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Modulation of clinical expression of plaque-induced gingivitis

II. Identification of "high-responder" and "low-responder" subjects

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Abstract

Aim: The aims of this study were to validate a randomized, split-mouth, localized experimental gingivitis model and to identify subjects with different gingivitis susceptibility.

Material and Methods: In each of 96 healthy subjects, one maxillary quadrant was randomly assigned as ''test'' (experimental gingivitis) and the contralateral quadrant as ''control''. Plaque index (PlI), gingival index (GI), gingival crevicular fluid volume (GCF), and angulated bleeding score (AngBS) were recorded in both quadrants at days 0, 7, 14, and 21. Cumulative plaque exposure (CPE), i.e. PlI over time, was calculated. Day-21 GCF was standardized according to CPE, and residuals of GCF on CPE were calculated. Two subpopulations were then defined, based on upper and lower quartiles of GCF-residual distribution and were, respectively, identified as ''high-responder'' (HR; $n = 24$) and ''low-responder'' (LR; $n = 24$).

Results: At test quadrants, all parameters significantly increased throughout the trial, while in control quadrants, PlI, GI, and AngBS remained low. Significant differences were noted between test and control quadrants on days 7, 14, and 21 for all parameters. Significant increases in GI, AngBS, and GCF were observed in test quadrants over the course of the study in both HR and LR groups. Significant differences were noted between HR and LR groups for all gingivitis parameters on day 21 in test quadrants, without any significant differences in PlI or CPE between the groups.

Conclusions: We identified two subpopulations characterized by significant differences in clinical parameters of plaque-induced gingival inflammation, despite similar amounts of plaque deposits and plaque accumulation rates.

Key words: dental plaque/diagnosis; gingival crevicular fluid/secretion; gingivitis/diagnosis; gingivitis/etiology; periodontal disease/gingivitis

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Dental plaque-induced gingivitis (Mariotti 1999) is the most prevalent disease of the periodontium (Oliver et al. 1998). The relationship between plaque accumulation and gingival inflammation was elegantly demonstrated by the studies of Löe and coworkers (Löe et al. 1965, 1967, Theilade et al. 1966) using their experimental gingivitis model. Since then, this model has been extensively used for the study of gingivitis pathogenesis and evaluation of therapeutic interventions.

Although several systemic and environmental factors (e.g. endocrine hormone status, medications, smoking) can modify the clinical expression of plaque-induced gingivitis (Mariotti 1999, Tatakis & Trombelli 2004), the majority of existing data indicate that the severity of gingival inflammation is primarily dependent on the quantitative (Löe et al. 1965, Breuer & Cosgrove 1989) and the inter-related qualitative (Theilade et al. 1966, Moore et al. 1982) aspects of the developing bacterial plaque.

Despite the universal effect of plaque accumulation on gingival status, there is evidence that the level and consistency of the gingival tissue response to plaque accumulation may vary significantly between individuals with neither quantitative nor qualitative differences in plaque accumulation (Abbas et al. 1986), suggesting that the level and consistency of the gingival tissue response to plaque accumulation is an individual trait possibly dependent on host-related factors, whether genetic or

environmental in origin (Wiedemann et al. 1979, Abbas et al. 1986, Tatakis & Trombelli 2004). This host-dependent variation in gingivitis susceptibility is supported by the results of a large-scale experimental gingivitis study found in the literature; Wiedemann et al. reported that in a group of 62 subjects, eight did not develop gingivitis within the time of the study (21 days), despite plaque accumulation (Wiedemann et al. 1979). Another group of 25 subjects exhibited substantial gingival inflammation within 2 weeks. The remaining 29 subjects formed an intermediate group, which developed gingival inflammation by day 21. In a study involving 45 subjects, van der Weijden et al. (1994a) found that 10 subjects consistently exhibited greater than average gingival inflammation, representing a ''susceptible'' group, while six subjects were consistently below average, representing a ''resistant'' group. From the data reported by Wiedemann et al. (1979) and van der Weijden et al. (1994a) one can estimate that approximately 13% of subjects represent a ''resistant'' group. These results suggest that host (genetic) factors may be one reason that account for such differences. Although the above studies suggested a difference in gingivitis susceptibility among various subjects, the issue has not been investigated any further.

The aims of the present study were (a) to validate the use of a randomized, split-mouth, localized experimental gingivitis model in a large sample of young adults, and (b) to identify, within such a sample, subjects with different severity of inflammatory response to plaque deposits. Identification of individuals with different gingival response to plaque accumulation, as assessed by clinical parameters, is the necessary first step in the pursuit of the factors underlying such variability.

Material and Methods

Experimental design

The overall design (Fig. 1) was that of a randomized, split-mouth, localized experimental gingivitis clinical trial. After a period of professional and supervised tooth cleaning, in each subject one maxillary quadrant was randomly assigned as ''test'' (experimental gingivitis) and the contralateral quadrant as ''control''. After 21 days of oral hygiene withdrawal in the test quadrant, subjects were assigned to a self-performed oral hygiene regimen for 21 days. The study design was approved by the local ethical committee and was found to conform to the requirements of the ''Declaration of Helsinki'' as originally adopted and subsequently revised (www.wma.net/e/policy/17-c_e.html). All participants provided written informed consent.

Subject recruitment

Subjects were recruited among current and permanent residents in the metropolitan area of Ferrara. The recruitment strategy included specific advertisements on local newspapers, on the student web site of the University of Ferrara, and at the various University departmental bulletin boards. The number of subjects selected for participation was based on an anticipated 10% attrition rate (Doherty 1982) and the set goal of 100 subjects completing the study. This goal stemmed from the need to have identified at study completion at least 15–20 subjects ''resistant'' and ''susceptible'' to gingivitis for further evaluation (see below).

At recruitment, subjects were asked to complete a self-administered questionnaire on demographic characteristics, medical and dental history. An explanatory pamphlet, with details on study purpose and design, and informed consent were handed out. Subjects who

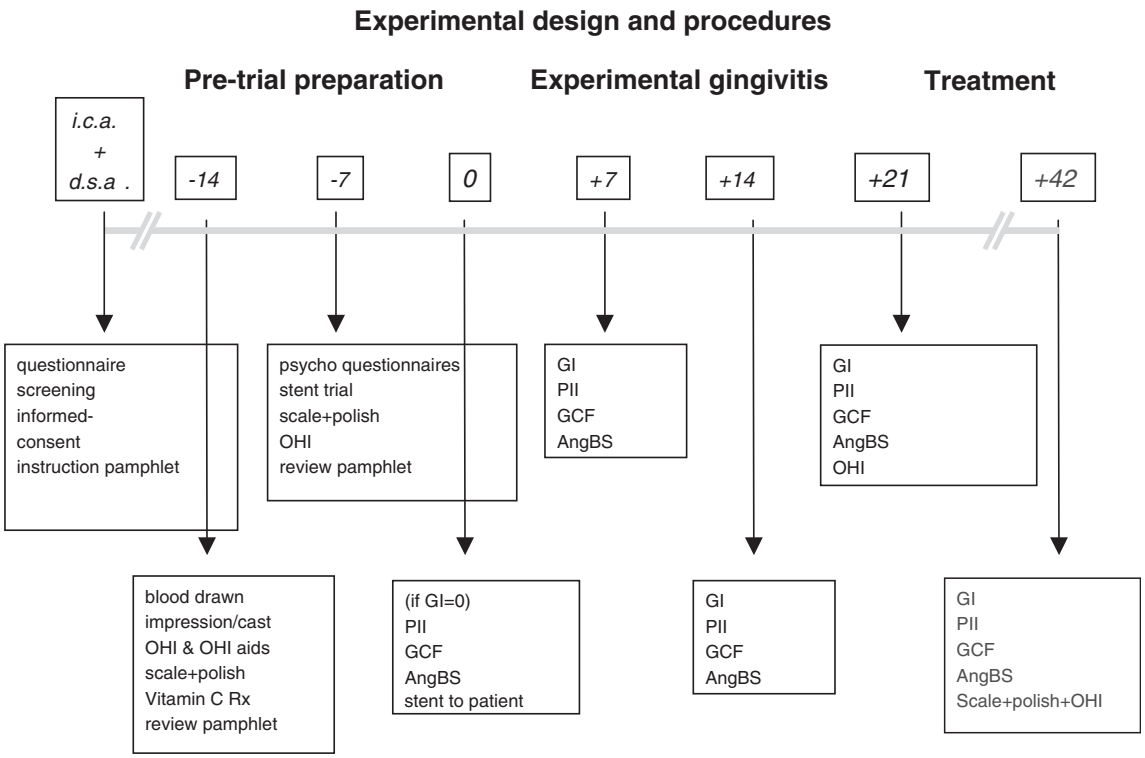

Fig. 1. Experimental design. i.c.a. = initial contact appointment; d.s.a. = dental screening appointment; AngBS = angulated bleeding score; GCF = gingival crevicular fluid volume; GI = gingival index; PlI = plaque index; OHI = oral hygiene instructions.

preliminarily met inclusion/exclusion criteria as reported on questionnaire were scheduled for a dental screening appointment. The screening procedure included verification of inclusion/exclusion criteria on a custom record form and verification of signed/dated/witnessed informed consent. A pamphlet containing specific instructions to be followed during study participation was dispensed at the end of the screening appointment.

Patient selection

Subjects were enrolled in the study if they met all of the following inclusion criteria:

- *Demographic*: 20–26 years of age; male or female (balanced groups); current and permanent residence in the major metropolitan area of Ferrara; non-smokers (never smokers or former smokers for at least 6 months).
- *Clinical* (*oral*): No probing pocket depth >3 mm at any site (except distal of second mandibular molars, where probing pocket depth $\leqslant 5$ mm was acceptable); no inter-proximal attachment loss >2 mm at any site; GI $= 0$ at study day 0 in teeth and sites of interest; maxillary lateral incisors, first or second premolars and first molars present and suitable for recordings; maxillary central incisors and second/third molars present and suitable for stent retention; able to have maxillary impression taken.
- *Other*: Able and willing to provide informed consent and to ensure compliance throughout the study.

