

Dynamics of red fluorescent dental plaque during experimental gingivitis—A cohort study

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ABSTRACT

Objectives: The dynamics of red fluorescent plaque (RFP) in comparison to clinical plaque and bleeding scores were studied during an experimental gingivitis protocol in a cohort of healthy participants.

Methods: Forty-one participants were monitored for RFP before (24 h plaque), during 14 days plaque accumulation (days 2, 5, 9, 14) and after 7 days recovery (24 h plaque). RFP was assessed on fluorescence photographs of the vestibular aspect of the anterior teeth (cuspid to cuspid) in the upper and lower jaw. Clinical plaque and bleeding were assessed at days −14, 0, 14 and 21.

Results: RFP of 24 h plaque was reproducible (days −14, 0), then increased during 14 days plaque accumulation and returned to baseline after 7 days recovery. Groups of low, moderate and high RFP formers were statistically significantly different at all times even already at baseline. The individual RFP response during 14 days plaque accumulation correlated well with RFP of 24 h plaque (days −14, 0). RFP correlated moderate to well with clinical plaque at days −14, 0, 14 and 21. From day 2 of the gingivitis challenge RFP correlated with bleeding at day 14.

Conclusions: RFP provided an objective measure of oral hygiene status. Given the correlation with clinical parameters found, the amount of RFP after 24 h plaque accumulation was indicative for the inflammatory response during a prolonged period of no oral hygiene. This trial was registered at the public trial register of the Central Committee on Research Involving Human Subjects (CCMO) under number NL51111.029.14

Clinical significance: This paper shows the association between RFP after 24 h plaque accumulation and inflammatory response after a prolonged period of no oral hygiene. Red plaque fluorescence can be used to identify subjects at risk for developing gingival inflammation.

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1. Introduction

Dental plaque and bleeding on probing indices are commonly used as indicators of oral hygiene and gingival health, respectively. Presence or absence of plaque is considered a measure indicating the current status of oral hygiene, which fluctuates per person per day. As reported in an experimental gingivitis study [1] bleeding on probing increases when plaque remains present during a period of three weeks refraining from all oral hygiene. Bleeding is therefore

often considered as an indicator of the average level of oral hygiene and gingiva inflammation.

While young plaque is considered healthy, old or matured plaque is considered to cause caries and/or gingivitis and to stimulate the development of periodontitis [2]. Hence, (re)viewing the presence or absence of matured plaque could provide a more reliable impression of the oral health risks in a mouth.

When an oral cavity is examined with quantitative light induced fluorescence (QLF), often red fluorescent plaque (RFP) is observed. This phenomenon is generally attributed to matured plaque and not young plaque [3,4]. In general matured plaque is considered to be old plaque (>48 h), however the definition of what constitutes matured plaque is not unambiguous. Recent *in vitro* studies on red biofilm fluorescence have reported a relationship with biofilm age and thickness, but more specifically with the cariogenicity of the biofilm related to level or frequency of sucrose

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availability in the growth media and mineral loss from the substratum [5–8]. Other studies have related red biofilm or bacterial fluorescence to the presence of metalloporphyrins such as heme [3,9]. The extent and level of RFP can be documented and quantified using a QLF-D biluminator camera system (Inspektor Research BV, Amsterdam, the Netherlands). This camera is designed to simultaneously capture both white-light, and fluorescence photographs of the oral cavity. Thus far only one cross-sectional clinical trial has been reported looking at RFP assessment with the QLF-D camera and its agreement with clinically recorded matured plaque and total plaque [10]. A moderate correlation between RFP and total plaque has been reported, while the correlation with matured or blue stained plaque was lower.

The aim of this study is to describe the dynamics of red fluorescent plaque during a two-week experimental gingivitis protocol and after a one-week recovery phase, where red fluorescent plaque parameters are compared to clinical plaque and bleeding on probing parameters recorded in time.

2. Materials and methods

2.1. Study design

A prospective cohort study was conducted at the Academic Centre for Dentistry Amsterdam between February and June 2015 to study the dynamic changes in red plaque fluorescence during an experimental gingivitis protocol. This study was performed as part of a randomized clinical trial exploring the dynamics of the oral ecosystem during a gingivitis challenge. The study was conducted in accordance with the ethical principles of the 64th WMA Declaration of Helsinki (October 2013, Brazil) and the Medical Research Involving Human Subjects Act (WMO), approximating Good Clinical Practice (CPMP/ICH/135/95) guidelines. The clinical trial was approved by the Medical Ethical Committee of the VU Medical Center (2014.505) and registered at the public trial register of the Central Committee on Research Involving Human Subjects (CCMO) under number NL51111.029.14.

