TATIANA MEULMAN LEITE BORTOLACI

"ESTUDO DO IMPACTO DE MODALIDADES TERAPÊUTICAS PERIODONTAIS NOS PARÂMETROS CLÍNICOS E MICROBIOLÓGICOS EM PACIENTES FUMANTES. ESTUDO PROSPECTIVO EM HUMANOS."

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FACULDADE DE ODONTOLOGIA DE PIRACICABA

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TESE DE DOUTORADO APRESENTADA A FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNICAMP PARA OBTENÇÃO DO TÍTULO DE DOUTORA EM CLÍNICA ODONTOLÓGICA NA ÁREA DE PERIODONTIA

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e orientada pelo Prof. Dr. Francisco Humberto Nociti Junior

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Prof. Dr. MÁRCIO ZAFFALON CASATI

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"Feliz aquele que transfere o que sabe
e aprende oque ensina".
(Cora Coralina)

RESUMO

O objetivo do presente estudo foi avaliar a resposta do paciente fumante com doença periodontal inflamatória crônica (DPIC) aos desafios bacterianos do biofilme dental e às diferentes modalidades de tratamento periodontal. Foram avaliados o perfil microbiológico dos fumantes comparado com os não-fumantes e a resposta aos tratamentos de raspagem e alisamento radicular (RAR), terapia *gold standard*, e debridamento periodontal (DBR) que envolve o tratamento da DPIC em sessão única.

O capítulo 1 teve como objetivo avaliar as alterações microbiológicas subgengivais ocorridas nos pacientes fumantes com DPIC severa em compração aos pacientes não-fumantes com mesmo padrão de doença periodontal após a terapia periodontal supragengival (TS). Os pacientes não-fumantes (n= 10) e fumantes (n = 10) selecionados deveriam apresentar no mínimo 9 dentes com profundidade de sondagem (PPD) (≥ 5 mm), sangramento à sondagem (SS), sem história de tratamento periodontal nos últimos 6 meses. Os parâmetros clínicos avaliados foram índice de placa (IP), SS, PS, a posição margem gengival relativa (PMGr) e nível clínico de inserção relativo (NICr). O biofilme subgengival foi coletado antes e 21 dias após a TS. O DNA foi extraído e do gene 16S RNAr foi amplificado com os pares de *primers* universais 27F e 1492R. Os genes amplificados foram clonados, seqüenciados e identificados por comparação com sequências conhecidas de 16S RNAr. A análise estatística foi realizada pelo teste t de Student e qui-quadrado (α = 5%). Clinicamente, TS promoveu uma redução significativa na IP e PPD, e ganho de NICr para ambos os grupos, com nenhuma diferença significativa entre eles. Microbiologicamente, no início do estudo, análise de dados demonstrou que os fumantes abrigavam uma maior proporção de *P.endodontalis*, *Bacteroidetessp.*, *Fusobacteriumsp.* e *T.forsythia* e um menor número de filotipos cultiváveis (p <0,05). Além disso, os não-fumantes apresentaram reduções

significativas nos filotipos-chave associados coma DPIC, enquanto que os fumantes apresentaram mudanças mais modestas. Dentro dos limites do presente estudo, TS promoveu comparáveis melhorias clínicas em fumantes e não-fumantes com DPIC severa. No entanto, em fumantes, TS alterou de maneira mais discreta a biodiversidadedo biofilme subgengival em comparação aos não-fumantes.

O capítulo 2 avaliou a hipótese de que o DBR pode ser uma forma efetiva para o tratamento periodontal em pacientes fumantes, portadores de DPIC severa. Para a realização deste estudo prospectivo, paralelo e cego, foram selecionados 20 pacientes fumantes e 10 não-fumantes, que deveriam ter um mínimo de 20 dentes na boca e pelo menos 9 dentes com profundidade de sondagem (PS) ≥ 5 mm com sangramento à sondagem (SS). Os pacientes foram divididos em 3 grupos experimentais: G1: pacientes não-fumantes que receberam tratamento com RAR (n= 10) (RARC); G2: pacientes fumantes que receberam tratamento de RAR semanal por sextante (RARF) (n= 10); G3: pacientes fumantes que receberam tratamento de DBR, consistindo de 1 sessão de 45 minutos por meio de instrumentação ultra-sônica (DBRF) (n= 10). Os parâmetros clínicos avaliados foram: Índice de Placa (IP), SS, PS e Nível de Inserção Clínico (NIC) para boca toda e para os 9 sítios selecionados. Essas avaliações foram realizadas na 1ª consulta da terapia inicial (PI) (Baseline), 21 dias após baseline e 45 dias, 3 e 6 meses após os tratamentos. Os resultados obtidos foram comparados estatisticamente utilizando análise de variância com medidas repetidas (ANOVA) ao nível de significância de 5%. Dentro dos limites desse capítulo, foi possível concluir que a terapia de DB apresentou resultados similares a terapia de RAR para os pacientes fumantes. No entanto, os fumantes apresentaram resposta menos favorável evidenciando a importante influencianegativa do fumo na saúde periodontal.

ABSTRACT

The aim of these studies was to evaluate the responses of smokers with chronic inflammatory periodontitis (CIP) to the challenges of dental biofilme and differents modalities of periodontal therapy, including scaling and root planning (SRP), gold standard therapy, and one session periodontal debridement (DB).

Chapter 1: The aim of this study was to assess subgingival microbiological changes in smokers versus non-smokers presenting severe chronic periodontitis after supragingival periodontal therapy (ST). Non-smokers (n=10) and smokers (n=10) presenting at least nine teeth with probing pocket depth (PPD) (≥5mm), bleeding on probing (BoP), and no history of periodontal treatment in the last 6 months were selected. Clinical parameters assessed were plaque index (PI), BoP, PPD, relative gingival margin position (rGMP) and relative clinical attachment level (rCAL). Subgingival biofilm was collected before and 21 days after ST. DNA was extracted and the 16S rRNA gene was amplified with the universal *primer* pair 27F and 1492R. Amplified genes were cloned, sequenced, and identified by comparison with known 16S rRNA sequences. Statistical analysis was performed by Student's t and Chi-Square tests (α=5%).Clinically, ST promoted a significant reduction in PI and PPD, and gain of rCAL for both groups, with no significant intergroup difference. Microbiologically, at baseline, data analysis demonstrated that smokers harbored a higher proportion of *P. endodontalis*, *Bacteroidetes sp.,Fusobacterium sp*. and *T. forsythia* and a lower number of cultivated phylotypes ($p<0.05$). Furthermore, non-smokers featured significant reductions on key phylotypes associated with periodontitis, whereas smokers presented more modest changes.Within the limits of the present study, ST promoted comparable clinical improvements in smokers and non-smokers with severe chronic periodontitis. However,

in smokers, ST only slightly affected the subgingival biofilm biodiversity as compared to non-smokers.

Chapter 2: Tobacco smoking is probably the most important, controllable environmental risk factor in periodontitis. DB has been characterized as a therapeutic approach for chronic periodontitis. The present study aims to examine the effect of DB on the clinical parameters of smokers with severe chronic periodontitis.Twenty smokers and ten non-smokers patients presenting at least nine teeth with a probing pocket depth (PPD) of ≥ 5mm and bleeding on probing (BOP) were selected and randomly assigned to G1(n=10): non-smokers treated with quadrant-wise SRP, G2 (n=10): smokers treated quadrant-wise SRP and G3(n=10): smokers treated with one session of full-mouth DB. The following clinical outcomes were assessed: plaque index, BOP, position of gingival margin, clinical attachment level (CAL) and PPD. All the parameters were evaluated at baseline, 45 days, 3 and 6 months after treatment.Both groups had a significant reduction in clinical indices (VPI and BOP) after therapy. In relation to clinical parameters, the three groups showed reduction of PPD and gain of RAL at 6 months, restoring periodontal health, however non-smokers showed better outcomes when compared to smokers.In the limits of this study, we conclude that DB results in similar outcomes when compared to traditional SRP to smokers. However, smokers showed outcomes less favorable than non-smokers, highlightingthe important role of tobacco smoking in periodontal health.

SUMÁRIO

INTRODUÇÃO

O objetivo principal no tratamento de pacientes com periodontite é estabelecer e manter um controle adequado da infecção na área dento-gengival. A instrumentação por meio da RAR combinada com uma medida efetiva do controle de biofilme supragengival pelo paciente pode alterar a microbiota subgengival através da ruptura do biofilme e suprimir a inflamação (Wennström et al., 2005). Neste contexto, o biofilme dental tem papel fundamental e é considerado o fator etiológico primário da DPIC. Este atua por meio de mecanismos diretos, causando destruição tecidual pela liberação de enzimas líticas e produtos citotóxicos; e indiretos, desencadeando as reações de defesa do hospedeiro que podem resultar em destruição progressiva do periodonto (Page et al., 1997). Tendo em vista este conceito, a terapia periodontal visa a biocompatibilização da superfície radicular por meio da desorganização do biofilme dental e remoção do cálculo, possibilitando o restabelecimento da saúde periodontal por meio da adesão epitelial sobre a superfície radicular (Waerhaug, 1978).

Um fator local que está presente também no biofilme dental, que contribui para o desenvolvimento da DPIC e pode interferir com os resultados do tratamento é a presença dos periodontopatógenos,que colaboram para a classificação desse biofilme como fator etiológico primário das doenças periodontais (Bergstrom et al., 2006). Um dos principais objetivos da terapia periodontal é modificar a microbiota das bolsas periodontais que esta associada com a destruição progressiva dos tecidos periodontais de suporte. Estudos tem documentado que a terapia subgengival convencional é efetiva para a biocompatibilização do ambiente da bolsa periodontal e que a terapia supragengival tem papel importante no sucesso da terapia periodontal devido ao seu impacto na recolonização do biofilme subgengival (Hellströmet al., 1996; Westfelt et al., 1998; Ribeiro Edel P et al., 2005). Estudo recente sobre a influência da terapia supragengival na microbiota subgengival de pacientes fumantes demonstrou que houve uma

redução nos microrganismo do complexo vermelho nas bolsas periodontais após o controle supragengival do biofilme (Gomes et al., 2008). Além disso, Shchipkova et al.(2010) demonstraram em seu estudo que outros microrganismos não-cultivaveis presentes na bolsa periodontal podem ter papel importante na etiologia da DPIC.

