# Non-invasive measurements of oral mucosa blood flow in patients with various clinical condition

Doctoral Thesis

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## INTRODUCTION

Periodontitis is an inflammatory disease of the tooth-supporting tissues of high prevalence. Surgical intervention is a common treatment in periodontitis, meanwhile wound healing could be difficult in patients with various microcirculatory alterations, such as Diabetes Mellitus or smoking. In order to investigate vasoregulatory changes in periodontitis or in systemic conditions, a vascular reactivity test has to be elaborated for the human gingiva.
Vascular reactivity could be assessed by various provocation tests including iontophoresis of vasoactive molecules, post-occlusive hyperaemia or heat challenge. The test should not interfere with wound healing. Among vascular reactivity tests the heat provocation test seems to be an appropriate method meeting these requirements.

In today's periodontal plastic surgery numerous flap designs with various grafting alternatives (autograft, allograft, or xenogeneic materials) are routinely applied. Graft exposure during soft and hard tissue augmentation might occur before there would be any chance for graft vascularization to take place due to wound healing disturbances and a lack of primary intention healing.

Only a few studies measured circulatory changes during wound healing in the human oral mucosa using the $^{133}$Xe clearance technique, Laser Doppler Flowmeter (LDF), or fluorescein angiography. The single-point laser Doppler technique is blind to spatial variations in microcirculation during wound healing. As both the progression and healing of periodontitis as well as periodontal surgery involve great anatomical reorganization of the affected tissue, only techniques capable of capturing the 2D heterogeneity of blood flow changes are suitable for follow-up.
The novel Laser Speckle Contrast Imager (LSCI) offers fast imaging and high spatial resolution and is, therefore, able to capture blood perfusion data over an entire mucosal surface in a non-invasive and

non-contact manner. Blood flow measurements on the oral mucosa by LSCI are inevitably accompanied by the retraction of the lips and cheeks with dental mirrors or retractors, which may disturb the microcirculation of the gingiva. Furthermore, direct view of the investigated field is frequently limited, therefore, a photo mirror should be used to capture the image. Clinical studies are suggesting that this technique may be a useful tool for assessing proper circulation during surgical intervention and evaluating wound healing, but it has not been tested in human oral mucosa yet.

## AIMS

The aim of our studies were as follows:

**I.** Develop a heat provocation test in clinical practice. Test the effect of warm saline on GBF as a function of time in the healthy gingiva with LDF.

**II.** To investigate the effect of light-induced heat on GBF in the healthy gingiva with LDF.

**III.** To compare the effect of periodontal inflammation on heat-induced hyperemia between non-smokers and smokers.

**IV.** To evaluate the intraday reliability of LSCI in oral mucosa measurement and investigate the effect of a change of the incidence angle.

**V.** To evaluate the effect of retraction on intraday reliability and the assessment of inter-day reproducibility.

**VI.** To investigate the effect of measurement based on reflected images on reliability.

**VII.** To evaluate the test-retest reliability of repeated LSCI measurements at the contralateral side of the oral cavity of patients involved in a surgical clinical trial described in experiment VIII.

**VIII.** To evaluate the capacity of LSCI to characterize the kinetics of blood flow after periodontal plastic surgery. As a further objective, comparison was made between the blood flow of Modified Coronally Advanced Tunnel (MCAT) flaps combined either with xenogenic (Geistlich Mucograft®) collagen graft material or the gold standard autogenic collagen tissue graft (CTG) harvested from the palate.

## METHODS

All participants were systemically healthy, the exclusion criteria were pregnancy, smoking (except in experiment III.), general diseases; furthermore, the subjects were not allowed to take any antibiotics before the investigation, anti-inflammatory drugs, systemic steroids, bisphosphonates and any other medicine possibly influencing mucosal wound healing, or any other products in the preceding three months. LDF was applied in experiment I, II, III and LSCI was used in experiment IV, V, VI, VII, VIII.