Subjects were excluded from the study if they met any of the following exclusion criteria:

- *Clinical* (*oral*): Carious lesions and/or inadequate restorations in maxillary lateral incisors, first or second premolars and first molars or adjacent teeth, interfering with dental plaque removal in the sites of interest; ongoing dental treatment; maxillary dental implants, other than central incisor and second molar; maxillary occlusal bite-guard; maxillary orthodontic appliances, fixed or removable (permanent retainers on palatal of maxillary incisors were acceptable); mouthbreathing.
- *Medical*: physical or mental handicap that can interfere with adequate oral hygiene performance; history of drug abuse; allergy to acrylic material (stent); contraindication for vitamin C supplementation; medications affecting the gingiva and/or oral mucosa (diphenylhydantoin, calcium channel blockers, cyclosporin A, immunostimulants/immunomodulators); systemic and/or topical medications (steroidal and non-steroidal anti-inflammatories in last 6 weeks, antibiotics in last 6 weeks, anti-bacterial agents such as chlorhexidine or listerine, any mouthrinse, use of oral irrigator even if only with water); systemic conditions (pregnancy, diabetes, quantitative, and/or qualitative polymorphonuclear cell defects, other immune system disorders, organ transplants, need for antibiotic premedication, known genetic defects, e.g. trisomy 21).

Participants were exited from the study immediately upon: requesting to withdraw from further participation; development of acute dental/oral conditions requiring treatment; development of medical conditions conflicting with the exclusion criteria listed above; development of conditions requiring treatment that was in conflict with the exclusion criteria listed above; failure to comply with study instructions/requirements.

Tooth and site selection

The following three maxillary teeth were used in each test and control quadrant: lateral incisor, first premolar (if missing, replaced by second premolar), first molar (if missing, replaced by second premolar). For each tooth, parameters were evaluated on the buccal and the mesiobuccal aspect.

Allocation and allocation concealment

In each subject, one maxillary quadrant was assigned as ''test'' (experimental gingivitis) and the contralateral quadrant as ''control'' according to a computer-generated randomization list. Examiners were kept unaware of randomization sequence and blinded as to which quadrant was either test or control. A study investigator not involved with examinations informed the participants of quadrant assignment, upon handing them their individual stent (see below). Because of the nature of the study, however, it is not possible to assume that examiners had not become aware of quadrant allocation over the 21-day experimental gingivitis period. Plaque accumulation and gingival status were clinically different between test and control quadrants on days 7, 14, and 21. Whether and to what extent this readily identifiable status of the quadrants may have biased the examiners has not been ascertained.

Clinical parameters

The following clinical parameters were obtained in the order listed below from the selected sites.

- Gingival index (GI), according to a modification of the method of Löe & Silness (1963) without the bleeding on probing component.
- Plaque index (PlI), according to Silness & Löe (1964) and modified as follows. Plaque formed on the various tooth surfaces was first categorized as either PlI score 0 or PlI score 2. Subsequently, plaque was stained by use of topically applied erythrosine solution (Red Cote, Butler, Chicago, IL, USA) to distinguish between PlI score 0 and PlI score 1 (Furuichi et al. 1992).
- Gingival crevicular fluid volume (GCF), collected as previously described (Fransson et al. 1999) and measured according to Periotron® 8,000 manufacturer's (OraFlow Inc., Plainview, NY, USA) instructions. The individual site was gently air-dried in an apico-coronal direction without removal of any visible supragingival plaque. The area was carefully isolated with cotton rolls, to avoid salivary contamination. A sterile paper strip (Periopaper®; OraFlow Inc.) was introduced into the crevice until mild resistance was felt. Attention was paid to avoid any mechanical injury to marginal tissues. The strip was left in place for 5 s and immediately transferred, for volume determination, to the calibrated, chair-side located Periotron® 8,000 (OraFlow Inc.). Paper strips contaminated by gingival bleeding during GCF determination were discarded and corresponding data were recorded as missing.

- Periotron® calibration: instrument calibration was accomplished as previously described (Ciantar & Caruana 1998, Deinzer et al. 1999), using human serum. On each study day, calibration was verified by re-assessing two of the standard volumes. If the mean of these assessments differed by more than 5 Periotron® units (i.e. approximately 0.03 μl) from the mean of the calibration data, calibration was repeated for all standard volumes and a new calibration curve was estimated (Deinzer et al. 1999).

- Angulated bleeding score (AngBS), which is a modification of the angulated bleeding index as reported by van der Weijden et al. (1994b). After lightly drying the gingiva with compressed air, a periodontal probe (PCP 12, Hu Friedy, Chicago, IL, USA) was held at an angle of approximately 60° to the longitudinal axis of the tooth and in contact with the sulcular gingival tissues. AngBS was scored as: 0: no bleeding; 1: bleeding upon probe stimulation; 2: spontaneous bleeding.

- Cumulative plaque exposure (CPE), a derived parameter, was calculated. CPE represents the area under the curve (AUC) of subject-specific PlI over a specific period of time (7, 14, or 21 days).

Two trained and calibrated examiners recorded all parameters. One examiner evaluated 56 subjects and the other evaluated 40 subjects. Each subject was randomly assigned to each of the examiners. Attempt was made to ensure that the originally assigned examiner evaluated a particular subject throughout the trial, thus limiting within-subject inter-examiner variability. Of the 96 subjects completing the study, 84 were evaluated by the same examiner throughout the study.

Assessment of inter- and intra-examiner reproducibility

Before starting the trial, inter- and intra-examiner agreement was assessed. Prior to the calibration sessions, the two examiners were given multiple sessions of training and calibration exercises during which they were asked to rate GI and PlI on a randomly selected group of patients referred to the Dental Clinic. Disagreement was resolved by discussion between examiners.

For the calibration sessions, 14 dental students, who were not included in the study population, were recruited after informed consent. They were screened to ensure they fulfilled inclusion/exclusion criteria, but no attempt was made to change their oral hygiene regimen. Students were evaluated in two different sessions: one for GI recordings and one for PlI recordings. The sessions were scheduled 2 weeks apart. In each session, the examiners were asked to separately rate either GI or PlI in the 12 sites of interest according to a randomly selected order. Duplicate examination was then performed after at least 1 h. The measurement of inter- and intra-examiner agreement on PlI and GI was based on unweighted κ-statistics at both site-specific and subject levels (Fleiss & Chilton 1983, Spolsky & Gornbein 1996).

For PlI, inter-examiner κ coefficient was 0.62 ± 0.05 (mean $\pm$ SE) for site-specific scores, and for subject scores was 0.57 ± 0.11. Intra-examiner κ coefficient was 0.74 ± 0.07 for both examiners for site-specific scores, and for subject scores was 0.74 ± 0.06. For GI, inter-examiner κ coefficient was 0.46 ± 0.05 for site-specific scores, and for subject scores was 0.41 ± 0.10. Intra-examiner κ coefficient ranged from 0.55 ± 0.07 to 0.77 ± 0.05 for site-specific scores, and for subject scores equaled 0.55 ± 0.07. Assessment of reliability for AngBS was not performed because intra- and inter-examiner agreement for bleeding indexes is affected by repeat examinations (van der Weijden et al. 1994c).

Clinical procedures

Pretrial preparation

To achieve optimum gingival health (GI = 0) and to standardize gingival baseline conditions all subjects participated in a pretrial period (Fig. 1). On day 14, after professional scaling and polishing a medium toothbrush (Elmex Inter X, GABA International AG, Münchenstein, Switzerland), unwaxed floss (Elmex, GABA International AG), and standard toothpaste (Aronal, GABA International AG), along with oral hygiene instructions, were provided. Toothpaste was provided in masked tubes. Subjects were taught the modified Bass technique or, if their hygiene regimen was judged sufficient, only few individual instructions were given on how to improve their performance. In addition, to exclude the possibility of subclinical ascorbate deficiency, all subjects were provided with a vitamin C (ascorbic acid, 500 mg; Vitamina C Angelini, ACRAF, Ancona, Italy) supplement to be taken once daily for the entire pretrial and trial period (56 tablets per subject). Polishing and oral hygiene instructions were repeated 1 week later (day 7) to achieve maximal plaque control and optimum gingival health.

Stent fabrication

Individual cast models were prepared on alginate impressions. The first cast model (master) was duplicated in order to fabricate two stents for each subject. A 2-mm thick film of light-curing flow composite material was set on the juxtagingival area at buccal and proximal surfaces of study teeth on test quadrant. This was done in order to eliminate stent contact with the cervical margin of the teeth, thus avoiding any disturbance to plaque accumulation and gingival condition during stent insertion/removal. The customized stents were then obtained by using 1.5-mm thick base plate material (LAS, Roveredo in Piano, PN, Italy) and a single-chambered vacuum machine (DR Pro-Form, Dental Resources Inc., Delano, MN, Italy). The stent was adapted and trimmed to fit the teeth (from central incisor to second/third molar) only in the test quadrant, extending at least 4–5 mm apical to the gingival margin on both buccal and palatal aspects. The stents were checked in the mouth and minor adjustments were performed chair side during pretrial visits. The two stents were delivered at day 0. Subjects were instructed to wear the stent prior to the oral hygiene session throughout the experimental gingivitis period to prevent plaque removal during brushing of the remaining dentition (Putt et al. 1993). The extra stent provided was to be used in the event of loss or fracture of the master stent.

Procedures by visit

The procedures by visit are outlined in Fig. 1. Additional information/explanations for specific visits are given below:

Day 14: The instruction pamphlet was reviewed chair side and detailed explanations were provided. A blood

sample (20 ml) was drawn. Blood samples were used to obtain serum for inflammatory mediator analysis and DNA for genetic studies (results to be reported elsewhere).

Day 7: The stent was tried, necessary adjustments were performed to avoid any disturbance on plaque accumulation during insertion/removal procedures. If needed, impression was repeated to prepare a new stent. Self-assessed psychological-/personality-/stress-level questionnaires were handed out and collected after completion (results to be reported elsewhere).