Partindo desses conceitos, o primeiro capítulo dessa tese foi desenvolvido com o objetivo de determinar o impacto da terapia supragengival na biodiversidade do biofilme subgengival de pacientes fumantes e não-fumantes com DPIC não tratada por meio de análise microbiológica usando o método de clonagem de 16S rRNA não-cultivável dependente e sequenciamento molecular.

Em relação às modalidades de tratamento subgengivais, no protocolo convencional de RAR por sextantes ou quadrantes, em intervalos de 1 a 2 semanas, os resultados demonstram a possibilidade de controle da DPIC e manutenção da saúde periodontal em longo prazo (Kaldahl et al., 1993). Entretanto, alguns estudos têm sido realizados avaliando uma nova proposta de tratamento não cirúrgico para a DPIC que consiste de uma única sessão de 45 minutos de debridamento ultrassônico (DBR) (Koshy et al., 2005; Wennström et al., 2005; Zanatta et al., 2006; Del Peloso Ribeiro et al., 2008; Ioannou et al., 2009). Essa nova proposta baseia-se em estudos experimentais mostrando que as endotoxinas estão fracamente aderidas ao cemento radicular e que não penetram em seu interior levando a alteração do conceito original sobre a contaminação do cemento radicular (Hughes & Smales, 1986; Moore et al., 1986;Cadosh et al., 2003). Com isso, houve também alteração no objetivo da RAR que envolvia não somente a remoção do biofilme e cálculo, mas também o cemento ou dentina contaminados com o objetivo de tornar a superfície radicular biocompatível para a cicatrização dos tecidos moles periodontais (Hatfield & Baumhammers, 1971, Aleo et al., 1974).

A partir desse conceito, a remoção intencional de estrutura dental durante a instrumentação radicular pode não mais ser considerada um pré-requisito para a cicatrização dos tecidos periodontais (Nyman et al., 1986; 1988; Gonçalves et al. 2006). Consequentemente pode-se considerar a realização da instrumentação radicular com instrumentos que causam remoção mínima de estrutura dental, mas que são efetivos em desorganizar o biofilme dental e remover cálculo. Alguns estudos avaliaram a quantidade de remoção de estrutura radicular por meio de instrumentos manuais e ultrassônicos, demonstrando menor remoção de estrutura dental para o ultrassom (Ritz et al., 1991; Busslinger et al., 2001, Schmidlin et al., 2001). Casarin e colaboradores (2009) mostraram que os defeitos na superfície radicular após a instrumentação são semelhantes para instrumentos manuais e ultrassônicos. Além disso, outros estudos demonstraram que não há diferença na eficácia das técnicas de debridamento usando instrumentos manuais ou ultrassônicos em relação à redução de bolsa e ganho de inserção clínica (Tunkel et al., 2002; van der Weijden & Timmerman 2002; Hallmon & Rees 2003).

Esses conhecimentos deram suporte biológico para o desenvolvimento do protocolo de DBR com ultrassom para boca toda em sessão única com 45 minutos de duração (Koshy et al., 2005; Wennström et al., 2005; Zanatta et al., 2006). O DBR consiste em uma instrumentação subgengival mais conservadora que visa à desorganização do biofilme dental bacteriano e remoção das toxinas bacterianas aderidas superficialmente ao cemento radicular (Nyman et al., 1986,1988). Apatzidou & Kinane (2004) não observaram, após 6 meses, diferenças entre a instrumentação de boca toda feita em única sessão e a RAR feita por quadrantes com intervalos de 2 semanas entre as sessões. Zanatta e colaboradores (2006) por meio de um estudo clínico e bioquímico compararam as terapias de RAR, DBR com ultrassom e DBR com ultrassom + irrigação com iodo-povidine e não encontraram diferenças entre essas modalidades de tratamento

para periodontite crônica moderada após 3 meses. Del Peloso Ribeiro e colaboradores (2008) realizaram um estudo clínico, microbiológico e imunológico comparando o tratamento de periodontite crônica severa com RAR com ultrassom por quadrante com intervalos semanais entre as sessões ou com DBR com ultrassom em sessão única de 45 minutos. Os autores mostraram que o DBR apresentou resultados clínicos, microbiológicos e imunológicos semelhantes à terapia convencional de raspagem e alisamento radicular, sendo o debridamento periodontal, portanto uma alternativa válida para o tratamento de periodontite crônica severa.

Fatores locais e sistêmicos têm sido listados entre aqueles que podem modificar a resposta do hospedeiro e até mesmo a progressão da DPIC, a severidade e a resposta ao tratamento periodontal (Bergstron et al., 2000) e também pela persistência ou recolonização de microrganismos (Drisko, 1998). Dentre eles, o consumo de cigarros tem sido um dos fatores mais investigados e é reconhecido como fator de risco local mais importante para a doença periodontal (Wendell et al., 2001). Em geral tem sido demonstrado que a perda óssea alveolar, mobilidade, aumento da profundidade de sondagem e perda dentária são mais severos em fumantes do que não-fumantes (Stoltenberg et al., 1993) e ainda que os pacientes fumantes apresentam uma resposta menos previsível às formas cirúrgica e não-cirúrgica da terapia periodontal (Preber & Bergstron, 1985; Kaldahl et al., 1996; Heasman et al., 2006). Pelos motivos listados acima o estudo do impacto de modalidades terapêuticas em pacientes fumantes é de relevância para a área e se constitui num significativo desafio. Até o momento, não há estudos que tenham avaliado sistematicamente e de forma controlada o efeito do DBR como forma de tratamento da DPIC em fumantes, portanto, o objetivo do segundo capítulo do trabalho foi avaliar clinicamente a hipótese de que o DBR pode ser uma forma efetiva para o tratamento periodontal em pacientes fumantes, portadores de periodontite crônica moderada.

CAPÍTULO 1: Artigo publicado pela Journal Oral Microbiology

Impact of supragingival therapy on subgingival microbial profile in smokers versus non-smokers with severe chronic periodontitis.

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Abstract

Background: The aim of this study was to assess subgingival microbiological changes in smokers versus non-smokers presenting severe chronic periodontitis after supragingival periodontal therapy (ST).

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Methods: Non-smokers (n=10) and smokers (n=10) presenting at least nine teeth with probing pocket depth (PPD) (≥5mm), bleeding on probing (BoP), and no history of periodontal treatment in the last 6 months were selected. Clinical parameters assessed were plaque index (PI), BoP, PPD, relative gingival margin position (rGMP) and relative clinical attachment level (rCAL). Subgingival biofilm was collected before and 21 days after ST. DNA was extracted and the 16S rRNA gene was amplified with the universal primer pair 27F and 1492R. Amplified genes were cloned, sequenced, and identified by comparison with known 16S rRNA sequences. Statistical analysis was performed by Student's t and Chi-Square tests (α=5%).

Results: Clinically, ST promoted a significant reduction in PI and PPD, and gain of rCAL for both groups, with no significant intergroup difference. Microbiologically, at baseline, data analysis demonstrated that smokers harbored a higher proportion of *P. endodontalis*, *Bacteroidetes sp.,Fusobacterium sp*. and *T. forsythia* and a lower number of cultivated phylotypes ($p<0.05$). Furthermore, non-smokers featured significant reductions on key phylotypes associated with periodontitis, whereas smokers presented more modest changes.

Conclusion: Within the limits of the present study, ST promoted comparable clinical improvements in smokers and non-smokers with severe chronic periodontitis. However, in smokers, ST only slightly affected the subgingival biofilm biodiversity as compared to non-smokers.

Keywords: Smoke habit, periodontitis, supragingival therapy, biodiversity, clonal analysis.

Introduction

Although smoking habit is a recognized risk factor for periodontitis (6) leading to an increase in periodontal tissues destruction due to altered production of MMP, interleukins and inflammatory markers release and host-cell function (7,10,11,18,43,54), biofilm still remains as

the primary etiologic factor for the development of a destructive periodontal disease (6). Thus, the primary goal of periodontal therapy is to target the subgingival biofilm present in periodontally diseased sites that is associated with the progressive destruction of the supportive periodontal tissues. It is well documented that conventional therapy, i.e., subgingival scaling and root planning, is effective in the achievement of this goal. Supragingival periodontal therapy (ST) has been shown to play a critical role for the success of periodontal therapy by its impact on the subgingival biofilm avoiding re-colonization to occur (25,42,55). However, conflicting results of the impact of supragingival dental biofilm control on the composition of established subgingival biofilm in untreated periodontal sites are found in the literature (2,5,28,29,33).

Smoking has been implicated as a factor that reduces the effectiveness of treatment. Smokers show less favorable responses to various kinds of periodontal treatments such as non-surgical, surgical, regeneration procedures, and mucogingival surgery (3,12,14,48,51,56). The mechanisms by which smoking affects the response to periodontal treatment might be related to the altered inflammatory and immune response that has been observed in smokers and/or to the persistence of pathogenic flora in smokers after treatment (7,8,17,40,50). Studies have, then, aimed to document a possible role of smoking on oral microbiota, and although no conclusive findings have been reported, some data have demonstrated that there are important differences in the composition of subgingival biofilm between smokers and non-smokers with chronic periodontal disease; which may, in fact, account for the lower response of smokers to therapy (19,35,44).

With the concepts discussed above in mind, there is an interest on the possible effect of supragingival biofilm control on the subgingival environment in untreated periodontitis sites in smokers. In smokers, in the only study available, supragingival periodontal therapy has been

shown to affect the total bacterial load in the subgingival biofilm with a tendency, not significant, of lower amounts of the red complex bacteria (*Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Tannerella forsythia)* in the treated sites (20). However, in a recent and pioneer study in the field, Shchipkova et al. (47) demonstrated that not only red complex bacteria, but several other uncultivated phylotypes, including "unsuspicious" species in the subgingival biofilm, may play a role in the disease etiology, remarking the necessity of expanding the analysis of the periodontal microbiome to open-ended techniques. In this sense, in the present study, it was hypothesized that ST, consisting of supragingival biofilm/calculus removal and dental surface polishing, removal of biofilm retainers, oral hygiene instruction and reinforcement after 7 days, would affect subgingival biofilm biodiversity in sites with untreated severe chronic periodontitis in smokers. Microbiological analysis were performed using the non-culture-dependent 16srRNA cloning and sequencing molecular approach, whereas clinically, smokers and non-smokers were followed with respect to the impact of the supragingival therapy on the tissue stability.