2.2. Study population

Males and females between 18 and 55 years of age, in good general health, who did not participate in a clinical study within the previous 30 days were eligible to participate. Dental students and employees from ACTA were excluded. Volunteers meeting these criteria were screened to determine eligibility. At screening volunteers received oral and written information about the study. They could join after signing the informed consent. Between the screening and the first visit a time span of 1–3 weeks was scheduled to allow volunteers time to reconsider their participation. Participants needed to have at least 20 natural teeth with first and second molars present, a regular check-up at the dentist within the last year and having finished any necessary dental treatment. Participants should be non-smokers, i.e., having refrained from smoking for at least a year.

Volunteers were excluded when having periodontitis as established by the Dutch Periodontal Screening Index (DPSI ≤ 3 minus) [11] or $>40\%$ bleeding on probing. Volunteers with untreated dental caries, removable partial dentures, night guards, (peri-)oral piercings, apparent oral lesions (besides aphthous ulcers) or presence of orthodontic appliances (except lingual retention wires) were also excluded. Additionally, smokers, volunteers with self-reported abuse of drugs or alcohol and pregnant or breastfeeding women were excluded. Further exclusion criteria were: use of antibiotics during the last 3 months, need of antibiotic prophylaxis prior to dental treatment, use of anti-inflammatory drugs on a regular basis or any adverse medical

history or (long-term) medication (except for contraceptives). The research coordinator (N.A.M.R.) randomly assigned the participants to an intervention or a control group. Since the research questions of the present cohort-study do not include this intervention, the intervention group was excluded for data analyses.

2.3. Study procedures

First, participants were monitored at days –14 and 0 when performing normal oral hygiene. They were asked to refrain from oral hygiene 24 h before these baseline appointments as well as before the recovery appointment (day 21). To induce gingival inflammation, participants were requested to refrain from any form of oral hygiene for two weeks (days 0–14; the gingivitis challenge), resulting in plaque accumulation. During this experimental period, visits were planned at days 2, 5, 9 and 14. After one week of recovery with normal oral hygiene, a final visit was planned (day 21). All participants were instructed not to eat and drink (except water) two hours before any study appointment.

2.4. Assessment of red plaque fluorescence

Fluorescence photographs of the vestibular aspect of the anterior teeth (cuspid to cuspid, upper and lower jaw) in end-to-end position were taken at every study appointment using a QLF-D camera (Inspektor Research Systems BV, Amsterdam, the Netherlands) and cheek retractors (Henry Schein, Gillingham, UK, Double end large, 106-7079) via image capture software on the PC (C3 1.25 Inspektor Research Systems BV, Amsterdam, the Netherlands) [10]. Fluorescence photographs were assessed planimetric for the percentage RFP coverage (RF%) using RFP analysis software (QA2 V1.25, Inspektor Research Systems BV, Amsterdam, the Netherlands). Fluorescent photographs were also analyzed for the amount of RFP on the vestibular aspects of the anterior teeth from cuspid to cuspid in upper and lower jaw using a modified Quigley and Hein index as described by Paraskevas et al. [12] and adapted for use on fluorescence photographs (RF-mQH) [10]. RF-mQH (six point scale 0–5) was scored at three sites of the vestibular aspects of the teeth by two trained and calibrated examiners independently (C.M.C.V. & M.H.V.) and at separate times for the separate days in the experiment. Scores were totaled and divided by the total number of sites scored. The average score was used as consensus score.

2.4.1. Clinical procedures

Plaque was assessed clinically in a half mouth randomized contralateral model, using a modified Sillness & Loe Plaque Index (mS&L) [13] on a four point scale (0–3) scored at six sites of the buccal and lingual aspects of all present teeth [14] at days –14, 0, 14 and 21. The mS&L was assessed by the same independent calibrated examiner throughout the experiment (J.M.V.). Scores were totaled and divided by the total number of sites scored. Also the number of sites with plaque are totaled and divided by the total number of sites scored ($P\%$).

