Measuring changes in blood volume fraction during induced gingivitis of healthy and unhealthy populations using hyperspectral spatial frequency domain imaging: a clinical study

Ben E. Urban*, Hrebesh M. Subhash & LaTonya Kilpatrick-Liverman

This investigation aimed to quantitatively measure the changes in inflammation of subjects with healthy and unhealthy gums during a period of induced gingivitis. A total of 30 subjects (15 healthy, 15 with gum inflammation) were enlisted and given oral exams by a dental hygienist. Baseline measurements were acquired before a 3-week period of oral hygiene abstinence. The lobene modified gingival index scoring was used for inflammation scoring and hyperspectral spatial frequency domain imaging was used to quantitatively measure oxy- and deoxygenated blood volume fraction at two time points: at Baseline and after 3 weeks of oral hygiene abstinence. We found that abstaining from oral hygiene causes a near proportional increase in oxygenated and deoxygenated blood volume fraction for healthy individuals. For individuals who started the study with mild to moderate gingivitis, increases in blood volume were mainly due to deoxygenated blood.

Gingivitis is a form of gum disease that causes inflammation of the gingival tissue. If left untreated, gingivitis can lead to periodontitis. Periodontitis is an irreversible destructive tissue disease which causes detachment of gingival tissue, deepening of gingival sulcus tissues, formation of periodontal pockets, and bone loss. Gingivitis is a major global burden and a link between periodontal disease with other debilitating diseases, such as diabetes, atherosclerosis, cancers, and Alzheimer's has been suggested. An experimental model for investigating inflammation of gingival tissue is induced gingivitis. The induced gingivitis model allows investigators to study the transition of healthy to diseased tissue over short time scales. In the induction phase, gingivitis is created by eliminating all oral hygiene practices over a period of up to 3 weeks. Disruption of oral hygiene causes dental plaque accumulation and affects the microbiome homeostasis of the oral cavity. Accumulation of plaque and subsequent disruption of homeostasis causes an inflammatory response of the tissue.

Induced gingivitis has been shown to cause a diverse range of inflammatory responses among individuals. The inflammation response for subjects generally peaks in the third week of oral hygiene disruption. However, the magnitude and the rate of the inflammatory response can be different between people. A visual based scoring system is generally used to measure gingival inflammation differences between populations; and in the study described in this manuscript, the lobene modified gingival index (MGI) was employed. Measuring inflammation changes via clinical indices, however, does not provide information on differences in oxy- and deoxygenated blood volumes. In fact, ratiometric differences in oxy- and de-oxygenated blood volume can affect the results of color-based scoring, especially when performed visually. An objective method to quantify changes in inflammatory responses as reflected by changes in blood volumes is, thus, needed to objectively investigate the progression of gingivitis.

Spectral imaging techniques have been successfully applied to map tissue chromophores, such as melanin, blood, and water. For mapping tissue chromophores, non-contact imaging techniques are preferable since...
gingival inflammation (MGI ≥ 2). The large difference in MGI between the healthy and inflamed group was chosen to ensure distinction between the two populations using the MGI scoring system. This manuscript will compare a history of infectious disease (i.e. Hepatitis B or C, HIV, oral herpes); had contagious illness (i.e. upper respiratory infection, oral herpes, sinuses); had enteric related illness; had medications known to affect the gingival tissues (i.e. calcium channel blockers, phenytoin, cyclosporine); had used medication that could affect salivary flow; had used anticonvulsants, antihistamines, antidepressants, sedatives, tranquilizers, antibiotics, antimicrobial, anti-inflammatory medication or daily analgesics within 30 days prior to the start of the study or started such intake during the course of the study; had an ongoing use of medications known to affect the gingival tissues (i.e. calcium channel blockers, phenytoin, cyclosporine); had a history of infectious disease (i.e. Hepatitis B or C, HIV, oral herpes); had contagious illness (i.e. upper respiratory infection, oral herpes, sinuses); had dentures; had < 20 natural teeth; had periodontal pockets ( > 4 mm deep); had carious lesions requiring immediate restorative treatment; had evidence of periodontal disease; had history of allergy to consumer or personal care products or dentifrice ingredients as determined by the dental professional monitoring the study; was pregnant or nursing; was participating in another oral clinical study or test panels that required oral ingestion or testing of a product within a 1 month prior to entering the study; had received dental treatment during the study dates; was a smoker or used tobacco or e-cigarette products; had braces or aligners.