Day 0: Presence of healthy gingival condition (i.e. GI = 0) in all sites of interest was verified. If sites of interest were GI0, oral hygiene instruction and polishing were repeated, and subjects were asked to return in 1 week.

Day 21: Subjects were asked to reinstitute the oral hygiene regimen (modified Bass brushing technique, flossing) in the test quadrant. All subjects received the same treatment regimen for 21 days (see below for details).

Day 42: After recording of clinical parameters, oral hygiene instruction and an additional session of supragingival scaling, as needed, and polishing were given.

Treatment regimen

At completion of the experimental gingivitis period (day 21), all subjects were prescribed the same treatment regimen consisting of amine/stannous fluoride-containing toothpaste (meridol® toothpaste, GABA International AG) and mouthrinse (10 ml t.i.d; meridol® mouthrinse, GABA International AG). Results related to treatment are reported in a companion paper (Trombelli et al. 2004).

Data analysis

General

The subject was regarded as the statistical unit. For each clinical parameter, the recordings from the six selected sites for either test and control quadrants were added and divided by 6 to give the mean value for each subject. Therefore, for each parameter at each observational period, the subject was represented by a single test and a single control value.

Kolmogorov–Smirnov goodness-of-fit tests were computed for each variable to assess whether the variables were normally distributed. Data were expressed by either median and interquartile range (IR) for non-parametric variables (GI, AngBS), or mean ± standard deviation (SD) for parametric variables (PlI, CPE, GCF).

To test the effect of ''time'' and ''quadrant'' on response variables ANOVA for repeated measures or Friedman's test for parametric and non-parametric variables, respectively, were used. Post hoc comparisons were performed to explore intra- and inter-quadrant differences. Comparisons between ''high-responder'' (HR) and ''low-responder'' (LR) groups were performed by using χ^2 test for dichotomous variables, and unpaired *t*-test and Mann–Whitney *U*-test for parametric and non-parametric variables, respectively. The level of significance was set at 5%.

Identification of ''HR'' and ''LR'' groups

To identify ''HR'' and ''LR'' subjects among the 96 individuals who completed the experimental gingivitis trial, we first determined which clinical parameter of gingival inflammation showed the highest correlation with plaque-related variables, i.e. PlI and CPE. CPE represents the AUC of PlI over a specific time period of the experimental gingivitis trial. Correlation analysis showed that GCF-day 21 presented the highest correlation with both PlI-day 21 (Pearson $r_p = 0.56$, $p \ll 0.001$) as well as CPE-day 21 ($r_p = 0.62$, $p \ll 0.001$), compared with GI (Spearman $r_s = 0.48$ and $r_s = 0.41$ for PlI and CPE, respectively) and AngBS (Spearman $r_s = 0.31$ and $r_s = 0.30$ for PlI and CPE, respectively). Therefore, GCF was chosen as the primary outcome variable to express the severity of plaque-induced gingival inflammation.

GCF showed a statistically good fit to Gaussian distribution in both test and control quadrants over the whole 21-day experimental period (data not shown). In order to determine the extent of individual variability in GCF that could be explained by plaque accumulation, a linear regression analysis was performed between GCF-day 21 and either PlI-day 21 or CPE-day 21. Comparison between the two regression analyses showed that the variability observed for the outcome variable was better explained by CPE-day 21 ($R^2 = 38\%$) than PlI-day 21 ($R^2 = 30\%$). Statistical analysis showed a significant difference between the two regression coefficients ($t = 6.14$, $p < 0.001$). Therefore, GCF-day 21 was standardized according to CPE-day 21, and residuals of GCF-day 21 on CPE were calculated. The analysis of standardized residuals from the regression line is a procedure widely used in the study of complex diseases (Demissie et al. 2002, Tang et al. 2002). It allows for subtraction from the total variability

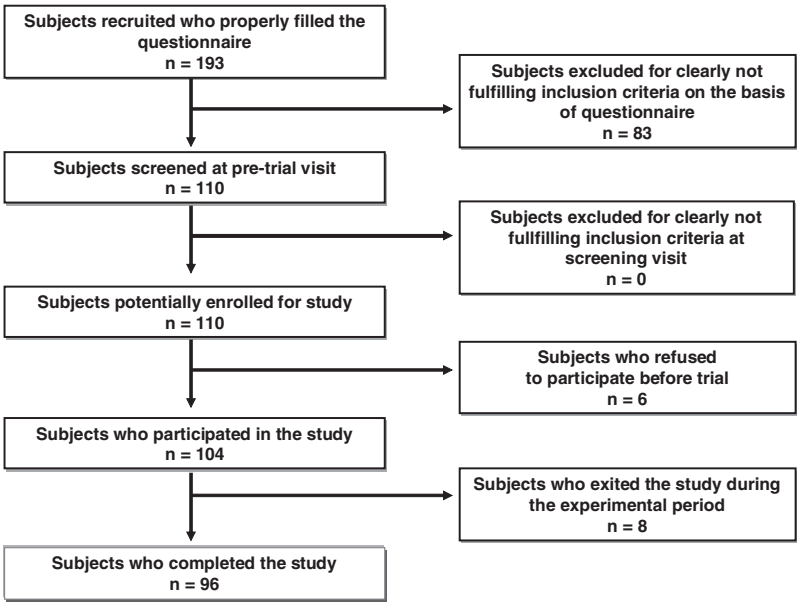

Fig. 2. Flow chart of subjects assessed and excluded at various stages of the trial.

observed for the response variable of the effect of those predicting factors for which the relationship with the primary outcome variable is well established. This would permit the identification of other factors potentially involved in the determination of the disease.

To estimate regression parameter, extreme variates were excluded when the absolute standard residual value was greater than +2. The exclusion of outliers was carried out because, within a relatively small sample size, the regression coefficient estimates are not very stable and a single extreme observation may considerably affect the final estimate of the β coefficient and may inflate or deflate the statistical significance tests. After estimation of the regression parameters, outliers were re-included in the study population and then standardized.

Subject distribution of GCF-day 21 residuals after standardization for CPE-day 21 did not differ from a normal distribution (Kolmogorov–Smirnov $d = 0.11$; $p < 0.20$). Two subpopulations were selected on the basis of upper and lower quartiles of this GCF-residual distribution. These subpopulations were, respectively, defined as ''high-responder'' (HR; $n = 24$) and ''low-responder'' (LR; $n = 24$) groups.

Results

Entire study population

Demographics and protocol deviations

Recruitment resulted in 193 subjects who properly filled the prescreening questionnaire. Eighty-three subjects were excluded for clearly not fulfilling inclusion/exclusion criteria, 110 were screened and enrolled for participation. At study initiation, six subjects refused to take part in the experimental trial. Of the remaining 104 subjects, eight volunteers exited the study during the experimental period for reasons not related to the experimental protocol. Ninety-six subjects (mean age: 23.6 ± 1.7 years), 46 males (mean age: 23.9 ± 1.7 years) and 50 females (mean age: 23.3 ± 1.6 years), completed the study. Fig. 2 is a flow chart of subjects assessed and excluded at various stages of the trial.

All subjects stated they had properly complied with study instructions, including treatment regimen. Of the 96 subjects completing the experimental

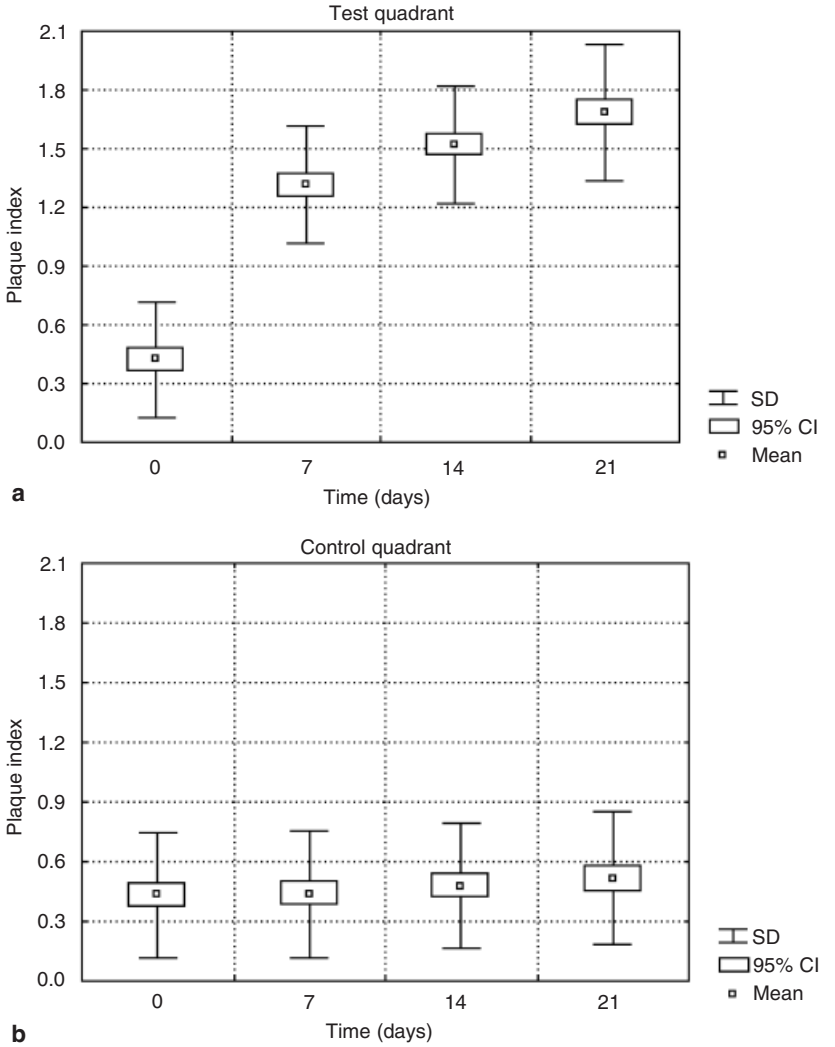

Fig. 3. Descriptive statistics (Box–Whisker plot) for plaque index over experimental gingivitis period in test (a) and control (b) quadrants ($n = 96$).

