Archives of Oral Biology 85 (2018) 51–57

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Research Paper

The effect of supragingival biofilm re-development on the subgingival microbiota in chronic periodontitis

Fátima Aparecida Rocha Resende Hartenbach[a,b], Carina Maciel Silva-Boghossian[a,b,c], Ana Paula Vieira Colombo[a,b,*]

[a] School of Dentistry, Department of Clinics, Rio de Janeiro, RJ, Brazil
[b] Institute of Microbiology, Department of Medical Microbiology, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[c] Department of Periodontics, and Postgraduate Program in Translational Biomedicine, University of Grande Rio, Duque de Caxias, RJ, Brazil

ARTICLE INFO

Keywords:
Dental plaque
Gingivitis
Chronic periodontitis
Oral hygiene
Periodontal microbiota
DNA probe

ABSTRACT

Objective: In this study, we hypothesized that in the absence of oral hygiene, re-growth of the climax microbial communities of supra and subgingival biofilm happens in a faster and more intense fashion in individuals with chronic periodontitis (CP) compared to periodontally healthy controls (PH).
Design: Thirty patients (PH = 15 and CP = 15) received professional supragingival prophylaxis, and were asked to refrain from oral hygiene for 7 days. Supra and subgingival biofilm samples and GCF were collected from randomly selected quadrants at baseline (before prophylaxis), immediately after prophylaxis, 2 h, 6 h, 24 h, and 7 days after prophylaxis. The composition of the biofilm was determined by the checkerboard method.
Results: All subjects developed gingivitis at the end of 7 days without oral hygiene. GCF mean volumes were significantly higher in CP than PH patients at baseline, but they started decreasing 2 h after prophylaxis, returning to baseline levels after 24 h in both groups. Significant increases in mean counts for most of the species evaluated were observed in both groups and biofilms over time ($p < 0.05$). Few hours after prophylaxis, a more marked reduction in microbial counts happened in the supragingival biofilm of the CP group, and re-development of biofilm started later than in the PH group. At 7 days, no differences were seen between groups. Significant differences in kinetics of re-colonization between groups were observed only in the subgingival biofilm for *T. denticola* and *F. nucleatum ss vicentii* (increased in the CP), and *N. mucosa* (increased in the PH group; $p < 0.05$).
Conclusions: Biofilm re-development was very similar between CP and PH individuals, although microbial re-growth occurred few hours earlier in PH than PC. Only 3 species in the subgingival biofilm differed in re-colonization between groups. Thus, we reject the hypothesis that re-colonization of biofilm in CP patients is more intense and faster than in individuals with PH.

1. Introduction

Dental plaque or biofilm is a sophisticated structure comprising a large variety of inter-related oral species that develops on the teeth surface. Depending on several local and/or systemic modulator factors, these structures may acquire pathogenic features such as a cariogenic or a periodontal pathogenic profile (Kolenbrander et al., 2006; Marsh, 2006; Socransky & Haffajee, 2002). Accumulation and persistence of a biofilm comprising high proportions of periodontal pathogens on teeth will lead to the development of gingivitis (Loe, Theilade, & Jensen, 1965), an inflammation of the marginal gingiva characterized by edema, redness and gingival bleeding (Armitage, 1999). Adequate biofilm removal results in resolution of inflammation and re-establishment of periodontal health (Chapple et al., 2015; Loe et al., 1965; Needleman, Suvan, Moles, & Pimlott, 2005). However, long-term biofilm accumulation combined to chronic inflammation may progress to periodontitis, an irreversible more severe periodontal inflammation that will lead to periodontal attachment loss and alveolar bone resorption (Marsh, 2006; Marsh & Devine, 2011; Page, Offenbacher, Schroeder, Seymour, & Kornman, 1997). The mechanisms involved in dental biofilm pathogenicity and induction of periodontal inflammation and destruction are complex and not fully understood. Studies on dental biofilm development and maturation have shown an orchestrated microbial colonization that is closely associated with specific micro-