Methods
All experiments in the study were approved by the U.S. Investigational Review Board, Inc. (IRB#U.S.URB2019CP/13) and were conducted at Colgate Technology Campus in Piscataway, New Jersey. All participants provided written informed consent before participating in the study. The clinical study strictly followed the approved protocol without deviation and all methods were performed in accordance with the relevant guidelines and regulations. None of the subjects communicated that they were diabetic or had abnormal sugar levels, high blood pressure, or heart disease.

Participant recruitment. Participants were respondents to the Colgate Technology Campus (Piscataway, NJ) clinical site's advertised announcement for a clinical study. Participants lived in the New Jersey area and were selected based on the inclusion and exclusion criteria defined in the below sections.

Inclusion criteria. A summary of participants in the clinical study is shown in Table 1. The criteria for inclusion were as follows: male or female, 18–65 years of age; subject in good general health; available for the duration of the study; willing to sign the consent form; at least 20 natural teeth; Modified Gingival Index (MGI) < 0.5 or MGI ≥ 2 for the healthy and unhealthy groups, respectively. 29 subjects completed the study. One person dropped out for personal reasons. There were no adverse events reported.

Exclusion criteria. Subjects were excluded if they met the following criteria: the subject had medical conditions which required premedication prior to dental visits/procedures; had knowledge of impaired salivary function; used medication that could affect salivary flow; had used anticonvulsants, antihistamines, antidepressants, sedatives, tranquilizers, antibiotics, antimicrobial, anti-inflammatory medication or daily analgesics within 30 days prior to the start of the study or started such intake during the course of the study; had an ongoing use of medications known to affect the gingival tissues (i.e. calcium channel blockers, phenytoin, cyclosporine); had a history of infectious disease (i.e. Hepatitis B or C, HIV, oral herpes); had contagious illness (i.e. upper respiratory infection, oral herpes, sinuses); had dentures; had < 20 natural teeth; had periodontal pockets ( > 4 mm deep); had carious lesions requiring immediate restorative treatment; had evidence of periodontal disease; had history of allergy to consumer or personal care products or dentifrice ingredients as determined by the dental professional monitoring the study; was pregnant or nursing; was participating in another oral clinical study or test panels that required oral ingestion or testing of a product within a 1 month prior to entering the study; had received dental treatment during the study dates; was a smoker or used tobacco or e-cigarette products; had braces or aligners.

| Variable            | Analyzed group (Healthy N = 9) | Unhealthy N = 9 | Total group (Healthy N = 15) | Unhealthy (N = 15) |
|---------------------|-------------------------------|-----------------|-----------------------------|-------------------|
| Average age         | 43.3 (3.6)                    | 41.4 (4.1)      | 40.5 (2.52)                 | 43.3 (3.07)       |
| Male                | 3                             | 6               | 6                           | 8                 |
| Female              | 6                             | 3               | 9                           | 7                 |
| Average incisor baseline MGI | 0.194 (0.018) | 2.833 (0.050) | 0.142 (0.027) | 2.417 (0.043) |

Table 1. Demographic data table of the subjects participating in the clinical study at baseline. Data are not representative of the full month MGI scores. The MGI scores capture the measurements from the upper and lower incisors (tooth # 7–10 and 23–26) and are expressed as mean (standard error of the mean) values. MGI modified gingival index.
Study visits and clinical procedures. Participants were first screened to assess whether they met the inclusion/exclusion criteria. At the screening, participants’ demographics and medical history were recorded, followed by an oral examination including a gingival assessment (described below in the "Health determination" section). Assessment results were recorded. After signing an Informed Consent Form, being provided a Privacy notice, and meeting all inclusion requirements, subjects were enrolled in the study. 30 participants were enrolled in the study (15 with MGI < 0.5 and 15 with MGI ≥ 2). After enrollment, these participants received a prophylaxis cleaning to remove all plaque and calculus. They were given a soft-bristled toothbrush and sodium fluoride (0.76%) containing toothpaste and instructed to brush twice a day for 2 min for 14 days. After this 14 day period, the participants returned for their baseline visit. They were asked to abstain from brushing 12 h prior to the baseline and for every successive visit to the clinical site. At each visit during the induced gingivitis stage (2, 7, 14, and 21 days post Baseline), participants received full oral soft tissue examinations and assessments of gingival inflammation (MGI). Hyperspectral Spatial Frequency Domain Imaging measurements were only conducted at Baseline and at the Day 21 visit. After the Baseline visit, the participants were instructed to abstain from performing any oral hygiene (i.e. toothbrushing, use of mouthwash, flossing, etc.) for 3 weeks. After the 3 weeks visit, subjects were instructed to return to their normal oral hygiene regimen, brushing twice a day for 2 min for 14 days with the provided fluoride containing toothpaste. After the conclusion of the 14-day period, the subjects returned to the clinic for a final clinical assessment of MGI and health.