Research points:

***I.*** *The effect of warm saline on GBF in the healthy gingiva*

This experiment was performed on nine non-smoking volunteers with a healthy gingiva. Blood flow was recorded before, during (30 s) and after dropping 2 ml of pre-warmed (44 °C) sterile saline solution on the marginal gingiva right next to the laser Doppler probe. Baseline GBF was recorded for 1 minute. The recording of gingival perfusion was continued for an additional 5 minutes after carrying out the test.

***II.*** *The effect of light-induced heat on GBF in the healthy gingiva*

This experiment was performed on twelve non-smoking volunteers with healthy gingiva. Heat was generated on the gingiva using a dental curing light (IvoclarVivadent AG, Liechtenstein, 35W) from which the light filter was removed. The light guide was directed to the marginal gingiva at a distance of 1.5 cm. GBF was recorded before and 5 minutes after heat provocation which was applied for 80 seconds on the marginal gingiva around the Laser Doppler Flowmeter probe.

***III.*** *The effect of periodontal inflammation on heat-induced hyperaemia in non-smokers and smokers*

This group was composed of twenty-nine volunteers with a periodontal condition of varying severity, from healthy to suffering from a moderately severe inflammation assessed by a GCF reading

(0–71). These patients were also systemically healthy based on the same exclusion criteria as above and separated into two groups: smokers (n = 11) and non-smokers (n = 18). As in the previous set of experiments, GBF was recorded for at least 1 minute before and 5 minutes after the application of heat induced by light.

***IV.*** *The effect of the incidence angle on reliability*

Twenty-two participants were involved in this series. Lips were retracted by a lip retractor (Spandex®, Hager & Werken, Germany). The LSCI device was centered perpendicular to the keratinized gingiva above tooth 12 for the first snapshot. Then the subject's head was turned right as much as possible for tooth 12 to be seen on the side of the 2x3 cm wide LSCI snapshot picture. The incidence angle was recorded by a protractor. After GBF measurement the same procedure was performed with a turn to the left and then all three types of measurements were repeated. Overall six snapshots were taken.

***V.*** *The effect of retraction on reliability and the assessment of inter-day reproducibility*

Twenty-two participants were involved in this series. Participants' upper lips were carefully retracted by two dental mirrors. The LSCI device was centered perpendicular to the keratinized gingiva above tooth 12 for the first snapshot. The procedure was repeated twice more. In-between the patients closed their mouth. This protocol was suitable to assess intra-day reliability, i.e. repeatability within one session. After one week, the whole experiment was repeated in order to assess inter-day reliability, i.e. reproducibility.

***VI.*** *The effect of mirrors on reliability*

Twenty-five patients were recruited. The LSCI device was centered perpendicular to the keratinized gingiva below the mandibular central incisors. Six snapshots of GBF were alternately taken either directly using a dental mirror for retraction of the lips or a silhouette-free dental photographic mirror, placed in the mandibular vestibulum to

reflect the same region of interest. The distance measurement of the LSCI was set to manual in PimSoft. ROIs were defined around tooth 31.

***VII.*** *The reliability of repeated measurements long term*

This analysis was done on data from the experiment VIII. Eight subjects (four women and four men) exhibiting multiple Miller Class I and II gingival recessions had undergone periodontal plastic surgery in order to cover the exposed tooth surface. During this trial, the gingiva of 2–4 teeth in the non-operated area were selected as reference sites in each subject in order to control the possible systemic variation of GBF during the six months' follow-up. Measurements were taken twice preoperatively and on the following days postoperatively: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 17, 30, 60, 90, 120, 150 and 180. On each day, the measurements on each site were repeated 2–4 times in a randomized manner by retracting the lips carefully by dental mirrors. Regions of interests were defined on the keratinized gingiva at each reference site.

***VIII.*** *Periodontal plastic surgery for root coverage*

Eight subjects (four women and four men) exhibiting multiple Miller Class I and II gingival recessions (Multiple Adjacent Recession Type Defects, MARTD) were recruited. All subjects had a thin gingival biotype. MARTDs were treated with modified coronally-advanced tunnel technique (MCAT) by an experienced periodontist. Two types of grafts were used during the surgeries: either a subepithelial connective tissue graft (CTG) removed from the palate or a xenogeneic collagen matrix (Geistlich Mucograft®). Five patients received both grafts in a split-mouth design. Three patients were treated only at one surgical site (two of them received Geistlich Mucograft®, one received CTG.