Table 1. Post-hoc multiple comparisons among mean plaque index measured at different observation intervals in test and control quadrants (*p*-levels)

	Day 0		Day 7		Day 14		Day 21	
	test	control	test	control	test	control	test	control
Day 0								
test	–							
control	*	–						
Day 7								
test	†	†	–					
control	*	*	†	–				
Day 14								
test	†	†	†	†	–			
control	*	*	†	*	†	–		
Day 21								
test	†	†	†	†	†	†	–	
control	*	*	†	*	†	*	†	–

*Not significant.
†Significance level: <0.001.

gingivitis period, two were available for evaluation on day 15 (instead of day 14), and five on day 22, two on day 23, and one on day 24 (instead of day 21). After treatment period, 15 subjects presented for evaluation on day 42 ± 3 days.

Plaque accumulation

ANOVA revealed a statistically significant effect of ''time'' ($F = 360.25$; $p < 0.001$) and ''quadrant'' ($F = 844.41$; $p < 0.001$) on PlI score. On day 0, PlI was 0.42 ± 0.30 in test quadrants and 0.43 ± 0.31 in control quadrants ($p = 0.99$). A statistically significant increase in PlI was observed in test quadrants, but not in control quadrants (Fig. 3a and b) over the course of the experimental gingivitis period. In test quadrants, PlI increased from 1.31 ± 0.30 on day 7 to 1.52 ± 0.30 on day 14, the difference being statistically significant ($p < 0.001$). Significant differences were also observed between days 14 and 21 (1.69 ± 0.35) (Table 1). In control quadrants, PlI remained similar to baseline condition on days 7, 14, and 21. Significant differences were noted between test and control quadrants on days 7, 14, and 21 (Table 1).

A statistically significant effect of ''time'' ($F = 1858.43$; $p < 0.001$) and ''quadrant'' ($F = 755.17$; $p < 0.001$) on CPE (Fig. 4) was observed. In test quadrant, CPE varied from 6.07 ± 1.62 on day 7 to 15.98 ± 3.22 on day 14, the difference being statistically significant ($p < 0.001$). Significant differences were also observed between days 14 and 21 (27.20 ± 4.97) ($p < 0.001$). CPE was significantly higher in test compared with control quadrants at each observation interval ($p < 0.001$ for all comparisons).

Gingival inflammation

On day 0, all clinical parameters related to gingival status were similarly low in both test and control quadrants ($p > 0.05$). Statistically significant increases in GI (Friedman ANOVA $\chi^2 = 214.63$; $p < 0.001$) and AngBS (Friedman ANOVA $\chi^2 = 167.36$; $p < 0.001$) were noted in test quadrants over the course of the experimental gingivitis period (Figs. 5a and 6a). On day 21, GI (0.67; IR 0.42–0.83) and AngBS (0.50; IR 0.17–0.92) were two- and threefold higher, respectively, than those recorded on day 14 ($p < 0.001$). In control quadrants, GI and AngBS remained close to zero throughout the trial (Figs. 5b and 6b). Significant differences were noted between test and control quadrants on days 7, 14, and 21 for both GI and AngBS ($p < 0.001$).

Twelve paper strips were contaminated by gingival bleeding during GCF determination and, therefore, were excluded from analysis. ANOVA revealed a statistically significant effect of ''time'' ($F = 280.25$; $p < 0.001$) and ''quadrant'' ($F = 432.59$; $p < 0.001$) on GCF. GCF varied from 0.06 ± 0.03 μl on day 0 to 0.33 ± 0.12 μl on day 21 in test quadrants ($p < 0.001$) (Fig. 7a), and from 0.06 ± 0.04 to 0.10 ± 0.05 μl in control quadrants ($p < 0.001$) (Fig. 7b). Significant differences were noted between test and control quadrants on days 7, 14, and 21 (Table 2). On day 21, GCF was threefold higher in test quadrants compared with control quadrants ($p < 0.001$). The distribution of the 96 subjects according to GCF values as recorded in test quadrants at completion of the experimental gingivitis trial (day 21) did not differ from a normal distribution (Kolmogorov–Smirnov $d = 0.074$, $p > 0.20$).

HR and LR subpopulations

Demographics and protocol deviations

The HR group comprised of 13 males and 11 females (mean age: 24.1 ± 1.6 years), and the LR group comprised of 11 males and 13 females (mean age: 23.4 ± 1.9 years). No significant differences were found in age (t-test = −1.31, $p = 0.51$) and gender ($\chi^2 = 0.33$, $p = 0.56$) between groups. There was no protocol deviation for the subjects in the HR and LR groups.

Plaque accumulation

PlI and CPE results for the two groups are presented in Tables 3 and 4, respectively. Statistically significant increase of PlI was observed in test quadrants over the course of the trial for both groups ($F = 142.8$, $p < 0.001$ for the HR group; $F = 154.3$, $p < 0.001$ for the LR group). In control quadrants, PlI remained similar to baseline condition on days 7, 14, and 21 for both groups ($F = 0.74$, $p = 0.534$ for the HR group; $F = 0.64$, $p = 0.592$ for the LR group). No significant differences in PlI were noted between groups on days 7, 14, and 21 in either test or control quadrants (Table 3).

On day 21, CPE was significantly increased in test compared with control quadrants in both the HR group (t-test = 17.1, $p < 0.001$) and the LR group (t-test = 15.0, $p < 0.001$). Differences in CPE were not statistically significant between groups in either test or control quadrants, at any period during the trial (Table 4).

Gingival inflammation

On day 0, all clinical parameters related to gingival status were similarly low in

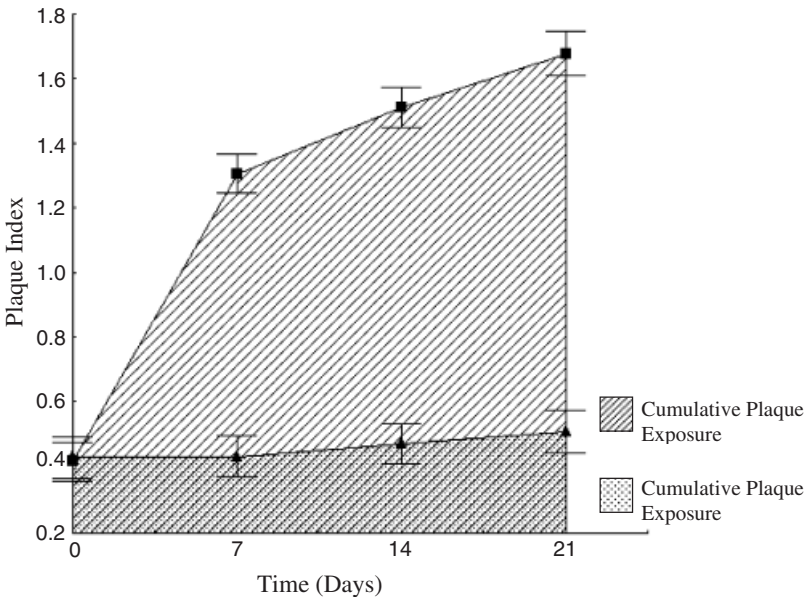

Fig. 4. Cumulative plaque exposure (i.e. plaque index over time) in test and control quadrants. Mean ± SD ($n = 96$) is presented.

test and control quadrants for both HR and LR groups ($p > 0.30$ for all clinical parameters). Statistically significant increase in GI was noted in test quadrants (ANOVA $\chi^2 = 62.9$, $p < 0.001$ for the HR group; ANOVA $\chi^2 = 50.8$, $p < 0.001$ for the LR group) over the course of the experimental gingivitis period. Significant differences in GI were noted between test and control quadrants on days 14 and 21 for the LR group ($p = 0.021$ and $P < 0.001$, respectively) and on days 7, 14, and 21 for HR ($p = 0.023$ on day 7, $p < 0.001$ on days 14 and 21).

For AngBS, a statistically significant increase was observed from baseline to day 21 in test quadrants (ANOVA $\chi^2 = 49.0$, $p < 0.001$ for the HR group; ANOVA $\chi^2 = 39.1$, $p < 0.001$ for the LR group). Significant differences were noted between test and control quadrants on days 14 and 21 for the LR group ($p < 0.001$ for both comparisons), and on days 7, 14, and 21 for HR ($p < 0.009$ on days 7 and 14 and $p < 0.001$ on day 21).

Tables 5 and 6 summarize, respectively, median levels of GI and AngBS measured for the HR and LR groups at different times in test and control quadrants. In test quadrants, significant differences were noted between the HR and LR groups on days 7, 14, and 21 for GI, and on days 7 and 21 for AngBS. In control quadrants, no significant differences in GI and AngBS were noted between the HR and LR groups over the course of the experimental gingivitis period.

Table 7 summarizes mean GCF values for the HR and LR groups at different times in test and control quadrants. ANOVA revealed a statistically significant effect of ''time'' and ''quadrant'' on GCF for both HR and LR groups. In test quadrants, GCF on day 21 was significantly increased from day 0 for both HR and LR groups ($p < 0.001$). Significant differences were noted between test and control quadrants on days 7, 14, and 21 for both groups ($p < 0.001$ for all comparisons). When the two groups were compared, significant differences in GCF were found at each observation interval in both test and control quadrants (Table 7).