* Corresponding author at: UFRJ/CCS – Instituto de Microbiologia Paulo de Góes – Bloco I, lab. I2-03; Av. Carlos Chagas Filho, 373 Cidade Universitária – Rio de Janeiro, RJ, CEP: 21941-902, Brazil.
E-mail address: apcolombo@micro.ufrj.br (A.P.V. Colombo).

http://dx.doi.org/10.1016/j.archoralbio.2017.10.007
Received 30 July 2017; Received in revised form 8 October 2017; Accepted 9 October 2017

environmental changes and host susceptibility (Marsh & Devine, 2011; Page et al., 1997). During biofilm formation, early colonizers adhesion provides substrates for subsequent colonizers to co-aggregate in this structure (Kolenbrander et al., 2006; Li et al., 2004; Marsh, 2006). Increase in biofilm microbial density will elicit a marginal gingival inflammation and a significant increase in gingival crevicular fluid (GCF) volume and flow (Goodson, 2003; Grant et al., 2010; Ngo et al., 2010). In turn, the elevated levels of inflammatory mediators and serum proteins in GCF will favor the overgrowth and establishment of more pathogenic species, including the orange and red complexes (Marsh & Devine, 2011; Socransky & Haffajee, 2002, 2005). Although the main stages of biofilm formation and gingival inflammation are observed in most people, the rate of microbial remodeling, host response and tissue destruction may vary (Marsh & Devine, 2011). For instance, in the experimental gingivitis model in humans (Loe et al., 1965), the onset of gingivitis varied among individuals, indicating that biofilm composition and host-related factors may determine biofilm pathogenicity and disease progression. In individuals with periodontitis, periodontal pockets constitute reservoirs of periodontal pathogens that may promptly colonize other sites of the oral cavity, including healthy sulci (Colombo et al., 2002; Riviere et al., 1996; Socransky & Haffajee, 2005). Thus, increased intraoral microbial transmission associated to a probable host immune susceptibility may explain in part the higher risk of periodontitis patients for further attachment loss compared to individuals with periodontal health. In fact, studies have indicated that differences in microbial colonization during biofilm formation between subjects with periodontitis and periodontal health do exist (Socransky & Haffajee, 2005; Teles et al., 2012; Uzel et al., 2011). Therefore, we hypothesized that biofilm re-development and composition, as well as the establishment of gingival inflammation occurs earlier and faster in individuals with chronic periodontitis (CP) compared to periodontally healthy (PH) individuals during withdraw of oral hygiene and supragingival plaque accumulation.

2. Material and methods

2.1. Sample population

The study protocol was approved by the Human Research Ethics Committee of the Hospital of the Federal University of Rio de Janeiro (UFRJ), Brazil (CEP approval n° 707.355). Participants were recruited from the Division of Graduate Periodontics of the School of Dentistry at UFRJ, between February and December of 2014. Patients were individually informed about the nature of the study, its risks and benefits, and signed an informed consent form. To participate in the study, subjects had to have at least 18 years of age and 16 teeth (at least 4 in each quadrant). Patients were diagnosed as having CP or PH according to Da Silva-Boghossian et al. (2011). Briefly, CP was defined as ≥10% of teeth with PD and/or CAL ≥5 mm or ≥15% of teeth with PD and/or CAL ≥4 mm and BOP, and PH was defined as < 10% of sites with BOP, no PD or CAL > 3 mm, although PD or CAL = 4 mm in up to 5% of the sites without BOP was allowed. Exclusion criteria included smoking, diagnosed inflammatory systemic diseases, autoimmune diseases, aggressive periodontitis, use of topical or systemic antimicrobials in the last 6 months, periodontal therapy in the last year, orthodontic treatment, antibiotic prophylaxis, pregnancy or nursing.

