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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 indicatory 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.

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 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 $\leq$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 & Löe 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.).