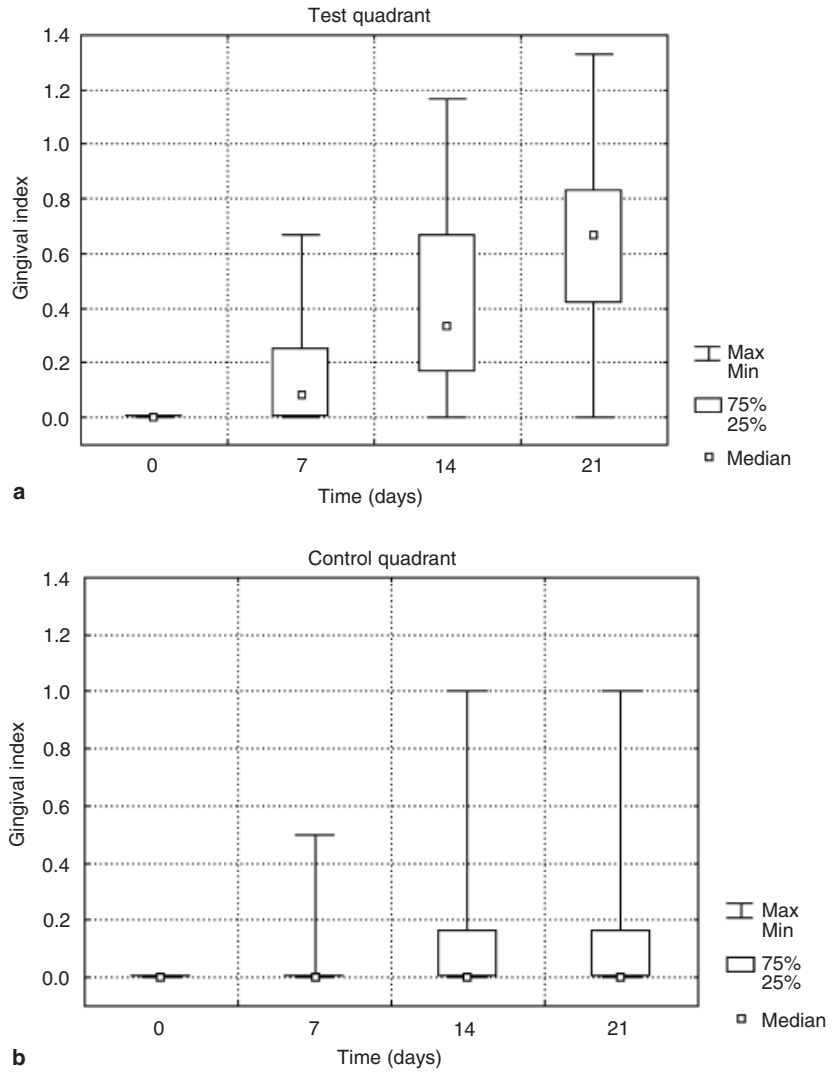

Fig. 5. Descriptive statistics (Box–Whisker plot) for gingival index over experimental gingivitis period in test (a) and control (b) quadrants ($n = 96$).

Discussion

The first aim of the present study was to validate the use of a randomized, controlled, split-mouth, localized experimental gingivitis model to determine the impact of supragingival plaque on gingival status over a 21-day period. A statistically significant increase of PlI, GI, AngBS, and GCF was observed in test quadrants, but not in control quadrants, over the course of the experimental gingivitis period. In test quadrants, PlI was fourfold higher on day 21 compared with day 0. Significant differences were noted between test and control quadrants on days 7, 14, and 21 for all clinical parameters.

An important modification of the present model has been the use of partial mouth experimentation (one quadrant, three teeth, six sites) as opposed to total oral hygiene abstinence. This modification made the model easier to accept by the prospective participants, therefore more feasible from a practical perspective. It also lessened the probability of adverse effects by limiting the number of teeth on which plaque was allowed to accumulate, rendering the model preferable from an ethical perspective. Previous reports included use of two quadrants (Deinzer et al. 1999), one quadrant (Putt et al. 1993, van der Weijen et al. 1994a), and any number of contiguous teeth (Bosman & Powell 1977, Matheny et al. 1993, Daly & Highfield 1996), extending over one (Bosman & Powell 1977, Daly & Highfield 1996) or two quadrants (Matheny et al. 1993). The results of partial mouth (''localized'') experimental gingivitis studies have always been similar to the results of full-mouth studies, making the ''localized'' model equivalent to the original full-mouth one. In the present trial, plaque scores revealed that the rate of plaque accumulation at test quadrants was

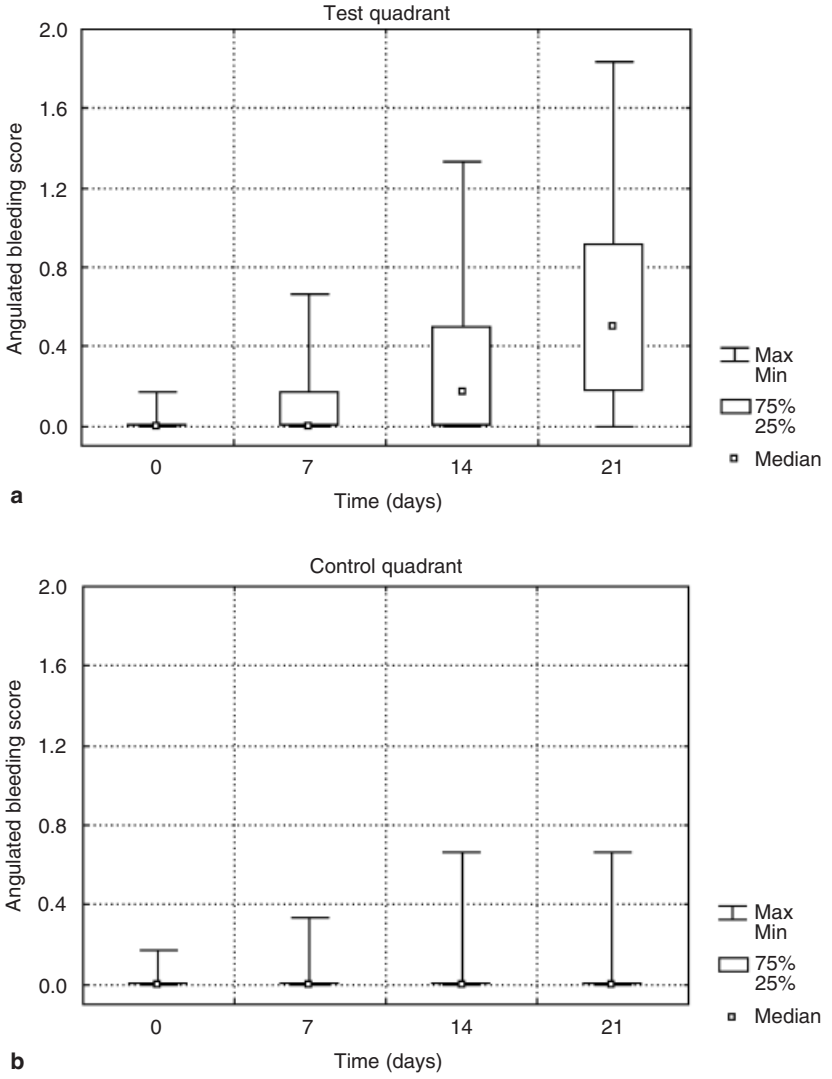

Fig. 6. Descriptive statistics (Box–Whisker plot) for angulated bleeding score over experimental gingivitis period in test (a) and control (b) quadrants ($n = 96$).

consistent with that observed in conventional full-mouth experimental gingivitis trials (Löe et al. 1965). Consistent with previous studies where a toothshield was used (Putt et al. 1993, Preshaw et al. 2001), plaque accumulation did not seem to be impaired by the use of a stent.

The three teeth chosen appeared to provide adequate representation for different tooth types and anatomic locations. The buccal and mesio-buccal sites were chosen because of the reported patterns of plaque accumulation (Furuichi et al. 1992) and the need to include sites where ''adequate'' amounts of plaque accumulated during the study. Inclusion of different tooth types and surfaces is also critical for evaluation of gingivitis treatment regimens (Ramberg et al. 1992).

The use of a ''static'' recording, such as plaque score at completion of accumulation period (i.e. PlI-day 21), may not adequately express the dynamics of plaque accumulation over the course of an experimental gingivitis trial. Therefore, the variable CPE (the PlI AUC during a specific experimental period) has been introduced to account for inter-individual differences in plaque accumulation rate. The fact that CPE-day 21 exhibited a higher correlation than PlI-day 21 with GCF-day 21 levels suggests that CPE may offer a better means of quantifying the biological challenge exerted by plaque on gingival tissues over a specific period of time.

The inclusion of a ''control'' quadrant in the examination and analysis was made in order to obtain information on the normal within-subject variation of gingival conditions without abstinence from oral hygiene procedures, thus better determining the specific effects of plaque accumulation. In test quadrants, an increasing amount of supragingival plaque was associated with a significant increase of gingival inflammation, as assessed by all clinical parameters. However, GCF significantly increased from day 0 to day 21 in control quadrants as well, despite the fact that plaque deposits, although present, remained almost unchanged over time. These observations support the suggestion that CPE, rather than PlI score, may be a better measure of the impact of accumulated dental plaque.

Another plausible explanation for the GCF increase in control quadrants may be the lack of independence between quadrants in the same individual, and may involve contralateral effects of plaque-induced gingivitis in test quadrants. In animal models, localized inflammation induced by bacteria or bacterial products has been shown to result in contralateral, i.e. distant inflammatory reactions (Terashima et al. 1996, Decaris et al. 1999). The mechanisms implicated in such responses include systemic release of cytokines (Terashima et al. 1996) and neurogenic transmission via neuropeptides (Decaris et al. 1999). Although some systemic effects of experimental gingivitis have been documented (Gaumer et al. 1976, Norman et al. 1979, Henry et al. 1987, Danielsen et al. 1993), there appear to be no studies on the effect of experimental gingivitis on systemic cytokine levels. The presence of neuropeptides implicated in neurogenic inflammation has been well documented in human gingiva (Bartold et al. 1994, Linden et al. 1997, Hanioka et al. 2000). Further studies are necessary to determine the reasons for and the mechanisms behind the small but significant GCF increase in control quadrants.