Materials and Method

Ethics

This study was designed as a parallel, single-arm and controlled study to evaluate the clinical and microbiological effects of supragingival therapy (ST) on the biodiversity of subgingival biofilm collected smokers and non-smokers with chronic periodontitis. Subjects included in the present study were examined, treated by ST and re-evaluated 21 days later. The study was IRB approved and patients received a detailed description of the proposed treatment and gave their informed and written consent.

Patient selection and groups

Potential patients were selected from those referred to the Graduate Clinic of Piracicaba Dental School, University of Campinas - UNICAMP, Brazil. All patients received a complete periodontal examination, including a full-mouth periodontal probing, radiographic examination and complete anamnesis.

The study inclusion criteria were

(i) diagnosis of Chronic Periodontitis, according to the criteria of the 1999 International classification (4).

(ii) presence of at least 20 teeth;

(iii) at least 9 teeth presenting probing pocket depth (PPD) ≥ 5 mm with bleeding on probing (being at least 2 with PPD ≥ 7 mm);

(iv) > 35 years of age.

Patients who (i) were pregnant or lactating; (ii) required antimicrobial pre-medication for the performance of periodontal examination and treatment; (iii) were suffering from any other systemic diseases (cardiovascular, pulmonary, liver, cerebral diseases or diabetes); (iv) had received antimicrobial treatment in the previous 3 months; (v) were taking long-term anti-inflammatory drugs; and/or (vi) had received a course of periodontal treatment within the last 6 months; were excluded from the study. It was designed a parallel, non-blinded and prospective study enrolling the following two groups: *Group Non-Smokers (n=10):* patients diagnosed as generalized and severe chronic periodontitis and that had never been smokers; and *Group Smokers (n=10):* patients diagnosed as generalized and severe chronic periodontitis and that have had the smoke habit for at least 10 years and smoke at least 20 cigarettes per day. Sample

size was determined by Bioestat program, using a standard deviation of 1.0, a power value of 80% to detect a difference between groups of 1.0 mm.

Supragingival therapy (ST)

After full mouth examination and consent in participation, patients of both groups received a full mouth prophylaxis, supragingival calculus and biofilm removal, using Gracey curettes, ultrasonic scaler, bicarbonate spray and floss. Extraction of condemned teeth and biofilm retentive factors removal also were performed. Moreover, the patients were individually instructed on how to perform oral self-care, including Bass technique, inter-dental flossing, and tongue brushing. All the subjects of the study received a standard fluoride dentifrice, toothbrushes and floss as necessary; and were asked to perform complete oral self-care hygiene at least twice a day. A week after this first instruction session, patients returned to a reinforcement of oral self-care instructions. Twenty-one days after ST, clinical re-evaluation was performed and subgingival samples were collected for microbiological analysis. The same individual (DP) was trained to perform ST in all the patients, whereas another individual was trained to perform baseline and post-therapy clinical assessments (TM).

Clinical parameters

The following clinical parameters were assessed immediately before therapy: full-mouth plaque index (FMPI), according to Ainamo and Bay (1), and full-mouth bleeding score (FMBS), according to Muhlemann and Son (34); these were calculated after assessing dichotomously the presence of dental biofilm at the site or bleeding on probing from the bottom of the pocket when probing with a manual probe[K]. The percentage of total sites that revealed the presence of plaque or bleeding was calculated. All teeth presenting at least one site with PPD $\geq$ 5 mm were selected

[K]PCP-15, Hu-Friedy, Chicago, IL, USA.

for clinical evaluation. The sites to be analyzed were standardized using an acrylic stent individually manufactured in which a groove was made to determine the site. For this accomplishment, one site from each tooth was selected for accomplishment during the period of the study. It results in a mean of 10.0±1.8 sites accomplished per patient. For the selected sites, clinical parameters were:

- Probing Pocket Depth (PPD – Distance from the bottom of pocket to margin);
- Relative Clinical Attachment Level (rCAL – distance from the bottom of the pocket to the stent margin);
- Relative Gingival Margin Position (rGMP – distance from the gingival margin to stent margin).

All parameters were evaluated using a periodontal probe at baseline and 21 days after ST. The examinations employed a calibrated examiner (TM). For this calibration, 3 patients were selected and full mouth rCAL and PPD were measured, twice, within 24 hours, with at least 1 hour between the examinations. The intra-class correlation was calculated for each parameter, resulting in 93.5% reproducibility for rCAL and 94.3% for PPD.

Subgingival biofilm analysis

Subgingival biofilm collection

After a full-mouth examination, all sites previously selected to clinical accomplishment were included to subgingival biofilm analysis, as described. Following the careful removal of supragingival biofilm, the areas were washed with a water spray, isolated with cotton rolls and

gently dried. A sterile paper point[¶] was inserted into the bottom of the periodontal pocket for 30 s. The paper points were placed into sterile tubes containing 300ul of reduced transport fluid. Each paper point was placed separately in plastic tubes containing 0.01M Tris Edta solution, pH 8 (TE). DNA collection and extraction was done as previously described by Casarin et al (9). For each patient, samples from the selected sites were pooled together to allow the 16S cloning sequencing.

Cloning and Sequencing

Firstly, the 16S rRNA gene was amplified using universal primer set (27f and 1492r) as described in de Lillo et al (15). Cloning procedures were performed using TOPO-TA cloning kit[#]. Initially, the amplicons resulted from universal amplification were cloned into *E. coli* and then cultured in Luria Bertani plates and after Luria Bertani broth media[**]. After vectors extraction, the product were purified[††] and sequenced[‡‡].After sequencing, a partial sequence of 600 bp was generated. They were initially aligned and a similarity matrix was constructed from the alignments by the method of Jukes and Cantor (27) (Bioedit 7.0 Program (24)). Phylogenetic trees were constructed by the neighbor joining method (Dotur Program (46)). Sequences were compared using HOMD database (13) using a level of 98.5% sequence identity as cut-off. Sequences presenting 98% or greater similarity within a genus were considered the same species.

Data management and statistical analysis

Clinical parameters were analyzed by Student's t test (for baseline intergroup comparisons) and repeated-measures ANOVA/Tukey (for clinical changes occurring after ST),

[¶] #35, Tanari, Manaus, AM, BR
[#]Invitrogen, San Diego, CA, USA
[**]LB-Top Agar, Sigma-Aldrich, Buchs, Switzerland
[††]QIAprep miniprep Spin®, Qiagen, Quebec, QC, Canada
[‡‡]CHUQ, Centre Hospitalier Universitaire de Québec, Université Laval, Québec, QC, Canada

using PROC GLM procedure of SAS program[§§]. For microbiological data, a variance-stabilizing transformation described in Shchipkova et al. (47), was used, promoting a normal distribution of the data. After data transformation, Student's t test was used to data analysis. For distribution and frequency analysis, Chi-Square test was employed. A 5% of significance was considered for clinical and microbiological data analysis.

Results

Demographical and clinical data

Demographic characteristics and baseline comparisons between groups are displayed in Table 1. There were no statistical differences between groups regarding age or gender, as well as in relation to full mouth clinical parameters: plaque, bleeding on probing, periodontal probing depth and clinical attachment level ($p>0.05$). Table 2 illustrates the effect of ST on the clinical parameters assessed in the selected sites (PPD $\geq$ 5 mm). At baseline, intergroup analysis (i.e., non-smokers versus smokers) showed no significant differences for any of the assessed parameters ($p>0.05$). In contrast, intragroup analysis (i.e., baseline versus after ST, within the same experimental group) demonstrated that ST led to a significant reduction in PI, PPD and rCAL ($p<0.05$); whereas no significant changes were found with respect to BoP and GMP, in smokers and non-smokers ($p>0.05$).

Microbiological data

A total of 45 clones per patient were analyzed in each period of evaluation resulting in a total sequenced clone of 1800. In non-smokers subjects, it were identified 78 different species at baseline and 73 after supragingival plaque control, while in smokers, a total of species identified at baseline was 71 and after ST, 70 phylotypes. At Table 3, are shown the distribution of clones

[§§]SAS Institute Inc. release 9.02, Cary, NC, USA

in Phylo and cultivation status (cultivated and not-yet-cultivated). No difference in Phylo distribution was observed between smokers and non-smokers, neither at baseline nor after plaque control. Moreover, no impact of the ST was observed in both groups, with no difference between periods of evaluation. In regards of cultivation status, non-smokers subjects presented a higher prevalence of cultivated taxas compared to smokers at baseline as well as after ST ($p<0.05$). Additionally, 21 days after ST, an increase in cultivated phylotypes was seen only in non-smokers ($p<0.05$).Figure 1 illustrates the distribution of 16S clonal analysis by genera in non-smkers and smokers, before and after ST, with regard to their percent of total clones. As noted, differences were observed at baseline, indicating dissimilarity between subgingival biofilm composition of smokers and non-smokers. Smokers presented a higher percentage of *Fusobacterium* and *Bacteroides* genera while non-smokers presented higher values of *Eubacterium, Synergistetes* and *Streptococcus* (Chi-Square test, $p<0.05$). At figure 1 it also could be seen the microbiological effect of strict supragingival plaque control on genus distribution. In non-smokers, a significant reduction in *Eubacterium, Filifactor, Tannerella, Treponema* and *Fusobacterium* genus was observed, whereas only *Filifactor* and *Fusobacterium* were reduced in smokers after ST ($p<0.05$). After the period of supragingival plaque control, some differences between smokers and non-smokers were still observed, being *Streptococcus* genera more present in non-smokers ($p<0.05$) and *Bacteroides* and *Porphyromonas* genus in smokers ($p<0.05$). The 20 most detected phylotypes were separately analyzed regarding its frequency and proportion in the subgingival biofilm (Table 4). Subgingival biofilm showed distinct patterns between smokers and non-smokers. Although *Filifactor alocis, Tannerella forsythia* and *Porphyromonas gingivalis* were the most prevalent species found in both groups at baseline ($p>0.05$), smokers presented higher levels of *Fusobacterium nucleatum ss. vincentii* and

Bacteroidetes [G-2] sp. | *Oral Taxon 274* | *Clone AU126 (p<0.05),* whereas non-smokers showed higher levels of *Sphingomonas sp. Oral Taxon 006* | *Clone FI012, Streptococcus constellatus* and *Eubacterium [11][G-6] nodatum Oral Taxon 694*. ST led to a more expressive alteration in subgingival composition in non-smokers. Twenty-one days after ST, reduced levels of *Filifactor alocis, Tannerella forsythia, Eubacterium [XI][G-5] saphenum* | *Oral Taxon 759, Porphyromonas endodontalis Oral Clone P2PB_52* and *Eubacterium [XI][G-3] brachy* were found for non-smokers ($p<0.05$), whereas only *Tannerella forsythia* presented reduced levels in smokers after ST as compared to baseline. Interestingly, a significant increase in *Synergistetes [G-3] sp. Oral Clone BH017* and *Porphyromonas endodontalis Oral Clone AJ002* was observed in smokers after ST ($p<0.05$), highlighting the dissimilarities between smokers and non-smokers with regards to their microbial profile. In addition, post ST data analysis showed that some phylotypes were found to be in higher levels in smokers, including *Bacteroidetes [G-2] sp.* | *Oral Taxon 274* | *Clone AU126* (which could be seen in higher amounts at baseline in smokers), *Porphyromonas endodontalis Oral Clones P2PB_52* and *AJ002* and *Synergistetes [G-3] sp. Oral Clone BH017* ($p<0.05$).