Clinical data collection was carried out at baseline and six months postoperatively: gingival recession depth (GRD0, GRD6), gingival recession width (GRW0, GRW6) and the width of the keratinized

tissue (KT0, KT6). The change of these parameters was calculated as follows: recession depth reduction (REC), recession width reduction (RW) and increase of the keratinized tissue in width (KT). Photo documentation was prepared at all visits. Blood flow was measured at the gingiva of 52 teeth in total. Blood pressure and wound fluid (WF) measurements were done before the operation (baseline) and postoperatively on the following days: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 17, 30, 60, 90, 120, 150 and 180.

## RESULTS

### *I. The effect of warm saline on GBF in the healthy gingiva*

The average MAP of this group of patients was 107 ± 4 mmHg. The Flux value represents GBF and in our experiments we recorded the components of the flux (CMBC and Speed) separately as well in order to better characterize the vascular changes in the gingiva after the heat challenge. The application of warm saline to the buccal gingiva resulted in a quick increase in CMBC for periods of 20 s. These values within this period were very noisy, therefore we excluded from the statistical analysis as they suggest an artefact due to the reflection of the laser light from the surface of the saline flow and/or mechanical irritation of the dropping. At the end of heat provocation CMBC dropped to the baseline level and remained there while the Speed and the Flux increased rapidly in parallel. Flux reached its peak response (76 ± 6.0%) 21 seconds after completing the application of heat. The percentage mean changes during saline application. Increased GBF after heat application was due solely to the increase in the average speed of blood cells without any change in CMBC. In further experiments only the Flux was used to estimate GBF.

### *II. The effect of light-induced heat on GBF in healthy gingiva*

The average MAP of this group of patients was 109 ± 5 mmHg, and it did not differ statistically from the warm saline group. The recording

of GBF (Flux values) was started just after the halogen light had been switched off as it interfered with laser Doppler measurements. The changes in GBF after the application of light were expressed as a percentage of the baseline. The GBF values for each minute recording were averaged and tested for statistical differences to the baseline values. The value of averaged GBF was significantly elevated at minutes 1 and 2 (80 ± 12%, $p < 0.001$ and 44 ± 10, $p < 0.001$, respectively). After 2 minutes GBF returned to baseline values (min 3: 15 ± 5%, NS; min 4: 8 ± 4%, NS; min 5: 7 ± 5%, NS). The mean peak GBF value was 89 ± 15% at 30 s after heat application had been finished which is very close to the value obtained in case of warm-saline. The average RT time was 110 s. Both methods were effective in inducing a rapid increase in GBF and even after provocation was finished GBF remained at an increased level long enough for data acquisition. This was an important criterion as both methods interfered with laser Doppler measurements, hindering recording during the provocation test. The application of warm saline was technically more demanding and sometimes run-off fluid caused discomfort to the patient resulting in movement artefacts. Therefore, in further experiments we opted for heat-induced light for the heat test.

***III.*** *The effect of periodontal inflammation and smoking on heat-induced hyperaemia*

There was no correlation observed between the baseline GBF values and the MAP of patients in either the non-smoking or the smoking group. As there was no change in MAP before and after heat provocation, the GBF values were used for further comparison instead of vascular resistance or conductance.

There were no significant differences observed between the non-smoking and the smoking group in terms of most of the baseline values such as age, MAP, GCF GBF-bsl, MAX, MAX% and Area.

On the other hand, there was a significant difference in RT values between the two groups (85 s (55–105) vs 115 s (75–155), $p < 0.05$).

GFPA was 68 ± 7 Flux at baseline and increased to 114 ± 10 Flux ($p < 0.001$) after heat provocation in non-smokers and similarly changed from 79 ± 9 Flux to 117 ± 12 Flux ($p < 0.001$) in smokers. Smoking itself did not influence absolute GFPA values. No change was observed in relative GFPA values (GFPA/GBF) after the application of heat in the non-smoking group, but in the smoking group relative GFPA decreased significantly after the heat test (44 ± 5% vs 37 ± 4%, $p < 0.05$), indicating a significant ($p < 0.05$) interaction between the effect of smoking and heat test.