The extent of gingival bleeding was measured using the bleeding on marginal probing (BOMP) index as previously described [15] in a half mouth randomized contralateral model. A bleeding score was given to six gingival areas of the buccal and lingual sides of all present teeth. For each subject the number of bleeding points elicited are totaled and divided by the areas probed ($B\%$). The index used a three point scale (0–2) to describe the bleeding tendency per probing site. Bleeding was assessed by the same independent calibrated examiner throughout the experiment (S.B.).

2.5. Study outcomes

The primary outcome was the amount of RFP on the vestibular surfaces of the front teeth from cuspid to cuspid in upper and lower jaw, assessed planimetric (RF%) and visual (RF-mQH). Secondary outcomes were clinical plaque and bleeding on probing parameters. For the plaque and bleeding scores (mS&L, *P*% and BOMP, *B*%, respectively) the full mouth scores were used as well as the scores for vestibular aspects of cuspid to cuspid in upper and lower jaw (mS&L front and BOMP front respectively). The disto-vestibular aspects of the cuspids were excluded for comparison with the RFP assessment on the photographs.

2.6. Statistical analysis

The dynamics of RFP (RF% and RF-mQH) and clinical plaque and bleeding were assessed using a Friedman test for multiple dependent groups. Post hoc analysis with Wilcoxon signed ranks test was performed using a Bonferroni correction for multiple comparisons, resulting in a significance level set at $p < 0.0023$. The correlation for RFP with time during the gingivitis challenge (days 0–14) was assessed using a Spearman rank correlation test.

The cohort was separated into three groups of low, moderate and high responders for RFP formation. The RF% at day 14, the last day of the gingivitis, challenge was used to determine cut-offs at 0–1.7% (14 participants); 1.8–6.0% (14 participants) and >6.0% (13 participants).

Differences between the groups of low, moderate and high responders at different days during the gingivitis experiment were assessed using a Kruskal Wallis test. The correlation of RFP (RF% and RF-mQH) in time during the plaque accumulation and recovery periods with baseline RFP (days –14 and 0) was assessed using a Spearman rank correlation test. To compare RFP data with clinical parameters Spearman rank correlation tests were performed. All statistical analyses were performed using SPSS (version 21, IBM Inc., USA).

3. Results

A total of 77 subjects were screened, of which 70 met the inclusion criteria and 63 were enrolled after informed consent. A total of 43 participants were enrolled in the control group of the study. Two participants stopped between the study start at day –14 and the start of the gingivitis experiment at day 0. Results are reported for the 41 participants who followed the entire protocol consisting of 15 men (mean age 26.0, SD 6.9, min 18–max 45) and 26 women (mean age 23.8, SD 4.0, min 18–max 34).

Box plots describing the dynamics of RFP (RF% and RF-mQH) during the experimental gingivitis protocol are presented in Fig. 1a and e. Statistically significant differences in RFP were found in time during the experimental gingivitis protocol ($\chi^2(6) = 160.6$, $p < 0.001$ RF%; $\chi^2(6) = 145.2$, $p < 0.001$ RF-mQH). Post hoc analysis with Wilcoxon signed-rank tests with Bonferroni correction showed no significant differences between days –14, 0 and 21 when normal oral hygiene was performed (p -values between 0.3 and 0.7 RF% and RF-mQH). A gradual increase in RFP was observed resulting in significant differences between days –14, 0, 21 and days 2 through 14 ($p < 0.001$ RF% and RF-mQH), RFP at days 2 through 14 were significantly different ($p < 0.001$ RF% and RF-mQH) except for days 5 and 9 ($p = 0.003$ RF%; $p = 0.006$ RF-mQH) and days 9 and 14 ($p = 0.007$ RF%; $p = 0.005$ RF-mQH) (Fig. 1a and e). A moderate correlation was found between the number of days of plaque accumulation and RFP ($r_s = 0.42$, $p < 0.001$ RF%; $r_s = 0.48$, $p < 0.001$ RF-mQH).