Health determination. All panelists were given an oral exam by a dental hygienist. The examination included evaluation of the soft and hard palate, gingival mucosa, buccal mucosa, mucogingival fold areas, tongue, sublingual and submandibular areas, salivary glands, and the tonsillar and pharyngeal areas. The panelists also completed a Medical History form to determine if they met the inclusion/exclusion requirements. Panelists that met the inclusion criteria were classified as healthy or unhealthy based on MGI scoring. Chosen panelists were not known to be susceptible to inflammation other than what would be driven by having poor oral hygiene habits.

Modified gingival index scoring. Non-invasive MGI scoring was chosen to prevent bleeding and disturbing plaque growth during the longitudinal investigation. MGI was scored at the mesial, middle and distal locations on the buccal tooth surface for the upper and lower incisors (8 total teeth), as shown in Fig. 1. The MGI of the sites were recorded at baseline (normal hygiene) and again at 2, 7, 14, and 21 days of abstaining from brushing (induced gingivitis). A description of MGI scoring is detailed in Table 2. Since this report captures the changes in oxygen and de-oxygenation at only two time points: Baseline and at Day 21, only the MGI data at these two timepoints will be presented in this manuscript. See all of the MGI data in the Supplemental Information.

Hy-SFDI image acquisition and processing. Functional blood information was measured using a hyperspectral spatial frequency domain imaging (Hy-SFDI) system. The location of data collection is shown in Fig. 1. Example images of the data are shown in Supplementary Fig. S1. The details of the system and data processing have previously been reported. Briefly, a compact LED projector (P2-A, AAXA Technologies) was
used to capture all images for Hy-SFDI. Due to significant spectral cross-talk, only 12 channels (502, 512, 524, 538, 562, 573, 586, 598, 611, 620, 632, 636) of the hyperspectral images were used for chromophore calculation. Captured images were first corrected using a company provided correction matrix. A modified open-source SFDI program was used in addition to a custom generated Monte Carlo look-up table for determining the absorption and scattering coefficients. Finally, the absorption coefficient at each wavelength was used to quantitatively map volumetric blood concentrations in the oral cavity (see Supplementary Fig. S1).

Subjects were given a cheek retractor to expose their gingival tissue for imaging. After the gingival tissue was well exposed, the subjects placed their head in a chin rest with forehead support for stability. Hy-SFDI images were then acquired for analysis. The acquisition time was approximately 300 ms. The imaging protocol was executed at baseline (normal hygiene) and again after 3-weeks of abstaining from brushing (induced inflammation). The measurements were taken only at these two time points because the inclusion of the Hy-SFDI system was for exploratory purposes in this study.

Exclusion of panelists from data. One panelist dropped out of the study before the 3-week imaging session. Their data was not used in the evaluation of this report. 11 of the Hy-SFDI sets (6 at baseline and 5 at the 3-week imaging session) presented frequency artifacts due to mis-matched calibration and imaging distances. Therefore, Hy-SFDI data with frequency artifacts were also removed. Data from the remaining 18 panelists were used in the evaluation and presented in this report.

Longitudinal measurement of oxy- and deoxy-blood volume fraction. After Hy-SFDI image acquisition, images were used to generate oxy- and deoxygenated blood volume fraction maps of the gingival tissues of the oral cavity of the 18 subjects without Hy-SFDI frequency artifacts. The maps were then used to select a 10X10 pixel area (approximately 1 x 1 mm²) on the gingival tissue approximately 1 mm above the central crest of the upper incisors and 1 mm below the trough of the lower incisors. The area was then averaged to get the oxy- and de-oxygenated blood volume fraction for the selected region. The base and 3-week time point images for each subject were co-registered to acquire the blood volume fraction from the same location at the different time points.

Longitudinal measurement of modified gingival index score. To compare MGI score and Hy-SFDI measurements, the anterior facial MGI measurements of the incisors were averaged and used for analysis.