No significant correlation was observed to age in any groups. No correlation was found between GCF and baseline GBF but a moderate positive correlation was found between GCF and the MAX value in the non-smoking group but not in the smoking group. Similarly, GFPA-bsl and GFPA-heat were highly correlated to GCF values both only in the non-smoking group. No correlation was observed between GCF and relative GFPA in either period or group. A strong negative correlation was found between GCF and RT ($r = –0.64$, $p < 0.01$) in non-smokers, but not in the smoking group.

***IV.*** *The effect of the incidence angle on reliability*

The effect of turning was found to be significant ($p<0.05$). Pairwise comparison showed that GBF was slightly but significantly higher (3.8%) during the turn to the left (196±7.0) than in the central position (189±6.3, $p<0.05$). When turned to the right, there was no statistical difference in GBF from the central position and the left turn. No significant change was observed in GBF means between the two repeats.

Repeatability was good with and without turning. After removing the outlier, statistical difference between the left and the central position disappeared and the divergence in means has decreased (2.8%) as well as the CV values.

The between-subject CV was 20.0% with the outlier and 16.6% without the outlier. The ICC was found to be excellent with and

without turning (0.93 and 0.91). Removing the outlier influenced these values only slightly (0.88 and 0.91).

***V.*** *The effect of retraction on intra- and inter-day reliability*

No significant differences were found between the means of the three repeated retractions and between the means of the two measurement weeks. Intra-day repeatability with retraction was good and inter-day reproducibility including the intra-day repetitions was moderate. Up to four intra-day repeats, the calculated standard error (Bland and Altman 2007, Brown and Prescott 2014) between two different days decreased noticeably.

The between-subject CV was 11.0%. The intra-day ICC was 0.70. Using only a single measurement within a day, the inter-day ICC was 0.56, but increased to 0.66 by averaging the three repeats.

***VI.*** *The effect of using a mirror on reliability*

No significant differences were found either between the means of the three repeats or between the GBF means measured by a mirror versus directly.

The CV values calculated from the three repeats carried out with the same method (repeatability) and the CV value of the method were both good. The CV values of the three repeated measurements using the mirror method (9.5%) were similar to the directly measured values (10.3%). Overall reproducibility without distinguishing between the capturing methods was moderate. The between-subject CV was 16.2%. The intra-day ICC was 0.55. The overall ICC was 0.65 and increased to 0.73 if the same method was used for the repetitions.

***VII.*** *The reliability of repeated measurements in a clinical surgical trial*

Mean GBF at the non-treated sites was typically under the baseline values during the six months' healing period, however, this did not reach the conventional significance level. The between-subject CV was 12.8% and inter-site variation within subjects was 7.9%. The

intra-day CV and the inter-day CV of a site were both moderate with fair to good ICC values. The inter-day ICC of a site increased from 0.47 to 0.52 by averaging the intra-day repeats.

### *VIII. Assessment of Oral Mucosal Blood Flow following Periodontal Plastic Surgery*

The statistical analysis showed that not only the graft (graft x time: $p<0.001$), but also gender has a strong influence (gender x time: $p<0.001$) on the blood flow of the healing mucosa. Furthermore, a significant interaction was observed between gender, graft type and time ($p<0.001$). The data were therefore split into two subgroups based on gender in addition to the two graft types.

In females, blood flow at the treated teeth dropped significantly, approximately to half of the baseline values in the case of both Geistlich Mucograft® and CTG on the first day after the surgery. After day 2, blood flow increased towards the baseline but remained below it until day 12 in Geistlich Mucograft® patients and until day 7 in CTG patients. Over the six-month period, there was only a slight difference in flap circulation between the two graft groups.

