDF is that flow readings are not only dependent on the blood flow in the measurement volume but also on the scattering properties of the surrounding tissues. It has been reported that up to 80% of LD blood flow signal recorded from an intact human pulp is of non-pulpal origin [41]. The same could be anticipated for LDF measurements performed on the gingivae.

Originally, iontophoresis was used in conjunction with single-point LDF, as opposed to LDI systems, which measure perfusion over a larger area and produce a detailed perfusion map. Laser Doppler flowmetry typically measures within a small volume ($\sim 1 \text{ mm}^3$) and, as a result, has often suffered from poor reproducibility, mainly due to the spatial heterogeneity of tissue blood flow and movement artefacts [97, 98], although reproducibility has been improved recently by the use of 'integrated probes'. These use multiple collecting fibres positioned in a ring around a central light delivery fibre, thus increasing the spatial resolution. However, the use of LDI still provides a larger surface area measurement and should be the preferred choice in areas of tissues with high spatial variability, despite the significant difference in costs. This could be detrimental if one is interested in the dynamics of the dilator response. This problem can be partially solved by altering the time taken for a scan. This can be done in two ways: by reducing the area to be scanned and/or increasing the scanning speed of the laser. The latter has the slight disadvantage of producing a slightly less detailed image, but in most

cases, it is a compromise worth making. Many studies are not closely concerned with the dynamics of the cutaneous response and are instead focusing more on the maximum response at a given dose, in which case LDI is adequate. The line-of-sight velocity of the moving scatterers is directly proportional to the frequency of the fluctuations. This would suggest that both techniques are linear with respect to velocity. In the case of Doppler, however, it has been accepted for some 30 years that if you take the first moment of the power spectrum of the fluctuations, then it scales linearly with both velocity and concentration (number of moving scatterers) [99]. In the case of blood flow, this is a measure of perfusion. If a Doppler system uses this algorithm (first moment of the power spectrum), then it should be linear with respect to perfusion [100].

3. Conclusions

The major advantage of the laser Doppler techniques in general is their non-invasiveness and their ability to measure the microcirculation flux of the tissue and fast changes of perfusion during provocations. The LDF represents an important instrument to assess gingival and pulpal microcirculation in the oral cavity. In this respect, it enables monitoring of the tooth vitality, establishing the pulp revascularization before these data could be derived from traditional sensitivity tests, which can also add more inflammation to the already irritated pulp. LDF can be used to assess the degree and duration of the pulpal inflammation or ischemic episodes, thereby identifying patients at risk for adverse reactions such as irreversible inflammation, avascular necrosis and tissue loss. Further studies are warranted to assess the validity of pulpal blood flow measurements by comparing them with histological tooth pulp changes, and by determining how well the LDF diagnoses of pulp health may predict the course of pre-prosthetic treatment.

In conclusion, LDF is a suitable technique for determining pulp vitality in most clinical situations and can be used together with other indices to evaluate the marginal gingival health status.

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