Gingivitis development differed among the 96 subjects that completed this trial, as determined by subject distribution according to test quadrant GCF levels on day 21. Although earlier experimental gingivitis studies have alluded to possible subject differences in susceptibility to gingivitis, such differences have been usually ascribed to differences in plaque accumulation rates (quantitative plaque differences) and/or the associated differences in plaque species present (qualitative plaque differences) (Löe et al. 1965,

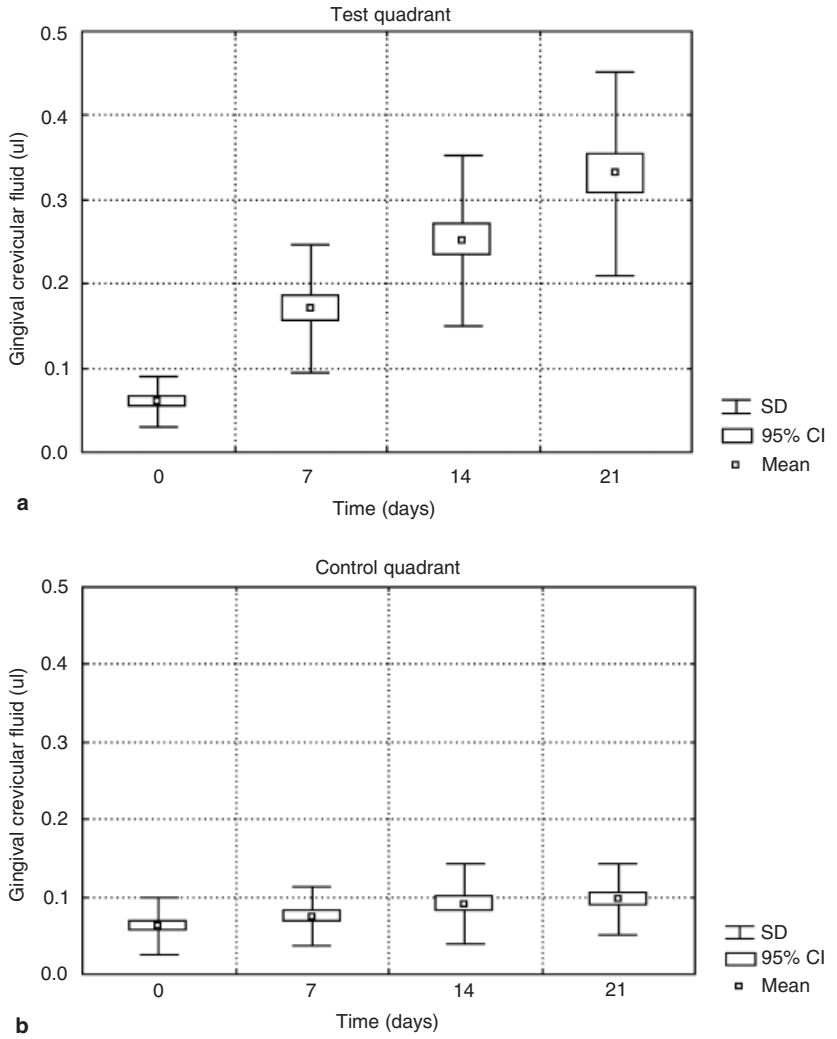

Fig. 7. Descriptive statistics (Box–Whisker plot) for gingival crevicular fluid volume (in μl) over experimental gingivitis period in test (a) and control (b) quadrants ($n = 96$).

Table 2. Post-hoc multiple comparisons among mean levels of gingival crevicular fluid volume (in μl) measured at observation intervals in test and control quadrants (p-levels)

	Day 0		Day 7		Day 14		Day 21	
	test	control	test	control	test	control	test	control
Day 0								
test	–							
control	*	–						
Day 7								
test	†	†	–					
control	*	*	†	–				
Day 14								
test	†	†	†	†	–			
control	†	†	†	*	†	–		
Day 21								
test	†	†	†	†	†	†	–	
control	†	†	†	*	†	*	†	–

*Not significant.
†Significance level: <0.001.

Theilade et al. 1966, Moore et al. 1982, Breuer & Cosgrove 1989). In the present study, such inter-individual differences in plaque accumulation (PlI and CPE) were also observed. However, the gingival response may vary significantly between individuals with neither quantitative nor qualitative differences in plaque accumulation (Wiedemann et al. 1979, Abbas et al. 1986, Tatakis & Trombelli 2004).

The second aim of the present study was, in fact, the identification of subjects with varying inflammatory response to similar plaque accumulation. According to CPE-standardized GCF values, as recorded on day 21 in test quadrants, we were able to discriminate two subpopulations (HR and LR groups) with significantly different severity of gingivitis to similar amounts of plaque deposits. When plaque accumulation in test quadrants is followed over time for the two subgroups, it is obvious that the behavior of the two groups is identical (Tables 3 and 4). Conversely, when GCF levels in test quadrants are followed over time, one observes a plateau for the LR group from day 14 on, while the HR group shows no such sign of having reached a maximum response (Table 7). In addition, after 21-day plaque accumulation HR individuals, compared with LR individuals, showed significantly higher values for all clinical parameters of gingival inflammation despite the lack of any difference in PlI or CPE between the two groups. The identification of individuals with different severity of gingival inflammation in response to similar plaque accumulation rate seems to confirm the hypothesis of a subject-specific effect of neglected oral hygiene on gingival tissues, as suggested by earlier studies on experimental gingivitis (Watts 1978, Wiedemann et al. 1979, Abbas et al. 1986, van der Weijden et al. 1994a) and recent studies on naturally occurring gingivitis (Müller et al. 2000). However, to the best of our knowledge, the present study is the first one demonstrating an individual pattern of gingival inflammatory response as assessed by an objective parameter (GCF) standardized for plaque accumulation over time (CPE).

Earlier studies have suggested that the bleeding on probing/plaque index ratio of an individual might be regarded as a prognostic indicator for the degree of experimentally induced gingival inflammation (van der Velden et al. 1985,

Table 3. Descriptive statistics and comparisons for plaque index (mean ± SD) in test and control quadrants of ''low-responder'' (LR) and ''high-responder'' (HR) subjects

	LR		HR		*t*-test (*p*-value)
	N	mean ± SD	*N*	mean ± SD	
Test quadrant					
day 0	24	0.34 ± 0.25	24	0.42 ± 0.32	0.32
day 7	24	1.28 ± 0.25	24	1.25 ± 0.39	0.77
day 14	24	1.55 ± 0.30	24	1.55 ± 0.33	1.00
day 21	24	1.65 ± 0.37	24	1.73 ± 0.33	0.41
Control quadrant					
day 0	24	0.35 ± 0.24	24	0.47 ± 0.37	0.23
day 7	24	0.40 ± 0.31	24	0.41 ± 0.33	0.94
day 14	24	0.42 ± 0.33	24	0.47 ± 0.35	0.62
day 21	24	0.42 ± 0.30	24	0.49 ± 0.37	0.48

Table 4. Descriptive statistics and comparisons for cumulative plaque exposure (mean ± SD) in test and control quadrants of ''low-responder'' (LR) and ''high-responder'' (HR) subjects

	LR		HR		*t*-test (*p*-value)
	N	mean ± SD	*N*	mean ± SD	
Test quadrant					
day 7	24	5.66 ± 1.20	24	5.86 ± 2.07	0.69
day 14	24	15.56 ± 2.69	24	15.65 ± 4.03	0.92
day 21	24	26.74 ± 4.61	24	27.13 ± 5.87	0.78
Control quadrant					
day 7	24	2.65 ± 1.76	24	3.06 ± 2.24	0.48
day 14	24	5.54 ± 3.55	24	6.15 ± 4.44	0.60
day 21	24	8.48 ± 5.38	24	9.50 ± 6.63	0.56

Table 5. Descriptive statistics and comparisons for gingival index (median, interquartile range) in test and control quadrants of ''low-responder'' (LR) and ''high-responder'' (HR) subjects

	LR		HR		Mann–Whitney test (*p*-value)
	N	Median (interquartile range)	*N*	Median (interquartile range)	
Test quadrant					
day 0	24	0.0 (0.0–0.0)	24	0.0 (0.0–0.0)	1.00
day 7	24	0.0 (0.0–0.0)	24	0.17 (0.0–0.33)	0.009
day 14	24	0.17 (0.0–0.33)	24	0.50 (0.33–0.67)	<0.001
day 21	24	0.50 (0.33–0.58)	24	0.83 (0.67–1.0)	<0.001
Control quadrant					
day 0	24	0.0 (0.0–0.0)	24	0.0 (0.0–0.0)	1.00
day 7	24	0.0 (0.0–0.0)	24	0.0 (0.0–0.17)	0.051
day 14	24	0.0 (0.0–0.17)	24	0.17 (0.0–0.25)	0.343
day 21	24	0.0 (0.0–0.17)	24	0.0 (0.0–0.17)	0.829

Abbas et al. 1986). However, subsequent studies indicated that bleeding, as elicited by probing to the bottom of the sulcus/pocket, appears to be an invalid indicator of early changes in the gingival condition (van der Weijden et al. 1994b). Among the clinical signs of gingivitis used in the present study, AngBS, designed to elicit bleeding from the marginal tissues rather than the bottom of the sulcus, showed the lowest correlation with plaque-related variables (PlI and CPE). In contrast, GCF showed the highest correlation with both plaque variables (see Materials and Methods). The choice of CPE-standardized GCF values as the discriminating parameter for the identification of the two subpopulations was based on (i) the reliability and accuracy of GCF as clinical indicator of gingival inflammation (Oliver et al. 1969, Borden et al. 1977, Poulsen et al. 1979, Engelberger et al. 1983), (ii) the strength of the correlation between GCF and CPE, and (iii) the need to limit the effect of differences in individual plaque accumulation rate on the variability of the inflammatory response to plaque. Taking into consideration the linear relationship between GCF-day 21 and CPE-day 21, a finding consistent with the linear relationship between GI and PlI (Breuer and Cosgrove 1989), we computed the residuals from the regression curve in order to determine the amount of individual GCF variability which could not be explained by the effect of plaque accumulation. Because the distribution of standardized GCF residuals was Gaussian, we had no reasons for rejecting the hypothesis that the biological factors influencing the residual variance are additive. We can reasonably hypothesize that if a multifactorial interaction could affect the clinical expression of plaque-induced gingivitis, the two tails of the GCF residuals distribution should present a very different content in factors related to gingivitis susceptibility. Consequently, we selected the 25th and 75th percentiles as an objective cutoff to isolate two sets (of equal and reasonable sample size) of individuals with clinically relevant differences in parameters of gingival inflammation.