In males, contrary to females, there were marked differences in blood flow between the two grafted sites. Blood flow at Geistlich Mucograft®-treated sites returned to baseline on day 2 and a hyperemic response occurred from day 4 to day 8. At CTG-treated sites, blood flow returned to baseline on day 3, and a reduced and shorter hyperemic response developed between day 5 and day 7. Perfusion at Geistlich Mucograft®-treated sites significantly exceeded the corresponding values for CTG on day 1, 2, 4 and 8 in males. The blood flow values of males significantly exceed those of females between days 1 and 10 in the case of Geistlich Mucograft® and from day 3 to day 6 in the case of CTG.

In the case of the WF the two main factors, graft type and gender were not significant but time ($p<0.001$) was. Interactions between graft x

time and graft x gender x time were not significant either, but the graft x gender interaction was significant ($p<0.001$). This means that, overall, the WF production of Geistlich Mucograft®-treated sites (13.8 PS) exceeded that of CTG-treated sites (10.7 PS) in females. On the other hand, in males, the opposite was found: Geistlich Mucograft®-treated sites had less WF (6.9 PS) than CTG-treated sites (10 PS). As time interacted with gender ($p<0.001$), pairwise comparisons were made at each time point. PSs increased dramatically in both genders on the first day after surgery. They remained significantly higher than the baseline until day 10 in females and until day 5 in males. On the first two days, WF looked similar in both genders but from day 3 the values in males dropped steeper than in females. One month after the surgery, WF tended to be lower than the respective baseline values in both genders. During the early healing period, blood flow showed a moderate inverse correlation with WF production on day 4 ($r=-0.55$, $p<0.05$), day 5 ($r=-0.49$, $p<0.05$), day 6 ($r=-0.51$, $p<0.05$) and day 7 ($r=-0.61$, $p<0.01$).

Baseline GRD0 and GRW0 were very similar in the Geistlich Mucograft®- and the CTG-treated groups, however, the initial KT0 was significantly less in the CTG-treated group. Gains in the depth (REC) and width (RW) of the recessions were similar in the two groups. The increase in KT at Geistlich Mucograft®-treated sites was significantly less than at CTG-treated sites. No statistically significant differences were observed between females and males either in the baseline values or in REC, RW and KT. REC and RW were positively correlated with the baseline values ($r=0.92$, $p<0.001$ and $r=0.64$, $p<0.001$). In contrast, KT was negatively correlated with KT0 ($r=-0.79$, $p<0.001$).

## CONCLUSIONS

**I.**: Local functional heat challenge tests could be useful methods to examine the vascular reactivity of the gingival tissue. Dropping saline on the gingiva may interfere with the LDF measurements.

**II.**: The light induced thermal test seems to be more advantageous under clinical conditions than the warm saline induced one. Despite similar peak responses, light resulted in a prolonged increase in GBF, which is possible due to the deeper penetration of heating light beams and to the longer stimulus.

**III.**: Periodontal inflammation promotes increased peak flow and faster restoration after heating in non-smokers, but not in smokers. Our data suggest that moderate periodontal inflammation may facilitate gingival vascular responsiveness which, however, is suppressed by smoking.

**IV.**: In the intraday repeatability of LSCI in oral mucosa measurement incidence angle has some effect on the mean of the measurements, therefore standardizing this angle under clinical circumstances would be demanding.

**V.**: Laser Speckle Contrast Imaging has good short- and long-term reliability. Inter-day variation was found to be higher than intra-day variation in spite of the standardized conditions. The effect of some intra-session components could be minimized by repeated intra-session measurements. It is recommended to repeat the measurement a couple of times.

**VI.**: It is preferable to use the lip retractors than dental mirror for LSCI measurements.

**VII.**: After surgery may have high individual variability. The effect of such systemic factors might be controlled by measuring GBF at the reference sites. This technique seems to be appropriate for the long-term clinical non-invasive follow-up of gingival microcirculation.

**VIII.**: The application of both grafts resulted in excellent recirculation patterns in the flap. Males showed a more rapid reestablishment of mucosal blood flow. It is conceivable that the opposite changes observed in blood flow versus vascular permeability are equally able to ensure the supply required for the tissues to heal. This, however, did not influence the apparent clinical outcome; favorable recession coverage could be achieved with both CTG and Geistlich Mucograft® in males and females.

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