Throughout the experiment individuals were observed with a low ($N = 14$; 1 male, 13 female; mean age 22.1 year, SD 3.0 year), moderate ($N = 14$; 8 male, 6 female; mean age 26.1 year, SD 7.1 year) or high ($N = 13$; 6 male, 7 female; mean age 25.7 year, SD 4.0 year) response to the gingivitis challenge. Time series of fluorescence images for typical examples of such low, moderate or high RFP formers are presented in Fig. 2 with line graphs of RF% and RF-mQH for the respective groups in Fig. 1b–d and f–h. The groups formed were statistically significantly different in amount of RFP at all time

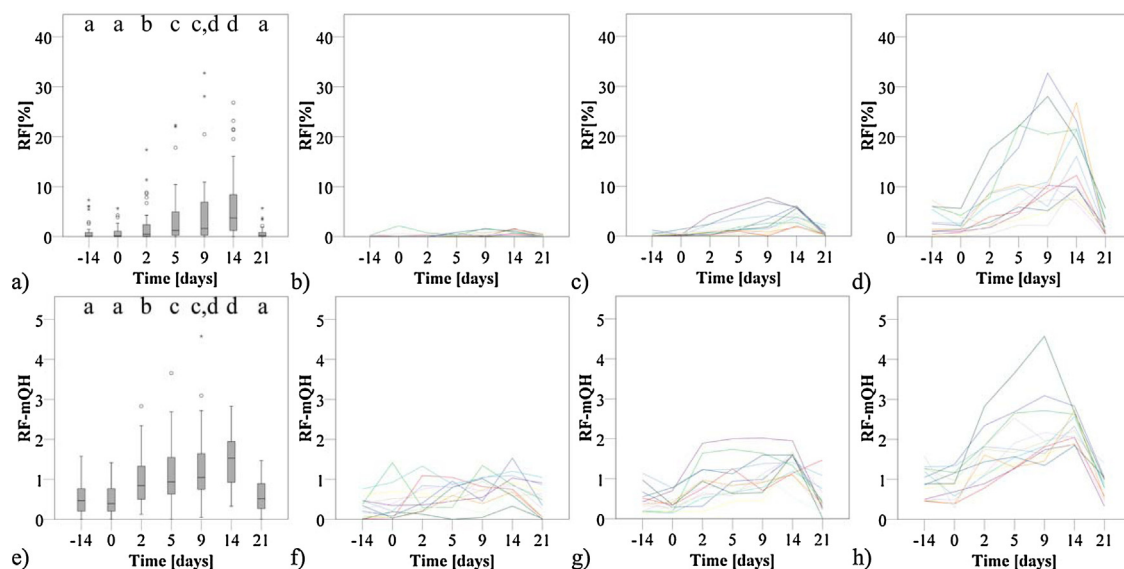


Fig. 1. Box plots and corresponding line graphs describing the dynamics of red fluorescent plaque (RFP) in time [days] from the start of the gingivitis challenge. The amount of RFP on the vestibular aspects of the front teeth from cuspid to cuspid in upper and lower jaw were assessed (a) planimetric for the percentage red fluorescent plaque coverage (RF%) and (e) using a Modified Quigley and Hein index adapted for use on fluorescence photographs (RF-mQH). Different index letters indicate statistically significant differences at $p < 0.0023$ (Friedman test and post hoc Wilcoxon signed ranks test using a Bonferroni correction). No significant differences were seen between days –14, 0 and 21 when normal oral hygiene was used (p -values between 0.3 and 0.7 RF% and RF-mQH). Line graphs for RF% and RF-mQH in time from the start of the gingivitis challenge are shown for groups of low (b and f), moderate (c and g) and high (d and h) red fluorescent plaque response. Statistically significant differences were found between the groups of low, moderate and high red fluorescent plaque response already at baseline (days –14 and 0). From the line graphs it becomes clear that RF% and RF-mQH increases during a period refraining from oral hygiene, but not always in a linear way.