Several extrinsic factors such as salivary contamination, evaporation, temperature, relative humidity, and strip placement have been reported to affect GCF recordings when the fluid is collected with filter paper strips (Lamster 1997). Every attempt possible was made to minimize the influence of these factors during GCF registration. Previous data have indicated that the presence of supragingival plaque can significantly elevate Periotron-based GCF measurements (Stoller et al. 1990). Because of the study design (effect of progressive plaque accumulation on gingival tissues) an influence of plaque deposits on GCF readings cannot be excluded. However, because similar

Table 6. Descriptive statistics and comparisons for angulated bleeding score (median, interquartile range) in test and control quadrants of ''low-responder'' (LR) and ''high-responder'' (HR) subjects

	LR		HR		Mann–Whitney test (*p*-value)
	N	Median (interquartile range)	*N*	Median (interquartile range)	
Test quadrant					
day 0	24	0.0 (0.0–0.0)	24	0.0 (0.0–0.0)	0.322
day 7	24	0.0 (0.0–0.0)	24	0.08 (0.0–0.25)	0.047
day 14	24	0.25 (0.0–0.42)	24	0.17 (0.0–0.42)	0.853
day 21	24	0.33 (0.0–0.75)	24	0.67 (0.42–1.0)	0.035
Control quadrant					
day 0	24	0.0 (0.0–0.0)	24	0.0 (0.0–0.0)	0.621
day 7	24	0.0 (0.0–0.0)	24	0.0 (0.0–0.0)	0.606
day 14	24	0.0 (0.0–0.0)	24	0.0 (0.0–0.0)	0.643
day 21	24	0.0 (0.0–0.0)	24	0.0 (0.0–0.0)	0.621

Table 7. Descriptive statistics and comparisons for gingival crevicular fluid volume (in μl; mean $\pm$ SD) in test and control quadrants of ''low-responder'' (LR) and ''high-responder'' (HR) subjects

	LR		HR		*t*-test (*p*-value)
	N	mean $\pm$ SD	*N*	mean $\pm$ SD	
Test quadrant					
day 0	24	0.06 $\pm$ 0.02	24	0.08 $\pm$ 0.04	0.025
day 7	24	0.15 $\pm$ 0.06	24	0.21 $\pm$ 0.09	0.018
day 14	24	0.21 $\pm$ 0.07	24	0.30 $\pm$ 0.12	0.002
day 21	24	0.22 $\pm$ 0.07	24	0.46 $\pm$ 0.13	$<$0.001
Control quadrant					
day 0	24	0.05 $\pm$ 0.03	24	0.08 $\pm$ 0.05	0.015
day 7	24	0.06 $\pm$ 0.03	24	0.09 $\pm$ 0.05	0.014
day 14	24	0.07 $\pm$ 0.04	24	0.10 $\pm$ 0.06	0.038
day 21	24	0.08 $\pm$ 0.03	24	0.11 $\pm$ 0.05	0.006

amounts of supragingival plaque were present on the test sites of both HR and LR groups, it is unlikely that the relative effect of supragingival plaque presence on GCF measurements would differ between the two identified subpopulations. Therefore, we have to conclude that the reported effect of supragingival plaque on GCF measurements (Stoller et al. 1990) may have altered the absolute GCF values recorded in the present study but not the relative differences in GCF between the two identified subpopulations. Because plaque levels were significantly lower in control quadrants compared with test quadrants throughout the study, we cannot exclude the possibility that the reported effect of supragingival plaque on GCF measurements may have contributed in part to the differences in GCF observed between test and control quadrants.

The fact that the present analysis identified two subpopulations with different response to experimental gingivitis in the absence of any discernible quantitative plaque differences does not rule out the possibility of differences in plaque composition between the two groups. However, the likelihood of such qualitative plaque differences is rather small, based on literature evidence (Abbas et al. 1986).

Previous studies investigated a possible relationship between age and the extent and severity of plaque-induced gingival inflammation (Holm-Pedersen et al. 1975, Winkel et al. 1987, Fransson et al. 1996). While some studies failed to identify age as a factor in the response of the gingiva to de novo plaque formation (Winkel et al. 1987), other reports showed that gingiva of older subjects responded earlier and in a more pronounced manner to plaque accumulation than younger subjects (Holm-Pedersen et al. 1975, Fransson et al. 1996). Therefore, discrimination of subjects in relation to gingival response to plaque could be affected by the age of examined subjects. In the present study, the age range of participants was limited to 20–26 years. The choice of such a narrow and young age range was made for three reasons. First, to effectively eliminate any age-dependent inter-individual susceptibility differences. Second, to include subjects presently free of chronic periodontitis who may still have the possibility to develop the disease in the future. Third, given current demographic patterns in Italy, this age range minimized the possibility of excluding large numbers of volunteers because of smoking or pregnancy.

The observed inter-individual variability in inflammatory response to an exogenous stimulus is not unique to humans and is not limited to bacterial stimuli. For example, studies indicate that genetic factors determine the susceptibility of different rat strains to a wide range of inflammatory diseases in response to a variety of pro-inflammatory stimuli, including bacterial components (Sternberg et al. 1989, Listwak et al. 1999). Introduction of a single immune response gene to animals naturally resistant to inflammation can render them prone to a variety of inflammatory conditions (Hammer et al. 1990, Tatakis et al. 2002). In humans, the studies of Löe et al. (1986) clearly demonstrate the existence of different subpopulations with significantly different susceptibilities to periodontitis. The data presented here suggest that the same holds true for gingivitis. The question that remains to be answered is which factors, be they environmental or genetic, govern susceptibility of periodontal tissues to plaque-induced inflammation (Tatakis & Trombelli 2004).

In conclusion, the results of the present study on 96 young adult subjects indicate that the randomized, split-mouth, localized experimental gingivitis model is an effective model to study the consequences of supragingival plaque accumulation on the inflammatory

status of the gingiva. Clinical parameters for gingivitis were significantly higher in test compared with control quadrants during the experimental period. On the basis of gingival crevicular fluid flow, as recorded on day 21 and adjusted for plaque exposure, we identified two subpopulations who differed significantly in severity of plaque-induced gingival inflammation despite similar amounts of plaque deposits and plaque accumulation rates. The identification of these two subpopulations should allow the pursuit of studies that are needed to investigate the host-related factors that may be implicated in this individual variation in gingival inflammatory response to dental plaque.

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References

Abbas, F., van der Velden, U., Hart, A. A., Moorer, W. R., Vroom, T. M. & Scholte, G. (1986) Bleeding/plaque ratio and the development of gingival inflammation. *Journal of Clinical Periodontology* **13**, 774–782.

Bartold, P. M., Kylstra, A. & Lawson, R. (1994) Substance P: an immunohistochemical and biochemical study in human gingival tissues. A role for neurogenic inflammation? *Journal of Periodontology* **65**, 1113–1121.

Borden, S. M., Golub, L. M. & Kleinberg, I. (1977) The effect of age and sex on the relationship between crevicular fluid flow and gingival inflammation in humans. *Journal of Periodontal Research* **12**, 160–165.

Bosman, C. W. & Powell, R. N. (1977) The reversal of localized experimental gingivitis. A comparison between mechanical toothbrushing procedures and a 0.2% chlorhexidine mouthrinse. *Journal of Clinical Periodontology* **4**, 161–172.

Breuer, M. M. & Cosgrove, R. S. (1989) The relationship between gingivitis and plaque levels. *Journal of Periodontology* **60**, 172–175.

Ciantar, M. & Caruana, D. J. (1998) Periotron 8,000: calibration characteristics and reliability. *Journal of Periodontal Research* **33**, 259–264.

Daly, C. G. & Highfield, J. E. (1996) Effect of localized experimental gingivitis on early supragingival plaque accumulation. *Journal of Clinical Periodontology* **23**, 160–164.

Danielsen, B., Wilton, J. M., Baelum, V., Johnson, N. W. & Fejerskov, O. (1993) Serum immunoglobulin G antibodies to *Porphyromonas gingivalis*, *Prevotella intermedia, Fusobacterium nucleatum* and *Streptococcus sanguis* during experimental gingivitis in young adults. *Oral Microbiology and Immunology* **8**, 154–160.

Decaris, E., Guingamp, C., Chat, M., Philippe, L., Grillasca, J. P., Abid, A., Minn, A., Gillet, P., Netter, P. & Terlain, B. (1999) Evidence for neurogenic transmission inducing degenerative cartilage damage distant from local inflammation. *Arthritis and Rheumatism* **42**, 1951–1960.

Deinzer, R., Forster, P., Fuck, L., Herforth, A., Stiller-Winkler, R. & Idel, H. (1999) Increase of crevicular interleukin 1beta under academic stress at experimental gingivitis sites and at sites of perfect oral hygiene. *Journal of Clinical Periodontology* **26**, 1–8.

Demissie, S., Cupples, L. A., Myers, R., Aliabadi, P., Levy, D. & Felson, D. T. (2002) Genome scan for quantity of hand osteoarthritis: the Framingham Study. *Arthritis & Rheumatism* **46**, 946–952.

Doherty, N. J. (1982) Economic costs of attrition in clinical trials. *Journal of Dental Research* **61**, 649–653.