Discussion

The smoking habit has been reported to negatively affect periodontal tissues, cell defense and host response, as well as microbiological evaluations indicate a possible influence also on subgingival microflora. However, very limited information is available with respect to the effect of supragingival therapy (ST) on the biodiversity of the subgingival biofilm in smokers with chronic periodontitis. With this in mind, here, it was hypothesized that ST, consisting of standard methods used for supragingival biofilm removal and control, including supragingival biofilm/calculus removal and dental surface polishing, removal of biofilm retainers, oral hygiene

instruction and reinforcement after 7 days, would affect subgingival biofilm profile in smokers with severe chronic periodontitis. Subgingival biofilm biodiversity was assessed by 16S gene cloning technique and clinical parameters were additionally used to illustrate clinical conditions at baseline and after ST in smokers and non-smokers. It is relevant at state that after the ST had been performed and the baseline and follow-up parameters assessed, all the patients enrolled in this study were then treated by the conventional subgingival scaling and root planing approach. Data analysis demonstrated that i) there were significant differences in the subgingival biofilm composition, at baseline, in smokers versus non-smokers, ii) subgingival biodiversity was significantly affected by ST in non-smokers, whereas only a slight effect was observed for smokers, and iii) clinical response was not affected by dissimilar microbiological outcomes to ST in smokers versus non-smokers.

With non-surgical subgingival therapy as the main treatment modality, most authors report greater reductions in probing depth in non-smokers compared with smokers (22,26,37-39,41). It is, therefore, important to note that although non-smokers universally respond better to periodontal treatment than do smokers, there is substantial evidence of clinical improvement in smokers after treatment, indicating that smoking, as a risk factor, will compromise rather than prevent tissue healing. In non-smokers, supragingival biofilm control results in a reduction of gingival bleeding, probing depth and a gain in attachment level, especially in shallow and moderate pockets in sites not treated by subgingival scaling and root planning (2,25,33,42); although some studies also shown a benefit in deep pockets (41). In smokers, however, there is a lack of information on the impact of ST on the periodontal tissues in untreated sites. Gomes et al, in 2007 (21), were the only group to assess the changes in the periodontal tissues after a supragingival biofilm control, consisting of supragingival calculus removal with hand curettes,

extractions, endodontic treatment, placement of temporary restorations and prostheses in smokers. Similarly to the findings of the present study, that only assessed sites with severe chronic periodontal disease, Gomes et al (21) demonstrated that a comparable clinical outcome was reached for smokers and non-smokers regardless the severity of the disease, featuring reduction in plaque and bleeding indices as well as reduction in probing depth and gain in clinical attachment level after 30 days of ST, that was maintained up to 180 days. Additionally, as previously reported by others, in the present study data analysis demonstrated that smokers presented a lower percentage of plaque index reduction than non-smokers (20% versus 30% for smokers and non-smokers, respectively), but both improved from baseline. In addition, as expected for deep pockets, in the present study ST promoted only a mild effect on bleeding on probing for both groups. In summary, clinical data available suggest that smokers and non-smokers with severe chronic periodontitis may similarly benefit from supragingival biofilm control.

Next, in the present study, it was aimed to determine whether or not ST affected subgingival biofilm biodiversity in both groups, towards a more proper environmental condition for appropriate tissue response to occur, and therefore, accounting for the findings of clinical improvements in smokers and non-smokers. Often, studies have focused on assessing, in smoking and non-smoking conditions, the effect of periodontal therapy on genus associated with periodontal disease, including *P. gingivalis*, *T. denticola*, and *T. forsythia* (5,20,23,25,28,49,52,53). However, an important study demonstrated that tobacco might also affect the levels of genus not always associated with periodontal disease (47). In the present study, using an open-ended approach, comparison between subgingival biofilm composition of smokers and non-smokers, at baseline (before ST), revealed a higher presence of certain genus,

such as *Fusobacterium* and *Bacteroides* (genus associated to periodontal disease); and lower levels of *Streptococcus, Synergistetes and Eubacterium*. These findings seem to confirm that tobacco exposure may lead to a subgingival biofilm composed not only in a higher proportion by pathogens associated to periodontal disease, but also some species, not commonly included as periodontal pathogens in target-ended or selective techniques; including *Filifactor alocis* that has been suggested as a potential marker for active disease (31,45). However, more than presenting a reduction in "health-associated" and increase in "disease-associated" biofilm, in the present study, smokers showed a lower response to ST compared to non-smokers subjects regarding the microbiological composition of the subgingival biofilm. Non-smokers had a significant reduction in 5 species commonly associated to periodontal disease following ST, whereas in smokers only the levels of *Filifactor alocis* were significantly reduced. Additionally, after ST in smokers, disease-associated species, including *P. endodontalis,* were found to be increased. *P. endodontali*s has been listed as one of the potential "new species" associated with periodontal disease (30), and the fact that *P. endodontali*s has additionally been shown to be reduced in subgingival biofilm when the smoking habit is quit (16), suggests this species as an important factor that might contribute for the pathogenesis of periodontal disease in smokers. Previous studies investigated the effect of active periodontal therapy, including subgingival scaling and root planning, on the subgingival microflora. Although using a different therapeutic approach and traditional methods for bacterial identification (for example, culturing or targeted DNA-based assays [PCR, real-time PCR, DNA-DNA hybridization]), a number of these studies have also suggested that smoking will increase the likelihood that patients will remain positive for periodontitis-associated species, including *P. gingivalis*, *T. denticola*, *T. forsythia, P. intermedia, F. nucleatum and P. micros* (23,49,52,53).

In the present study, the reduction in some species in the subgingival biofilm after ST may be explained by the intimate relationship between both, supra and subgingival environments (32), and also as a result of the mild, but statistically significant, clinical benefits promoted by ST after 21 days, as probing depth reduction, which may have led to significant changes in nutrients, oxygen and microorganisms disposable in periodontal pockets. In conclusion, in smokers, ST only slightly affected the subgingival biofilm biodiversity as compared to non-smokers, although clinically, the indication is that the response in smokers and non-smokers will be similar regardless the differential impact of ST on the subgingival biofilm composition on those groups of patients.

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Table 1.Clinical and Demographical characteristics of participants at baseline (mean ± standard deviation).

Characteristics	Non-smokers (n=10)	Smokers (n=10)
Age (mean/range)	45.6 ± 4.8	43.4 ± 7.4
% males	50%	55.6%
FMPI (%)	79.7% ± 0.1	73.0% ± 0.2
FMBOP (%)	76.8%± 0.1	78.0% ± 0.2
FMPPD (mm)	3.2 ± 0.5	3.5 ± 0.6
FMCAL (mm)	3.9 ± 0.8	4.3 ± 0.8
Tobacco exposure (number of cigarettes/day)	0 ± 0	20.8 ± 3.5*

* Indicates intergroup difference by Student's t test, $p<0.05$.

Table 2.Clinical parameters in Smokers and Non-smokers, at baseline and after supragingival therapy (ST), at the selected sites.

	Non-smokers		Smokers	
	Baseline	**After ST**	**Baseline**	**After ST**
PI (%)	89.2±0.1	63.0±0.2*	89.2±0.1	68.3±0.2*
BoP (%)	96.0±0.1	91.1±0.1	91.7±0.1	84.2±0.1
PPD (mm)	4.9±0.2	4.3±0.6$^{\#}$	5.0±0.8	4.4±0.6$^{\#}$
CALr (mm)	8.6±1.1	8.3±1.1*	9.1±1.0	8.7±0.9*
GMP (mm)	3.7±1.1	4.0±1.1	4.1±0.6	4.3±0.5

*Statistical difference between baseline and ST within groups (repeated measures ANOVA followed by Tukey test, *$p<0.05$, $^{\#}$ $p<0.01$).*

Table 3. Distribution by Phylo (total number of clones) and culture condition (% of cultivated and not-yet-cultivated phylotypes) in smokers and non-smokers, at baseline and after supragingival therapy (ST).

	Non-smokers		Smokers	
Phylo	**Baseline**	**After ST**	**Baseline**	**After ST**
Actinobacteria	3	4	1	3
Bacteroidetes	13	14	16	21
Firmicutes	27	24	22	19
Fusobacteria	6	4	11	6
Proteobacteria	8	12	6	5
Spirochaetes	11	6	8	9
Synergistetes	10	9	7	7
Total number of clones	**78**	**73**	**71**	**70**
% of Cultivated *†	61.5	77#	36	40
% of Not yet cultivated	38.5	23	64	60

** Indicates intergroup difference at baseline; † indicates intergroup difference after ST; # indicates intragroup difference (Chi-Square test, $p<0.05$).*

Table 4. TOP 20 most detected Phylotypes values (x value mean, median and range) in Smokers and Non-smokers, at baseline and after Supragingival Therapy.