Statistical analysis. For MGI measurements, the mesial, middle, and distal MGI measures from the eight incisors (four upper, four lower) were averaged for each person to give an average MGI score of (1) the upper incisors, (2) the lower incisors, and (3) total incisors. Each panelist received an average score, and the standard error of the mean was calculated for each group (healthy and unhealthy). For blood volume measurements, the sampling method is described in the “Longitudinal measurement of oxy- and deoxy-blood volume fraction” section. Oxy and Deoxy-blood volume fractions from the upper incisors and lower incisors were averaged to give an Oxy and Deoxy-blood volume fraction average of the total incisors. Each panelist received an average score, and the standard error of the mean was calculated for each group (healthy and unhealthy).

Results

Modified gingival index dynamics. MGI scores of panelists at baseline and after 3 weeks of abstaining from oral hygiene are plotted in Fig. 2. Clear changes in MGI were observed for the healthy group, whereas the unhealthy group had a smaller MGI change. For the healthy group, the average MGI score increased from 0.14 ± 0.02 to 1.07 ± 0.07 over the 3 week period, confirming the inflammatory response. For the unhealthy group,
the average MGI score increased from 2.19 ± 0.07 to 2.73 ± 0.08. Changes for both groups showed an increase in inflammation according to MGI score, but the healthy group showed a larger overall increase. Changes in MGI score for both groups for the top and bottom jaw had similar trends as compared to the averaged MGI score, however a single panelist did experience a decrease in MGI score for the lower incisors. Changes in MGI score were found to be statistically significant for both groups (see Supplementary Table S1).

**Blood volume fraction dynamics.** Figure 3 shows the blood volume fraction dynamics for the panelists. Similar to MGI scores, oxygenated and de-oxygenated blood volume fraction changes were observed in healthy and unhealthy groups. In general, changes in the blood volume fraction corroborated MGI measurements for the healthy group. Overall blood volume fraction increased for both healthy and unhealthy groups during three-weeks of non-brushing. The healthy group presented a larger and statistically significant increase in average oxygenated blood volume fraction (∼17%) compared to the unhealthy group, whereas the unhealthy group had a larger and statistically significant increase in de-oxygenated blood volume fraction (∼85%) (see Supplementary Table S1). On an individual level, a majority of the panelists in the healthy group showed an increase in both oxy- and de-oxygenated blood volume fractions. For oxygen blood volume fraction, the unhealthy group had a diverse response; five panelists presented a decrease and four panelists increased or remained the same. In contrast, blood oxygen fraction increased for all panelists in the healthy group. The unhealthy group had a less diverse increasing trend for de-oxygenated blood volume when compared to the oxygenated blood volume response.

**Adverse events.** No adverse events were reported by any of the panelists during the period of the investigation.
Discussion

Results show that both MGI and Hy-SFDI measured inflammatory responses of panelists in both the healthy and unhealthy groups after 3 weeks of oral hygiene abstinence. However, Hy-SFDI demonstrates that the blood component response is apparently different on average for the two groups. Among the blood components measured, de-oxygenated blood volume fraction for the healthy group and oxygenated blood volume fraction for the unhealthy group were not found statistically significant. Changes in MGI had low to moderate correlation with blood volume fraction components for the healthy group, but presented high correlation with all blood volume fraction components in the unhealthy group (see Supplementary Table S2).

MGI is a visual scoring scale, defined in Table 2. MGI evaluates the level of oral tissue inflammation and gingival health. Oral tissue inflammation is known to occur in healthy populations with immediate withdrawal of oral hygiene. Induced gingivitis has been shown to peak after 3 weeks of oral hygiene abstinence. Previous induced gingivitis investigations show a diverse inflammation response composed of low and high responders\(^7,12-14,34,35\). However, comparing MGI scoring and quantitative blood volume measurements during induced gingivitis of healthy people and populations with mild to moderate gingival inflammation has not been investigated.

Immediate withdrawal of oral hygiene leads to changes in gingiva tissue MGI score of healthy subjects as well as those with gum inflammation. Panelists in both groups showed an increase in MGI scoring, consistent with what has been observed for experimentally induced gingivitis. However, the unhealthy group had a less pronounced average MGI increase in response to induced gingivitis. Both groups had a prophylaxis cleaning and followed a designated oral cleaning routine (described in the “Study visits and clinical procedures” section) before the baseline measurements. The professional cleaning and hygiene routine reduced gum inflammation for the unhealthy group (average \(\Delta\text{MGI} = 0.52\)) and had no significant change for the healthy group (average \(\Delta\text{MGI} = 0.01\)). The average MGI score at Baseline for the subjects with gum inflammation still, however, fell in the mild to moderate range (Table 2). The application of prophylaxis cleaning with 2x/day brushing thus only slightly mitigated gum inflammation experienced by this population. During the 3-week brushing abstinence, the unhealthy panelists’ MGI score returned to the original pre-brushing routine homeostasis.