2.2. Sample size calculation

In a previous analysis of our microbial database, we computed the mean total counts of oral bacteria in 120 PH and 290 CP individuals. Considering total mean counts as the primary outcome variable, and assuming that CP would presented higher mean counts than PH patients, to detect a difference of 2.5×10^7 total mean counts between groups at 7 days with a one-sided significance level of 5% and power of 85% with equal allocation to two groups, a minimum sample size of 13 patients was required in each arm of the trial. To allow for 15% drop out, 15 patients were recruited in each clinical group. Secondary outcome variable were gingival bleeding (GI) and mean volume of GCF at day 7.

2.3. Clinical monitoring and biofilm development

At the screening visit, participants answered a questionnaire, and information about demographic features, medical and dental health history was obtained. For diagnosis of periodontal status, clinical measurements were performed by a single calibrated examiner (F.A.R.R.H) using a North Carolina probe (UNC-15, Hu-Friedy, Chicago, IL, USA). The intra-class correlation coefficients for probing depth (PD) and clinical attachment level (CAL) were 0.94 and 0.88, respectively. The periodontal parameters evaluated included PD and CAL (mm), and presence of supragingival plaque (PL), bleeding on probing (BOP), GI, suppuration (SUP) and calculus (CA), at 6 sites per tooth of all teeth, except third molars. Ninety-seven recruited patients who agreed to participate in the study (signed the consent form) were clinically examined. Of those, 67 did not meet the inclusion criteria. Therefore, 30 individuals, 15 with PH and 15 with CP entered and finished the study.

Patients selected for the study based on the inclusion/exclusion criteria and consent returned within one week at time -T1 (baseline) for sampling (described in Sections 2.5 and 2.6), and for full mouth measurements of PD, CAL, BOP, PL, GI, SUP and CA. Following baseline sampling and clinical evaluation (-T1), participants were submitted to supragingival ultrasonic and manual debridement, followed by dental polishing with rubber cup and prophylactic paste. At this moment, individuals were instructed to refrain brushing and flossing of their teeth for 7 days. GI was again measured at the seventh day of refraining oral hygiene procedures, after sampling (Fig. 1S). Patients were then referred to dental care in the different clinical specialties according to their treatment needs.

2.4. Supra and subgingival biofilm sampling

At baseline (-T1), 2 individual supragingival biofilm samples (from disto-buccal sites of one anterior and one posterior tooth) and 2 individual subgingival biofilm samples (from the same sites and teeth) were collected per quadrant, providing a total of 8 supra and 8 subgingival biofilm samples per patient. For biofilm re-development analysis, supra and subgingival samples were collected from the distal-buccal sites of all teeth of contralateral quadrants at T0 (immediately after prophylaxis) and T1 (2 h), T2 (6 h), T3 (24 h) and T4 (7 days) after professional prophylaxis and no oral hygiene (Fig. 1S). For instance, if quadrant 2 was drawn for sampling at T0/T1, all distal-buccal sites from anterior teeth and from posterior teeth would be pooled into 2 pools (1 anterior and 1 posterior) for supra and subgingival biofilm (total of 4 pooled samples). Then, at T2, the contralateral quadrant 4 would be sampled likewise. At T3, quadrant 1 or 3 would be drawn and sampled, followed by the contralateral quadrant at T4. Thus, at time from T0 to T4 a patient would provide 4 samples (2 supra and 2 subgingival) per quadrant. During sampling, the area was dried and isolated with cotton rolls. Supragingival samples were always collected first, followed by subgingival samples with sterile curettes (Hu-Friedy®). Samples were placed into microtubes containing 150 µl of TE buffer.

2.5. GCF sampling

Before removal of biofilm from distal-buccal sites, GCF was collected from the mesio-buccal sites of the same 8 teeth selected for biofilm sampling at baseline (-T1), following the same sequence of quadrants at T0 to T4. For post-prophylaxis sampling, GCF samples were obtained individually from the mesio-buccal sites of all teeth in that particular quadrant selected, and samples were not pooled. GCF samples were collected with paper strips (Periopaper, Oraflow Inc.,