Phylotype	Non-smokers				Smokers			
	Baseline		After ST		Baseline		After ST	
	Mean	Median (range)	Mean	Median (range)	Mean	Median (range)	Mean	Median (range)
Filifactor alocis	0.14	0.13 (0.1-0.2)	0.06#	0.08 (0-0.1)	0.11	0.14 (0-0.2)	0.05#	0.06 (0-0.1)
Tannerella forsythia	0.09	0.10 (0-0.2)	0.03#	0.00 (0-0.1)	0.09	0.10 (0-0.2)	0.06	0.06 (0-0.2)
Porphyromonas gingivalis	0.10	0.09 (0-0.2)	0.07	0.06 (0-0.2)	0.06	0.03 (0-0.2)	0.04	0.06 (0-0.1)
Eubacterium [XI][G-5] saphenum | Oral Taxon 759	0.09	0.08 (0-0.2)	0.01#	0.00 (0-0.1)	0.07	0.09 (0-0.2)	0.05	0.03 (0-0.2)
*Fusobacterium nucleatum ss. vincentii **	0.01	0.00 (0-0.1)	0.00	0.00 (0-0)	0.05	0.03 (0-0.1)	0.01	0.00 (0-0.1)
*Bacteroidetes [G-2] sp. | Oral Taxon 274 | Clone AU126 *†*	0.00	0.00 (0-0)	0.00	0.00 (0-0)	0.03	0.0 (0-0.1)	0.04	0.00 (0-0.2)
Parvimonas micra	0.02	0.00 (0-0.1)	0.02	0.00 (0-0.1)	0.03	0.0 (0-0.2)	0.02	0.00 (0-0.1)
Porphyromonas endodontalis Oral Clone BB134	0.02	0.00 (0-0.1)	0.02	0.00 (0-0.1)	0.03	0.0 (0-0.1)	0.04	0.03 (0-0.1)
Porphyromonas endodontalis Oral Clone P2PB_52†	0.02	0.00 (0-0.1)	0.00#	0.00 (0-0.1)	0.03	0.0 (0-0.1)	0.03	0.00 (0-0.1)
Fusobacterium sp. Oral Clone FL002	0.01	0.00 (0-0.1)	0.00	0.00 (0-0)	0.03	0.0 (0-0.1)	0.01	0.00 (0-0.1)
Eubacterium [XI][G-3] brachy	0.03	0.03 (0-0.1)	0.01#	0.00 (0-0.1)	0.03	0.0 (0-0.1)	0.01	0.00 (0-0.1)
Peptostreptococcus stomatis	0.00	0.00 (0-0)	0.00	0.00 (0-0)	0.02	0.0 (0-0.1)	0.01	0.00 (0-0.1)
Synergistetes [G-3] sp. Oral Clone BH017†	0.04	0.06 (0-0.1)	0.02	0.00 (0-0.1)	0.03	0.0 (0-0.1)	0.06#	0.06 (0-0.1)
Porphyromonas endodontalis Oral Clone AJ002†	0.02	0.00 (0-0.1)	0.02	0.00 (0-0.1)	0.02	0.0 (0-0.1)	0.08#	0.08 (0-0.2)
Treponema sp. Oral Clone P4GB_42	0.00	0.00 (0-0)	0.00	0.00 (0-0)	0.01	0.0 (0-0.1)	0.01	0.00 (0-0.1)
*Sphingomonas sp. Oral Taxon 006 | Clone FI012 **	0.03	0.00 (0-0.1)	0.01	0.00 (0-0)	0.00	0.0 (0-0)	0.00	0.00 (0-0)
*Streptococcus constellatus **	0.03	0.00 (0-0.1)	0.01	0.00 (0.0.1)	0.00	0.0 (0-0)	0.01	0.00 (0-0.1)
*Eubacterium [11][G-6] nodatum Oral Taxon 694 **	0.06	0.06 (0-0.1)	0.05	0.00 (0-0.1)	0.01	0.0 (0-0.1)	0.01	0.00 (0-0.1)
Fusobacterium naviforme	0.01	0.00 (0-0.1)	0.01	0.00 (0-0.1)	0.01	0.0 (0-0.1)	0.02	0.00 (0-0.1)
Treponema medium	0.02	0.00 (0-0.1)	0.01	0.00 (0-0.1)	0.01	0.0 (0-0.1)	0.01	0.00 (0-0.1)

**indicates statistical difference between Non-Smokers and Smokers at baseline; † indicates statistical difference between Non-Smokers and Smokers after ST; # indicates statistical difference between Baseline and After ST within group (ANOVA followed by Tukey test, $p<0.05$). ST: Supragingival therapy.*

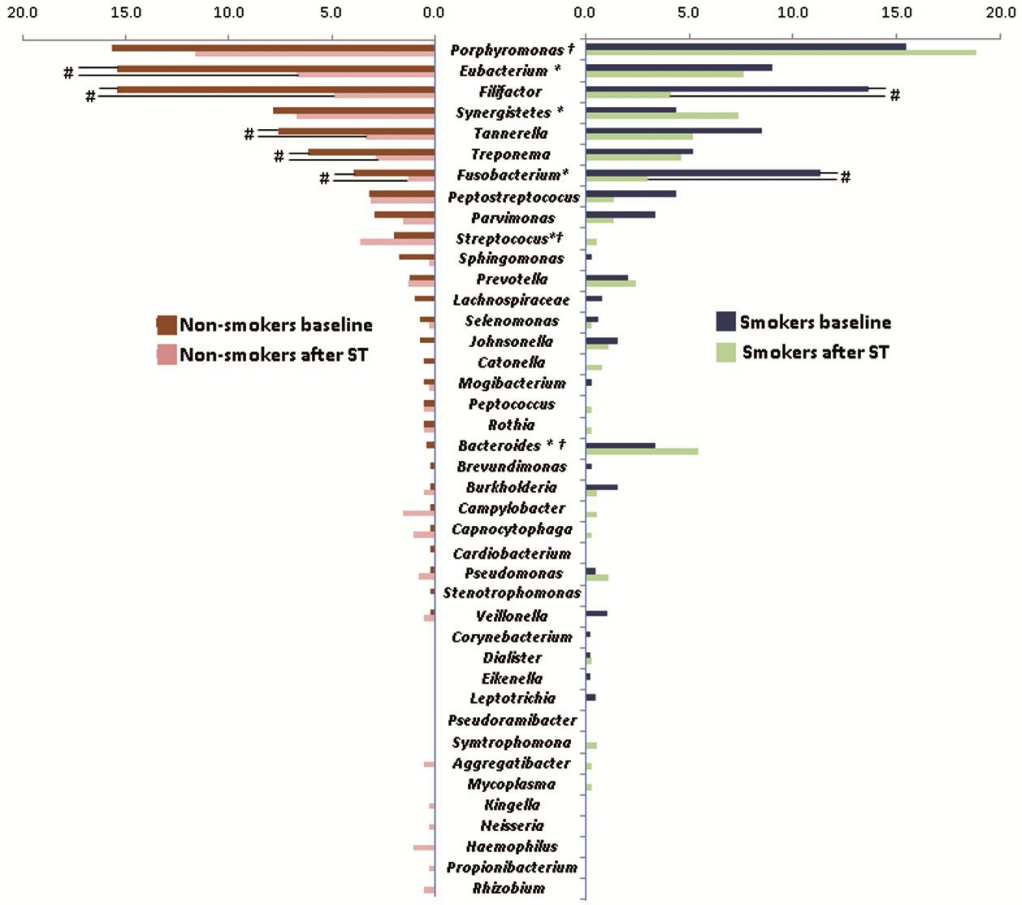

Figure 1. Distribution by Genus (percent total clones) in Smokers and Non-smokers, at baseline and after supragingival therapy (ST).**Indicates intergroup differences at baseline; † indicates intergroup differences after ST; # indicates intragroup differences after ST (Chi-Square test, $p<0.05$).*

CAPÍTULO 2: Artigo submetido à revista Quintessence International

One stage, full mouth, ultrasonic debridement in the treatment of severe chronic periodontitis in smokers.A preliminary, blind and randomized clinical trial.

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One stage, full mouth, ultrasonic debridement in the treatment of sevenic periodontitis in smokers.A preliminary, blind clinical trial.

Abstract

Objective: The aim of this parallel, single blinded and controlled clinical trial was to clinically assess the performance of a full mouth ultrasonic debridement protocol (FMUD) in the treatment

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of severe chronic periodontitis (ChP), in comparison with scaling and root planning (SRP) in a quadrant-wise procedure in smokers.

Materials and Methods: Twenty smokers presenting ChP were divided into two groups by a computer-generated list: Group FMUD (n=10), which received full-mouth ultrasonic debridement, i.e., one session of 45 minutes of ultrasonic instrumentation and Group SRP (n=10), which received full mouth scaling and root planning performed in a quadrant-wise manner. For Group Control (n=10), non-smokers with ChP were treated following the same protocol of SRP group. The following parameters were evaluated: plaque and bleeding on probing (BOP) indexes, probing pocket depth (PPD), relative gingival margin position (rGMP), and relative clinical attachment level (rCAL) at baseline, 45, 90 and 180 days after therapy.

Results: FMUD and SRP resulted in comparable gain of attachment 6 months after therapy, both groups presented PPD reduction at all experimental periods as compared to baseline. Smokers, however, presented a lower PPD reduction and rCAL gain compared to non-smokers, despite the mechanical protocol used ($p<0.05$). Moreover, at 180 days, non-smokers presented a lower number of sites requiring re-treatment (PPD>5mm and BoP) than smokers ($p<0.05$).

Conclusions: FMUD and SRP presented comparable clinical outcomes for the treatment of smokers with severe chronic periodontitis. Furthermore, despite the non-surgical technique used, smokers presented a less favorable clinical response than non-smokers.

Clinical relevance: The findings of the present study demonstrate that FMUD may be considered as an alternative approach to treat periodontal disease in smokers, as it presents comparable clinical outcome to “conventional” therapy. It may, therefore, represent a reduced “in-office” period and less post-visit discomfort for the patient.

Keywords: Full Mouth Ultrasonic Debridement, Randomized Clinical Trial, Severe Chronic periodontitis, Smoking habit.

Funding: CAPES, Brazil.