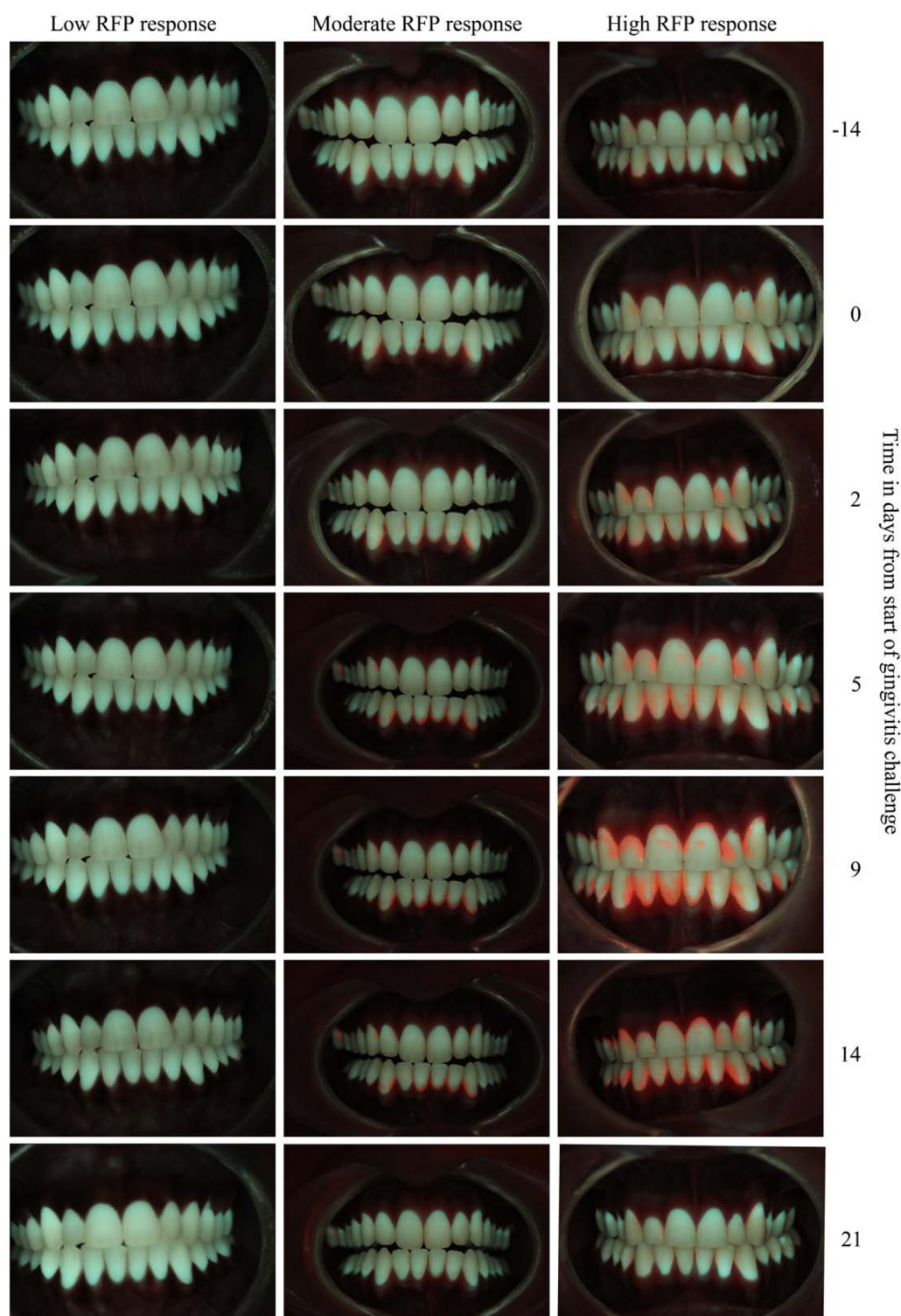


Fig. 2. Fluorescent photographs of three participants at the different time points during the gingivitis experiment are given for typical examples of low, moderate or high red fluorescent plaque (RFP) response.

points throughout the experiment (Kruskal Wallis test $p < 0.001$ RF % and $p < 0.05$ RF-mQH), i.e., already from baseline (24 h plaque accumulation). The line graphs indicate that individual RFP response is associated with the RFP levels after 24 h plaque accumulation.

The individual amount of RFP throughout the experiment correlated well (RF%) or moderate to well (RF-mQH) with the

baseline level, whereas clinical plaque (mS&L) and bleeding (BOMP) correlated moderate at best (Table 1).

Comparisons of RFP with clinical plaque and bleeding parameters were presented for two contralateral quadrants (full mouth), as well as the vestibular surfaces of cuspid to cuspid in upper and lower jaw of these two contralateral quadrants (front). The amount of RFP correlated moderate with $P\%$ and mS&L and

Table 1

Spearman rank correlations between the amount of red fluorescent plaque (RF% and RF-mQH); clinical plaque (mS&L) and bleeding (BOMP) during the gingivitis challenge and baseline levels at days –14 and 0.