Hy-SFDI functional blood volume measurements showed different inflammatory responses for the healthy and unhealthy groups. The healthy group had a larger increase in oxygenated blood volume at the 3-week time point. In the unhealthy group, the blood volume increase was mainly due to de-oxygenated blood. Increases in de-oxygenated blood volume cause tissue to appear darker, whereas increases in oxygenated blood cause a redder appearance to tissue\(^36\). The impact of blood oxygenation status on color may have led to an apparent smaller MGI reduction for the subjects with mild to moderate gingivitis at Baseline. Both healthy and unhealthy groups showed increased blood volume fraction after 3 weeks of abstaining from oral hygiene. The average blood volume fraction was similar between both groups after 3 weeks of oral hygiene abstinence. However, the de-oxygenated blood volume fraction increase was more pronounced and statistically significant for the unhealthy group (see Supplementary Table S1). Therefore, either the tissue-oxygen demand was higher for the unhealthy population or vasculature created by angiogenesis, which occurs in chronically inflamed tissues, feeds the tissue with a higher percentage of de-oxygenated blood\(^37,38\).

The current investigation demonstrates an apparent quantitative difference in gingival blood functional response of healthy and gingivally unhealthy groups due to withdrawal of oral hygiene. Strength of correlation between changes and MGI and blood volume fraction components differed depending on oral health. Large variation in response to experimental gingivitis has been observed in previous investigations. Therefore, to confirm the blood functional response difference between the two groups, a larger population is needed with measurements acquired at more time points. In addition, the current investigation is limited to the front of the incisors. Limited field-of-view also limits better understanding of inflammatory response, which can differ regionally in the oral cavity. Incorporating 3D depth measurements into the current Hy-SFDI system will greatly improve diagnostic capabilities.

Data availability

The datasets generated and/or analysed during the current study are not publicly available due to company confidentiality policy but are available from the corresponding author on reasonable request.

Received: 4 August 2022; Accepted: 25 October 2022
Published online: 01 November 2022

References

1. Grossi, S. G. et al. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. J. Periodontol. 65, 260 (1994).
2. Ge, Z. et al. Assessment of local hemodynamics in periodontal inflammation using optical spectroscopy. J. Periodontol. 82, 1161 (2011).
3. Loos, B. G. & Van Dyke, T. E. The role of inflammation and genetics in periodontal disease. Periodontology 2000 83, 26 (2020).
4. Könönen, E., Gursoy, M. & Gursoy, U. K. Periodontitis: A multifaced disease of tooth-supporting tissues. J. Clin. Med. 8, 1135 (2019).
5. Haumschild, M. S. & Haumschild, R. J. The importance of oral health in long-term care. J. Am. Med. Dir. Assoc. 10, 667 (2009).
6. Tonetti, M. S., Jepsen, S., Jin, L. & Ottoo-Corgel, J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. J. Clin. Periodontol. 44, 456 (2017).
7. Trombelli, L., Farina, R., Silva, C. O. & Tatakis, D. N. Plaque-induced gingivitis: Case definition and diagnostic considerations. J. Clin. Periodontol. 45, S44 (2018).
8. Trombelli, L. et al. Modulation of clinical expression of plaque-induced gingivitis: II. Identification of ‘high-responder’ and ‘low-responder’ subjects. J. Clin. Periodontol. 31, 239 (2004).
9. Lundmark, A. et al. Identification of salivary microbiota and its association with host inflammatory mediators in periodontitis. Front. Cell. Infect. Microbiol. 9, 216 (2019).


The authors declare no competing interests.

**Acknowledgements**

The authors are grateful to the dental hygienist Pamela Monty for collecting MGI data. The authors also acknowledge internal reviewers Zoe Scoullos, Silvana Barros and Juliana Gomez for their thoughtful comments on data and writing.

**Author contributions**

B.U. developed the Hy-SFDI system setup, control algorithm, data processing algorithm, as well as collected and processed the Hy-SFDI data. H.S. also developed the Hy-SFDI system, control software, and processed data. L.K.L. setup the investigation, developed the protocol, and did a thorough literature review. All authors interpreted the data and wrote the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-23115-x.

**Correspondence** and requests for materials should be addressed to B.E.U.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