Introduction

Smoking is considered as a recognized risk factor for periodontal disease[1], modifying the interaction between bacterial biofilm and host response by several ways. Studies suggested that smokers may present an altered subgingival biofilm, harboring higher amounts of periodontal pathogens[2-3]. At the same time, they may present impaired neutrophil function[4], altered production of metalloproteinases (MMP), interleukins and inflammatory markers[5-6] and vascular alterations in periodontal tissues[7]. Together, these alterations have been suggested to be responsible for the increased risk for periodontitis development and progression in smokers.

Smoking habit has been associated with 2- to 3-fold increase in the odds of developing clinically detectable periodontitis[8]. Smokers have both increased prevalence and more severe extent of periodontal disease, as well as higher prevalence of tooth loss, also post-treatment, as compared to non-smokers[9-10]. However, despite the intrinsic differences between smokers and non-smokers, periodontal treatment is based on microbial biofilm, calculus, ‘‘contaminated’’ root cementum and dentin removal, resulting in attachment level gain and periodontal probing depth reduction. The traditional mechanical treatment is based on scaling and root planning performed in a quadrant or sextant-wise manner with an interval of 1 or 2 weeks between appointments. Smokers, however, have been shown to respond less favorably to traditional periodontal therapy than non-smokers[11-12]. As an attempt to obtain a more reliable and predictable outcome for the treatment of smokers with periodontal disease, a number of alternative approaches have been

proposed, including surgical approach (open flap scaling and root planning)[13], systemic[14-15] and locally delivered antimicrobials[16-17], but no definitive conclusion can be taken.

Meanwhile, contrasting the traditional protocol to treat periodontal disease based on mechanical scaling of root surface, evidences show that an extensive scaling is not an obligate condition in order to allow periodontal tissues healing and adequate clinical response to occur following periodontal therapy[18-19]. FMUD, consisting of one stage, full mouth, ultrasonic debridement protocol, was then proposed based on the concept that bacterial lipopolysaccharides (LPS) are weakly adhered and easily removed from cementum[20]. Predictable clinical results have been reported for FMUD in treatment of non-smokers either with chronic or aggressive periodontitis[21-24]. However, no information is available regarding the use of FMUD in the treatment of chronic periodontitis (ChP) in smokers. Thus, the aim of the present study was to clinically assess the performance of the FMUD in the treatment of severe ChP in smokers, in comparison with the conventional procedure, e.g. scaling and root planning in quadrant-wise manner with an interval of 1 week between appointments, performed in smokers and non-smokers in comparable disease.

Material and Methods

Study design

The present study was designed as a parallel, single blinded and controlled clinical trial of 6 months duration to compare the performance of FMUD and conventional scaling and root planning (SRP) in the treatment of ChP in smokers. A ChP non-smoking group treated by SRP was used as the control group for FMUD and SRP in smokers. The study design was, before commencement, approved by the IRB of the University of Campinas – UNICAMP (121/2008).

All patients were individually informed about the nature of the proposed treatment and the risks of tobacco smoking, and an informed consent form was signed.

Population screening

Thirty subjects, including 10 non-smokers and 20 smokers from those referred for treatment to the Department of Prosthodontics and Periodontics in Piracicaba Dental School, University of Campinas – UNICAMP, Brazil, were recruited from March 2009 to December 2009, after a screening examination including a full medical and dental history, an intra-oral examination, a full-mouth periodontal probing and radiographic evaluation. Subjects who were invited to participate met the following inclusion criteria:(1) diagnosis of severe chronic periodontitis[25] by the presence of periodontal pockets with a clinical attachment loss of ≥5 mm, bleeding on probing (BoP) and radiographic bone loss; (2) at least nine teeth with a probing pocket depth (PPD) of ≥5mm and bleeding following pocket probing; (3) minimum of 20 teeth in both jaws (wisdom teeth excluded); (4) smokers must have consumed 20 cigarettes per day during at least 5 years.

Exclusion criteria were as follows: (1) periapical alterations on qualifying teeth; (2) medical disorders that required prophylactic antibiotic coverage or that could influence the response to treatment; (3) scaling and root planning in the preceding 6 months; (4) consumption of drugs known to affect periodontal status (antibiotic, anti-inflammatory, anticonvulsant, immunosuppressant and calcium channel blocker) within the past 6 months; (5) orthodontic therapy; (6) pregnancy. Sample size was determined by Bioestat program, using a standard deviation of 1.0, a power value of 80% to detect a difference between groups of 1.0 mm.

Randomization, allocation concealment and examiner calibration

Smokers were randomized into two groups according to a computer-generated list. The allocation concealment was secured by having a person not involved in the study performing the randomization. This person was different from the one responsible for the treatment (D.P.) and different from the examiner (T.M.). The randomization code was not broken until all data had been collected. Thus, the treatment group was not revealed to the clinical examiner or to the statistician.

Three non-study related patients with chronic periodontitis were used to calibrate the examiner (T.M.). Duplicate measurements for PPD and rCAL were collected with an interval of 24h between the first and second recording. The intra-class correlation coefficients, used as a measure of intra-examiner reproducibility, were 0.81 and 0.88 for mean PPD and rCAL, respectively.

Treatment

Patients initially received detailed information on the etiology of periodontal disease and instructions for proper, self-performed plaque control measures, including inter-dental cleaning with dental floss and inter-dental toothbrushes. In the initial sessions, patients also had plaque retentive factors (caries, excess of restorations and supragingivalcalculus) removed. Twenty-one days after oral hygiene instructions and supragingival plaque control, patients were subjected to one of the following treatment groups:

Group SRP (n=10): smokers treated with quadrant-wise scaling and root planning, with an interval of 1 week between quadrants, using Gracey curettes[7].

Group FMUD (n=10): smokers treated with one session of full-mouth periodontal debridement with a time limit of 45 min., using an ultrasonic scaler[8].

Group control (n=10): non-smokers treated with quadrant-wise scaling and root planning, with an interval of 1 week between quadrants, using Gracey curettes;

Specific tips for subgingival instrumentation[9]were used. In all three groups, local anesthesia was used as necessary. Only one clinician (D.P.) was responsible for the treating the patients throughout the study. Since the clinician in charge to treat the patients was not the same performing the clinical examinations, the calibrated examiner (T.M.) remained blinded throughout the study.

Clinical measurements

The following clinical parameters were taken at baseline,45, 90 and 180 days after treatment: Visible Plaque Index (VPI), dichotomously assessed in the full mouth at six sites per teeth[26]; Bleeding on probing (BoP) was also measured dichotomously in the full mouth at six sites per tooth[27]; Relative gingival margin position (rGMP) - measured from a specially and individually oriented stent to the gingival margin; Relative clinical attachment level (rCAL) - measured from the stent to the bottom of periodontal pocket; Periodontal probing depth (PPD) was calculated

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[8]Cavitron, Dentsply, York, PA, USA
[9]FSI-SLI, Dentsply, York, PA, USA

based on rCAL and rGMP. The parameters VPI, BoP, rGMP, rCAL and PPD were obtained using a standardized periodontal probe with 1mm markings[10].

Re-assessment examinations

After the proposed treatments, all subjects were included in a maintenance program composed of professional supragingival plaque control and reinforcement of oral hygiene instructions every month until the sixth month. At the sixth month recall visit, all sites exhibiting PPD≥5mm and BoP were re-instrumented, using scaling and root planning with Gracey curettes. The maintenance program also included an update of the medical and dental histories, extra-oral and intra-oral soft tissue examination, dental examination and periodontal evaluation.

Statistical analysis

The statistical analysis considered the Per Protocol population (subjects who completed the follow-up, n=30). The homogeneity of groups at baseline (PPD and rCAL – primary variable) was tested using the one-way ANOVA/Tukey test. For clinical parameters, a repeated-measures analysis of variance (ANOVA) was used to detect intra-group differences in clinical parameters (rGMP, PPD, rCAL), considering the patient as a statistical unit. The results of rGMP, PPD, and rCAL refer strictly to the qualifying sites. When a statistical difference was found, an analysis of the difference was determined using the Tukey method. The Student's t-test was used to determine the differences between groups regarding the percentage of residual pockets. The Friedman test was used to detect intra-group differences, and the Kruskall-Wallis test was used

[10]PCPUNC 15s Hu-Friedy, Chicago, IL, USA.

for inter-group analysis of full mouth plaque and bleeding index in all periods. The experimental level of significance was determined to be 5%.

Results

Study schedule

Data analysis at baseline indicated that the experimental groups were balanced for age, gender and clinical parameters (Table 1). Subject recruitment started in December 2008 and was completed by the end of December 2009. All the 6-month follow-up visits were completed in July 2010.

Subject accountability

Four smokers, two from group SRP and two from group FMUD, were excluded from the study because they need to take antibiotics during the experimental period; thus, a total of 30 patients completed the study (10 in the group control, 10 in the group SRP and 10 in the group FMUD). Figure 1 illustrates the study flowchart.

VPI and BoP

The oral hygiene status during the course of the study is illustrated in Figure 2. No intergroup differences between the groups were observed at any time point for VPI and BoP. Intra-group analyses further demonstrated that VPI and BoP were significantly reduced overtime as compared to the baseline for all the experimental groups, at 180 days and 90 and 180 days for VPI and BoP, respectively ($p<0.05$).

PPD, rCAL and rGMP

Intra-group analysis demonstrated that, except for FMUD, all the experimental groups presented a significant PPD reduction over the experimental period up to 180 days post-therapy ($p<0.05$). Additionally, inter-group analysis showed that smokers treated by FMUD, presented deeper PPD at the end of the experimental period as compared to non-smokers ($p<0.05$).

Regarding rCAL, intra-group analysis demonstrated that only non-smokers presented a significant increase of rCAL overtime, whereas for smokers, both, FMUD and SRP, did not significantly affect rCAL. Furthermore, inter-group comparisons showed a tendency of lower rCAL for non-smokers treated by the conventional therapy as compared to smokers, which was statistically significant at 180 days post-therapy between non-smokers and smokers treated by FMUD ($p<0.05$). No intra- and inter-group differences were found regarding rGMP among the experimental groups ($p>0.05$) (Figure 3).

The percentage of sites presenting PPD$\geq$5mm and BoP, the clinical parameters that would indicate re-treatment need, was statistically reduced at 180 days only for the non-smoking control group ($p<0.05$), which represented a statistically higher percentage of residual pockets in both smoking groups (FMUD and SRP) (17.67% and 23.14% for SRP and FMUD, respectively) than in non-smokers (5.37% - $p<0.05$) (Table 2). Consequently, the need for re-treatment was higher in smokers regardless the therapy used.