Day	Parameter	Time from start of the gingivitis challenge [days]					
		0	2	5	9	14	21
–14	RF%	0.852 ^b	0.819 ^b	0.811 ^b	0.803 ^b	0.762 ^b	0.739 ^b
	RF-mQH	0.715 ^b	0.696 ^b	0.632 ^b	0.651 ^b	0.695 ^b	0.702 ^b
	mS&L	0.486 ^b				0.366 ^a	0.393 ^a
	BOMP	0.310				0.250	0.637 ^b
0	RF%	–	0.885 ^b	0.788 ^b	0.787 ^b	0.705 ^b	0.723 ^b
	RF-mQH	–	0.623 ^b	0.479 ^b	0.633 ^b	0.546 ^b	0.568 ^b
	mS&L	–				0.398 ^b	0.590 ^b
	BOMP	–				0.547 ^b	0.528 ^b

^a Correlation is significant at the 0.05 level (2-tailed).

^b Correlation is significant at the 0.01 level (2-tailed).

moderate to well for the front at each time point (Table 2). The amount of RFP correlated moderate with B% and BOMP in both full mouth and front at day 14. No correlation between RFP and B% or BOMP (full mouth and front) was seen at the other time points.

Spearman rank correlations for RFP at the different days during the gingivitis challenge and bleeding parameters at day 14 were presented in Table 3. A weak correlation between RFP and bleeding at day 14 was found from day 2 of the gingivitis challenge onwards.

4. Discussion

In this study the amount of red fluorescent plaque proved an objective measure of oral hygiene status and reproducible in time when assessed after 24 h no oral hygiene (days –14 and 0). RFP increased in time when refraining from oral hygiene up to 14 days. A 7 days recovery period is adequate to return RFP levels back to baseline levels. During a period of up to 14 days plaque accumulation the individual RFP response correlated well with the baseline levels (24 h plaque accumulation). From day 2 of the gingivitis challenge RFP correlated with bleeding percentage at day 14, suggesting that RFP is indicative for the level of inflammatory response during a 14 days gingivitis challenge.

This is the first publication describing the dynamics of RFP accumulation during an experimental gingivitis protocol. While RFP only constitutes a portion of plaque, its dynamics follow that of total plaque as assessed by mS&L in this study. The amount of RFP correlated moderate to well with mS&L at all time-points. The pattern of RFP during the study is comparable to that described in earlier experimental gingivitis studies [1,16]. Clinical plaque and bleeding parameters were only assessed at days –14, 0, 14 and 21 and thus the dynamics of plaque and bleeding during the two weeks refraining from oral hygiene were not assessed.

Table 2

Spearman rank correlations between the amount of red fluorescent plaque with clinical plaque and bleeding parameters at –14, 0, 14 and 21 days from the start of the gingivitis challenge.

Day	Parameter	RF-mQH	P%	mS&L	mS&L front	B%	BOMP	BOMP front
–14	RF%	0.699 ^a	0.421 ^b	0.356 ^a	0.503 ^b	0.059	0.055	–0.025
	RF-mQH		0.590 ^b	0.565 ^b	0.718 ^b	–0.074	–0.078	–0.106
0	RF%	0.790 ^a	0.405 ^b	0.374 ^a	0.426 ^b	0.183	0.172	0.005
	RF-mQH		0.419 ^b	0.381 ^a	0.530 ^b	–0.068	–0.073	–0.073
14	RF%	0.897 ^b	0.310 ^a	0.411 ^b	0.711 ^b	0.418 ^b	0.366 ^a	0.518 ^b
	RF-mQH		0.343 ^a	0.425 ^b	0.653 ^b	0.347 ^a	0.302	0.384 ^a
21	RF%	0.674 ^b	0.411 ^b	0.419 ^b	0.600 ^b	–0.034	–0.005	0.154
	RF-mQH		0.418 ^b	0.436 ^b	0.604 ^b	0.004	–0.019	0.111

^a Correlation is significant at the 0.05 level (2-tailed).

^b Correlation is significant at the 0.01 level (2-tailed).