Engelberger, T., Hefti, A., Kallenberger, A. & Rateitschak, K. H. (1983) Correlations among papilla bleeding index, other clinical indices and histologically determined inflammation of gingival papilla. *Journal of Clinical Periodontology* **10**, 579–589.

Fleiss, J. L. & Chilton, N. W. (1983) The measurement of interexaminer agreement on periodontal disease. *Journal of Periodontal Research* **18**, 601–606.

Fransson, C., Berglundh, T. & Lindhe, J. (1996) The effect of age on the development of gingivitis. Clinical, microbiological and histological findings. *Journal of Clinical Periodontology* **23**, 379–385.

Fransson, C., Mooney, J., Kinane, D. F. & Berglundh, T. (1999) Differences in the inflammatory response in young and old human subjects during the course of experimental gingivitis. *Journal of Clinical Periodontology* **26**, 453–460.

Furuichi, Y., Lindhe, J., Ramberg, P. & Volpe, A. R. (1992) Patterns of de novo plaque formation in the human dentition. *Journal of Clinical Periodontology* **19**, 423–433.

Gaumer, H. R., Holm-Pedersen, P. & Folke, L. E. (1976) Indirect blastogenesis of peripheral blood leukocytes in experimental gingivitis. *Infection and Immunity* **13**, 1347–1353.

Hammer, R. E., Maika, S. D., Richardson, J. A., Tang, J. P. & Taurog, J. D. (1990) Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. *Cell* **63**, 1099–1112.

Hanioka, T., Takaya, K., Matsumori, Y., Matsuse, R. & Shizukuishi, S. (2000) Relationship of the substance P to indicators of host response in human gingival crevicular fluid. *Journal of Clinical Periodontology* **27**, 262–266.

Henry, C. A., Ungchusri, T., Charbeneau, T. D. & Winford, T. E. (1987) Relationships of serum opsonins and complement in human experimental gingivitis. *Journal of Periodontology* **58**, 177–186.

Holm-Pedersen, P., Agerbaek, N. & Theilade, E. (1975) Experimental gingivitis in young and elderly individuals. *Journal of Clinical Periodontology* **2**, 14–24.

Lamster, I. B. (1997) Evaluation of components of gingival crevicular fluid as diagnostic tests. *Annals of Periodontology* **2**, 123–137.

Linden, G. J., McKinnell, J., Shaw, C. & Lundy, F. T. (1997) Substance P and neurokinin A in gingival crevicular fluid in periodontal health and disease. *Journal of Clinical Periodontology* **24**, 799–803.

Listwak, S., Barrientos, R. M., Koike, G., Ghosh, S., Gomez, M., Misiewicz, B. & Sternberg, E. M. (1999) Identification of a novel inflammation-protective locus in the Fischer rat. *Mammalian Genome* **10**, 362–365.

Löe, H., Anerud, A., Boysen, H. & Morrison, E. (1986) Natural history of periodontal disease in man. Rapid, moderate and no loss of attachment in Sri Lankan laborers 14 to 46 years of age. *Journal of Clinical Periodontology* **13**, 431–445.

Löe, H. & Silness, J. (1963) Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* **21**, 533–551.

Löe, H., Theilade, E. & Jensen, S. B. (1965) Experimental gingivitis in man. *Journal of Periodontology* **36**, 177–187.

Löe, H., Theilade, E., Jensen, S. B. & Schiott, C. R. (1967) Experimental gingivitis in man. 3. Influence of antibiotics on gingival plaque development. *Journal of Periodontal Research* **2**, 282–289.

Mariotti, A. (1999) Dental plaque-induced gingival diseases. *Annals of Periodontology* **4**, 7–19.

Matheny, J. L., Abrams, H., Johnson, D. T. & Roth, G. I. (1993) Microcirculatory dynamics in experimental human gingivitis. *Journal of Clinical Periodontology* **20**, 578–583.

Moore, W. E., Holdeman, L. V., Smibert, R. M., Good, I. J., Burmeister, J. A., Palcanis, K. G. & Ranney, R. R. (1982) Bacteriology of experimental gingivitis in young adult humans. *Infection and Immunity* **38**, 651–667.

Müller, H. P., Heinecke, A. & Eger, T. (2000) Site-specific association between supragingival plaque and bleeding upon probing in young adults. *Clinical Oral Investigations* **4**, 212–218.

Norman, M. E., Baehni, P. C., Tsai, C. C., Stoller, N., McArthur, W. P. & Taichman, N. S. (1979) Studies of host responses during experimental gingivitis in humans. II. Changes in acute phase reactants, serum immunoglobulins and complement during

the development of gingival inflammation. *Journal of Periodontal Research* **14**, 361–369.

Oliver, R. C., Brown, L. J. & Löe, H. (1998) Periodontal diseases in the United States population. *Journal of Periodontology* **69**, 269–278.

Oliver, R. C., Holm-Pedersen, P. & Löe, H. (1969) The correlation between clinical scoring exudate measurements and microscopic evaluation of inflammation in the gingiva. *Journal of Periodontal Research* **4**, 160.

Poulsen, S., Holm-Pedersen, P. & Kelstrup, J. (1979) Comparison of different measurements of development of plaque and gingivitis in man. *Scandinavian Journal of Dental Research* **87**, 178–183.

Preshaw, P. M., Knutsen, M. A. & Mariotti, A. (2001) Experimental gingivitis in women using oral contraceptives. *Journal of Dental Research* **80**, 2011–2015.

Putt, M. S., van der Weijden, G. A., Kleber, C. J. & Saxton, C. A. (1993) Validation of a 21-day, partial-mouth gingivitis model for evaluating chemotherapeutic dentifrices. *Journal of Periodontal Research* **28**, 301–307.

Ramberg, P., Furuichi, Y., Lindhe, J. & Gaffar, A. (1992) A model for studying the effects of mouthrinses on de novo plaque formation. *Journal of Clinical Periodontology* **19**, 509–520.

Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* **22**, 121–135.

Spolsky, V. W. & Gornbein, J. A. (1996) Comparing measures of reliability for indices of gingivitis and plaque. *Journal of Periodontology* **67**, 853–859.

Sternberg, E. M., Hill, J. M., Chrousos, G. P., Kamilaris, T., Listwak, S. J., Gold, P. W. & Wilder, R. L. (1989) Inflammatory mediator-induced hypothalamic–pituitary–adrenal axis activation is defective in streptococcal cell wall arthritis-susceptible Lewis rats. *Proceedings of the National Academy of Sciences of the United States of America* **86**, 2374–2378.

Stoller, N. H., Karras, D. C. & Johnson, L. R. (1990) Reliability of crevicular fluid measurements taken in the presence of supragingival plaque. *Journal of Periodontology* **61**, 670–673.

Tang, W., Arnett, D. K., Devereux, R. B., Atwood, L. D., Kitzman, D. W. & Rao, D. C. (2002) Linkage of left ventricular early diastolic peak filling velocity to chromosome 5 in hypertensive African Americans: the HyperGEN echocardiography study. *American Journal of Hypertension* **15**, 621–627.

Tatakis, D. N., Guglielmoni, P. & Fletcher, H. M. (2002) Accelerated alveolar bone loss in HLA-B27 transgenic rats: an adult onset condition. *Journal of Rheumatology* **29**, 1244–1251.

Tatakis, D. N. & Trombelli, L. (2004) Modulation of Clinical Expression of Plaque-Induced Gingivitis. I. Background review and rationale. *Journal of Clinical Periodontology* doi: 10.1111/j.1600-051X.2004.00477.x.

Terashima, T., Matsubara, H., Nakamura, M., Sakamaki, F., Waki, Y., Soejima, K., Tasaka, S., Nakamura, H., Sayama, K., Ishizaka, A. & Kanazawa, M. (1996) Local *Pseudomonas* instillation induces contralateral lung injury and plasma cytokines. *American Journal of Respiratory and Critical Care Medicine* **153**, 1600–1605.

Theilade, E., Wright, W. H., Jensen, S. B. & Löe, H. (1966) Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation. *Journal of Periodontal Research* **1**, 1–13.

Trombelli, L., Scapoli, C., Orlandini, E., Tosi, M., Bottega, S. & Tatakis, D. N. (2004) Modulation of clinical expression of plaque-induced gingivitis. IV. Response of ''high responders'' and ''low responders'' to therapy. *Journal of Clinical Periodontology* doi: 10.1111/j.1600-051X.2004.00479.x.

van der Velden, U., Winkel, E. G. & Abbas, F. (1985) Bleeding/plaque ratio. A possible prognostic indicator for periodontal breakdown. *Journal of Clinical Periodontology* **12**, 861–866.

van der Weijden, G. A., Timmerman, M. F., Danser, M. M., Nijboer, A., Saxton, C. A. & van der Velden, U. (1994a) Effect of pre-experimental maintenance care duration on the development of gingivitis in a partial mouth experimental gingivitis model. *Journal of Periodontal Research* **29**, 168–173.

van der Weijden, G. A., Timmerman, M. F., Nijboer, A., Reijerse, E. & van der Velden, U. (1994b) Comparison of different approaches to assess bleeding on probing as indicators of gingivitis. *Journal of Clinical Periodontology* **21**, 589–594.

van der Weijden, G. A., Timmerman, M. F., Saxton, C. A., Russell, J. I., Huntington, E. & van der Velden, U. (1994c) Intra-/inter-examiner reproducibility study of gingival bleeding. *Journal of Periodontal Research* **29**, 236–241.

Watts, T. L. (1978) Variability of gingival bleeding in experimental gingivitis trials. *Community dentistry and oral epidemiology* **6**, 253–255.

Wiedemann, W., Lahrsow, J. & Naujoks, R. (1979) The effect of periodontal resistance on experimental gingivitis. *Deutsche Zahnaerztliche Zeitschrift* **34**, 6–9.

Winkel, E. G., Abbas, F., van der Velden, U., Vroom, T. M., Scholte, G. & Hart, A. A. (1987) Experimental gingivitis in relation to age in individuals not susceptible to periodontal destruction. *Journal of Clinical Periodontology* **14**, 499–507.

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