Discussion

Smoking habit is a well-established risk factor for periodontitis development and progression. Added to its influence on etiopathogenesis, tobacco smoking also impairs periodontal clinical response to mechanical therapy. The present study evaluated the clinical performance of full mouth ultrasonic debridement (FMUD) in the treatment of severe chronic

periodontitis in smokers. In general, the results of the present study showed comparable clinical changes occurring when smokers were treated by traditional scaling and root planning (SRP) or FMUD, featuring PPD reduction and a slight rCAL improvement. Additionally, regardless of the mechanical technique used, smokers consistently presented a less favorable response to therapy as compared to non-smokers.

FMUD protocol rose from two distinct points: i) the weak LPS adherence to root surface, what indicated no need for extensive scaling and root planning; and ii) the possibility of bacterial translocation from non-treated and/or other infected oral niches to periodontally treated sites[1,20,28]. FMUD has been reported to promote comparable clinical outcomes to conventional therapy in treatment of moderate and/or severe chronic periodontitis and aggressive periodontitis in non-smokers, with CAL gain ranging from 0.7 to 1.7 mm and 1.4 to 2.2 mm for chronic and aggressive periodontitis, respectively; and PPD reduction ranging from 1.1 to 1.5 mm and 0.9 to 1.8 mm in non-smoking groups with aggressive and ChP, respectively[21-24]. However, to the best of our knowledge, the clinical performance of FMUD on treating chronic periodontitis in smokers has never been determined.

In the present study, as previously reported for non-smoking groups, FMUD promoted an overall improvement in the periodontal conditions reducing BoP and PPD in smokers with ChP. Moreover, FMUD resulted in comparable clinical changes with a reduced “in-office” period as compared to SRP (PPD reduction of 1.01±1.13 and 1.61±0.81 mm and rCAL gain of 1.22±1.03 and 0.93±1.21 mm, for FMUD and SRP, respectively; at 180 days after therapy), reinforcing that FMUD may represent a good cost-benefit alternative to the conventional approach to treat chronic periodontitis. PPD reduction and rCAL gain values found in the present study for the smoking groups are in accordance with others that showed a PPD reduction ranging from 0.6 to

2.38 mm[14-15,27-29] in smokers treated by conventional therapy. Previous studies, corroborating with our study, indicate that smokers present a less favorable response to periodontitis therapy, as compared to non-smokers. In a meta-analysis, Labriola et al.[12] confirmed the negative influence of tobacco smoking on clinical results of scaling and root planning. The authors concluded that following non-surgical therapy, people who smoked experienced less reduction in PPD than non-smokers. Another clinical landmark that illustrates the reduced clinical response of smokers is the percentage of sites with PPD>5mm and BoP at 180 days after therapy. Such clinical conditions are considered predictors for future clinical attachment level and tooth loss[10]; and have been used to determine the efficiency of different periodontal therapies[21-23]. In the present study, data analysis demonstrated that neither FMUD nor SRP were able to significantly reduce the percentage of sites presenting PPD>5mm with BoP in smokes. In fact, a higher percentage of these sites were observed for smokers versus non-smokers ($p<0.05$), despite the therapy used. In summary, these findings reinforce smokers as a high-risk group presenting a less favorable clinical response to periodontal non-surgical therapy with a higher percentage of periodontal sites requiring re-treatment than non-smokers.

Interestingly, in the present study, non-surgical periodontal therapy in smokers did not result in a significant attachment level alteration throughout the study. In line with this finding, Labriola et al.[12], highlighted the fact that there were no evidences to support significant alterations of clinical attachment level in smokers, whereas according to Wan et al.[29] only PPD reduction seemed to be impaired by tobacco smoking.

Tobacco smoking is associated to imbalanced host-response, altered cytokines and MMP release and altered oral microbiota. Some other pathways associated with periodontal healing have also been reported to be impaired by tobacco and its metabolites, including phagocytic function of

alveolar macrophages that may result in an impaired healing capacity of epithelial wounds and accumulation of apoptotic and inflammatory cellular debris[30-31]. Moreover, nicotine as well as cotinine, the major nicotine metabolite, may adhere to root surface and alter fibroblasts function, reducing their growth and adhesion[32]. Recently, studies have correlated nicotine to the release of fibrotic markers, indicating that tobacco smoking may lead to a fibrotic periodontal tissue and a decreased periodontal healing quality[33]. Together, these observations may explain the reduced clinical response to periodontal therapy reported for smokers and should be considered in future studies as new protocols to overcome such limitations in smokers.

As previously mentioned in the present manuscript, smoking habit has a significant negative influence on periodontitis development and progression, mucogingival surgery and regenerative therapy outcomes, and dental implant success rate[34-36]. Moreover, smoking has also been associated as a factor that leads to a major rate of tooth loss, as well as other oral diseases[34,37]. Fortunately, most of the effects of smoking have been shown to be reverted after quitting it. Previous studies showed the clinical and microbiological positive effects of quitting smoking on the periodontal tissues and disease therapy outcome[38-39]. Preshaw et al.[40] stated that quitting smoking has an additional beneficial effect in reducing probing depths following non-surgical treatment. With that in mind, one can speculate that FMUD may also become a more predictable and realistic therapeutic method to deal with ChP in smokers who quit smoking.

Conclusion

In conclusion and within the limits of this preliminary study, full mouth ultrasonic debridement promote a similar clinical outcome in the treatment of smokers with chronic periodontitis than traditional scaling and root planning. However, despite the mechanical protocol, smokers presented an inferior clinical response to periodontal treatment than non-smokers.

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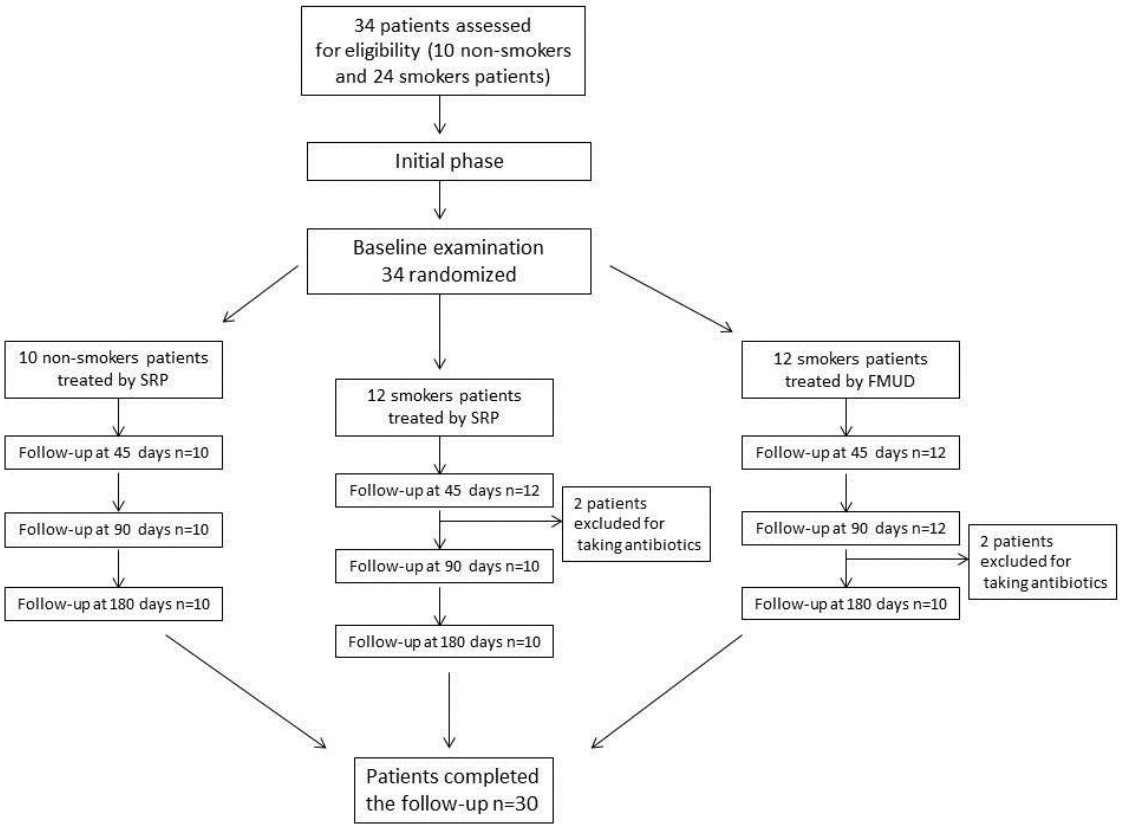
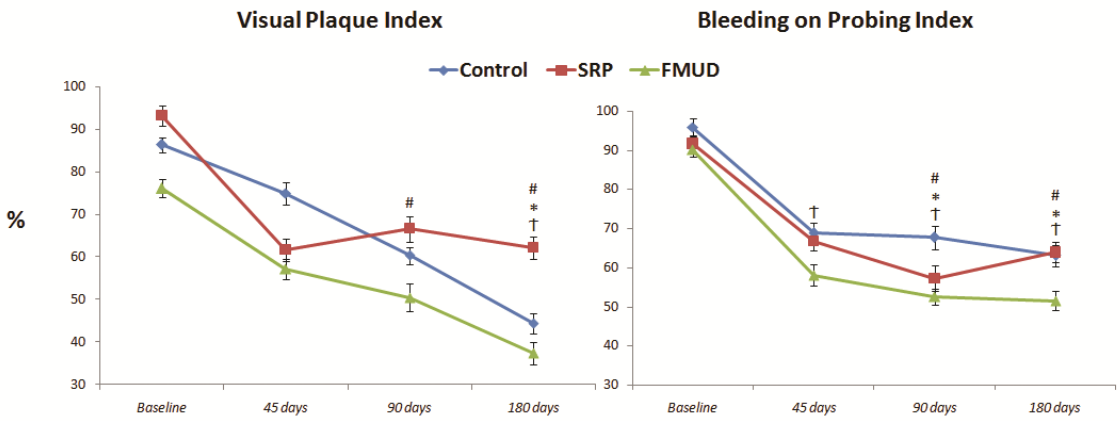


Figure 1. Flowchart for study patients.