Table 3

Spearman rank correlations between the amount of red fluorescent plaque (RF% and RF-mQH) during the gingivitis challenge and bleeding after 14 days gingivitis challenge.

Day	Parameter	Day 14		
		B%	BOMP	BOMP front
–14	RF%	0.267	0.230	0.300
	RF-mQH	0.084	0.057	0.104
0	RF%	0.162	0.138	0.204
	RF-mQH	0.058	0.044	0.060
2	RF%	0.335 ^a	0.300	0.387 ^a
	RF-mQH	0.350 ^a	0.324 ^a	0.300
5	RF%	0.331 ^a	0.300	0.341 ^a
	RF-mQH	0.399 ^b	0.369 ^a	0.294
9	RF%	0.394 ^a	0.356 ^a	0.465 ^b
	RF-mQH	0.361 ^a	0.341 ^a	0.346 ^a
14	RF%	0.418 ^b	0.366 ^a	0.518 ^b
	RF-mQH	0.347 ^a	0.302	0.384 ^a

^a Correlation is significant at the 0.05 level (2-tailed).

^b Correlation is significant at the 0.01 level (2-tailed).

Nevertheless, the clinical plaque and bleeding showed the same pattern, and clinical plaque parameters correlated with RFP at days –14, 0, 14 and 21. Equally so, the clinical bleeding parameters correlated with RFP at day 14.

The planimetric assessment of RFP (RF%) correlated well at all time points with visual assessment of RFP on photographs (RF-mQH) indicating that the planimetric assessment of fluorescence photographs is a valid way to measure RFP. The levels of 24 h RFP at days –14 and 0 were very similar (Fig. 1) indicating that the method is a robust measure reflecting the average day-to-day level of oral hygiene, unlike clinical indices assessing total plaque that indicate current oral hygiene status, which may fluctuate from day-to-day.

It is known that the rate of plaque accumulation and the subsequent gingival response varies per individual [1,17]. In general, elderly people respond more strongly to plaque accumulation; more gingival inflammation is seen in elderly during an experimental gingivitis experiment than in young individuals [16,18]. In a study comparing *de novo* plaque formation in young individuals, subjects with naturally occurring overt gingivitis formed on average more plaque than young individuals with healthy gingivae [19], suggesting that the rate of plaque formation may be indicative of gingival health.

In this study participants were all considered orally healthy at enrollment. Nevertheless great differences were observed in

individual responses of RFP formation during the experimental gingivitis challenge. The ability to separate individuals who responded with low, moderate or high RFP formation during the two weeks refraining from oral hygiene, could be a subject for further research. The correlation with bleeding indicates that subjects with high RFP response also have the strongest bleeding or inflammatory response. Whether caries activity as suggested in earlier *in vitro* and *in situ* research had an influence on RFP response during the gingivitis challenge is unknown. Caries did not fall under the scope of this study and was not recorded other than to determine in- or exclusion. Hence no information exists about current or past caries activity. Equally so no information is known about dietary patterns, such as sugar intake that could have impact on RFP formation as suggested from *in vitro* biofilm studies.

The red fluorescence of plaque has been assumed to be a property of matured plaque rather than young plaque [3,4], which is supported by the outcome of our study that on average the individual RFP increases in time during the experimental period of no oral hygiene. Also, *in vitro* studies report an association between red biofilm fluorescence and biofilm age, thickness and cariogenicity [5–8]. This red fluorescence has been attributed to the presence of metalloporphyrins such as haem [3,9] rather than the presence of specific species. In a clinical study, Han et al. [20] found an association between the presence of *Prevotella intermedia* and *Streptococcus anginosus* with RFP, however only six species were studied and not the entire microbiome.

The present study had a short follow-up period with a limited number of participants included. Therefore a future longitudinal diagnostic study should be performed including participants at varying stages of disease mimicking the actual prevalence of disease in the population to investigate the relation between red fluorescent dental plaque, progress of oral diseases and the inflammatory response. Given the correlation with clinical parameters found, in the current study the amount of RFP after 24 h plaque accumulation was indicative for the inflammatory response during a prolonged period of no oral hygiene.

Conflicts of interest

Monique van der Veen is co-inventor on several patents relating to quantitative light-induced fluorescence (QLF). The authors declare that otherwise, there are no conflicts of interest pertaining to the data presented in this article.

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