Figure

2.Visual Plaque Index (VPI) and Bleeding on Probing (BoP) values (%±SD) at baseline, 45, 90 and 180 days of follow-up for non-smokers (Control), and smokers treated by SRP and FMUD.

Symbols indicate significant intra-group difference by Friedman test ($p<0.05$) versus baseline: *Control group; # SRP group; and † FMUD group.

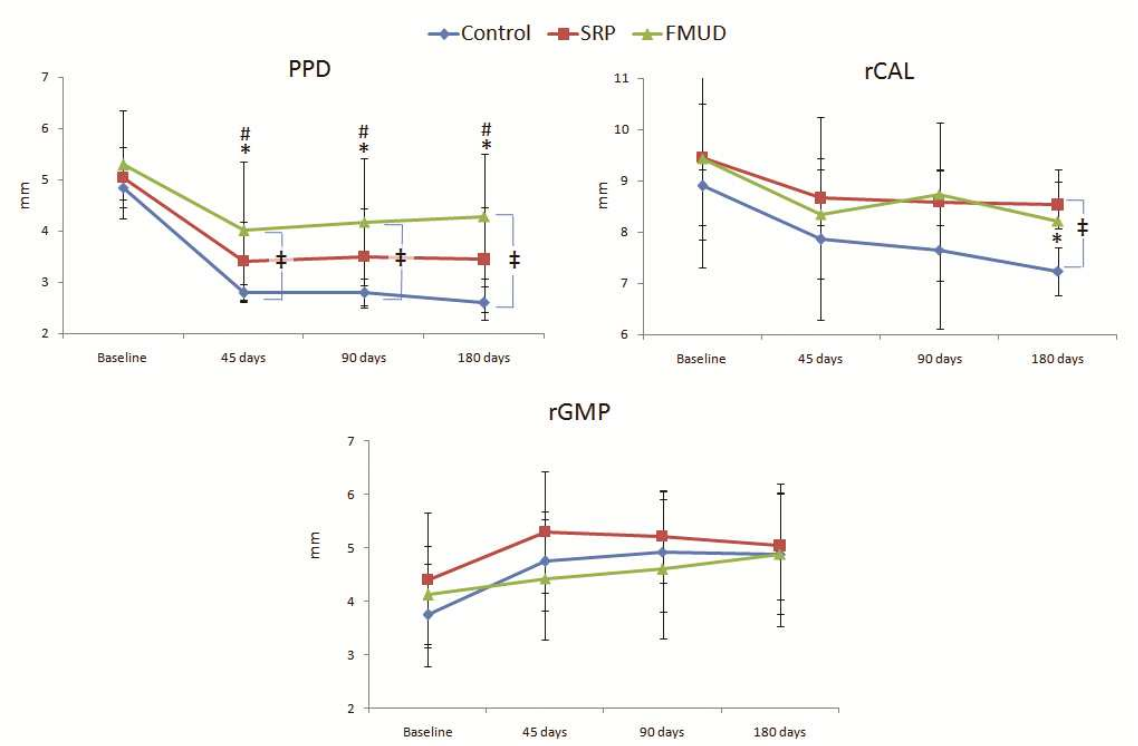


Figure 3. Periodontal Probing Depth (PPD), Relative Clinical Attachment Level (rCAL) and Relative Gingival Margin Position (rGMP) values (%+SD) at baseline, 45, 90 and 180 days of follow-up at Control, Scaling and Root Planning (SRP) and one stage, full mouth, ultrasonic debridement (FMUD) groups.

Symbols indicate significant intra-group differences versus baseline: *Control group; #SRP group, and † FMUD group; and ‡ indicate significant difference between Control and FMUD groups (ANOVA/Tukey, $p<0.05$).

Table 1. Baseline means (± SD) of age, gender and clinical parameters*.

Characteristic	Control (n=10)	SRP (n=10)	FMUD (n=10)
Age (years)	45.60±4.84	43.40±7.38	42.25±4.75
% males	50.00	55.55	50.00
VPI (%)[†]	79.69±0.12	75.46±0.18	58.87±0.13
BOP (%)[†]	76.75±0.14	75.49±0.16	66.82±0.19
PPD (mm)[†]	3.19±0.46	3.64±0.83	3.72±0.99
rCAL (mm)[†]	3.92±0.80	4.65±1.31	4.31±1.60

** At baseline, no significant differences were noted in the demographic and clinical parameters*
[†] Values of VPI, BOP, PPD and RAL refer to means of the whole mouth - VPI, visible plaque index; BoP, bleeding on probing; PPD, probing pocket depth; rCAL, relative clinical attachment level; SD – Standard deviation

Table 2. Percentage (±SD – Standard Deviation) of sites presenting PPD>5mm and BoP at Baseline and 180 days after treatment for non-smokers (Control), and smokers treated by SRP and FMUD.

	Control (n=10)	SRP (n=10)	FMUD (n=10)
Baseline	28.90±8.54 Aa	36.70±25.01 Aa	37.57±16.35 Aa
180 days	5.37±5.26 Bb	17.67±14.53 Aa	23.14±11.86 Aa

Different lower case and capital letter represent intra- and inter-group statistical differences (ANOVA/Tukey, $p<0.05$).

DISCUSSÃO

A principal causa da periodontite é o efeito acumulativo da interação entre o desafio bacteriano causado pelo biofilme e a resposta imune e inflamatória do hospedeiro. Algumas condições de risco para periodontite podem colaborar com esse processo interferindo no equilíbrio entre o desafio do biofilme e a resposta do hospedeiro, entre eles estão a pobre higiene oral, diabetes, idade, fatores genéticos e o fumo (Bergström 1989, Grossi et al., 1996; Hart et al., 1997; Ah et al., 1994). Dentre esses fatores de risco conhecidos, o fumo tem sido muito estudado e existem evidencias epidemiológicas, clínicas e microbiológicasde que os pacientes fumantes são mais susceptíveis a desenvolver periodontite, apresentar uma forma mais severa da doença periodontal e responder de forma menos favorável as formas de tratamento existentes. Além disso, alguns estudos têm sugerido que os pacientes fumantes apresentam maiores níveis de periodontopatógenos e certas espécies bacterianas quando comparados com indivíduos que nunca fumaram (Stoltenberg et al., 1993; Zambom et al., 1996; Darby et al., 2000; Shiloah et al., 2000; Haffajee et al., 2001; van Winkelhoff et al., 2001; Boström et al., 2001;Apatzidou et al., 2005; Salvi et al., 2005).Com esses conhecimentos em mente os objetivos dos nossos trabalhos foram avaliar a resposta microbiológica e clínica dos pacientes fumantes com DPIC frente a diferentes formas de tratamento periodontal.

O capítulo 1 avaliou o perfil microbiológico dos pacientes fumantes comparado com osnão-fumantes após o tratamento de RARS, com o intuito de verificar se apenas a remoção do biofilme supragengival seria capaz de interferir com a composição do biofilme subgengival e qual seria resposta dos pacientes fumantes comparado com os não- fumantes. A influência do controle supragengival do biofilme na manutenção da saúde gengival e periodontal já esta bem estabelecida na literatura (Axelsson et al., 2004). Listgarten e colaboradores (1975) mostraram

que a presença de biofilme supragengival e inflamação estavam relacionadas com o estabelecimento e desenvolvimento do biofilme subgengival (ten Napel et al., 1985; Ximénez-Fyvie et al., 2000; Weidlich et al., 2001). No entanto, não existem muitos trabalhos avaliando a influencia desse controle supragengival na presença e composição do biofilme subgengival. Katsanoulas et al. (1992), McNabb et al. (1992), al-Yahfoufi et al. (1995) e Hellström et al. (1996) conseguiram encontrar alterações tanto quantitativas quanto qualitativas na composição biofilme subgengival após o controle supragengival. Gomes e colaboradores (2008) avaliaram o efeito do controle de biofilme supragengival isolado na microbiota subgengival de fumantes e não-fumantes. Foi observada uma diminuição no número de bactérias subgengivais nos indivíduos com periodontite, a redução mais significativa ocorreu nos primeiros 30 dias após a realização do controle supragengival e esses resultados foram mantidos até o final do estudo (180 dias). Foi observado também que sítios com bolsas mais profundas e sangramento à sondagem estavam significativamente associados com alta contagem de bactérias. Os autores concluíram que tanto os pacientes fumantes quanto os não-fumantes foram beneficiados com esse protocolo de controle supragengival do biofilme.

Existem alguns trabalhos que relataram o efeito positivo da terapia mecânica na microbiota subgengival (Ximénez-Fyvie et al., 2000; Cugini et al., 2000; Doungudomdacha et al., 2001; Apatzidou et al., 2005; Van der Velden et al., 2003),a maioria dos autores relata grandes reduções na PS nos pacientes não-fumantes, comparados com os fumantes (Renvert et al., 1990; Preshaw et al., 1990; Preber, 1985; Preber et al., 1995; Grossi et al., 1997; Jin et al., 2000). No entanto quando limitamos a terapia ao ambiente supragengival os resultados tem sido contraditórios (Smulowet al., 1983;Kho et al., 1985; Beltrami et al., 1987; , Katsanoulas et al., 1992; McNabb et al., 1992; al-Yahfoufi et al., 1995; Hellström et al., 1996; Dahlén et al., 1992;

Westfelt et al., 1998; Nogueira Moreira et al., 2000). Possíveis razões para as divergências nesses achados envolvendo o controle supragengival podem ser justificadas pela ausência de critérios de inclusão bem estabelecidos e padronizados, pela maneira como o controle supragengival é realizado e pelos diferentes parâmentros clínicos avaliados por cada estudo.Além disso, existem poucas evidências do papel do controle do biofilme supragengival nos pacientes fumantes com DPIC. Esses pacientes fumantes apresentam uma resposta menos favorável as formas de terapia cirúrgica e não-cirúrgica e uma das possíveis causas é o comprometimentoda circulação sanguínea causada pelos componentes do cigarro, principalmente a nicotina e a cotinina. Essa vasoconstrição contribui para redução do fluxo sanguíneo e diminuição de oxigênio e componentes do sangue que chegam até o tecido gengival, ao mesmo tempo em que colabora para a destruição tecidual ou interfere com a resposta imune do hospedeiro (Palmer, 1999). No entanto é importante observar que embora os pacientes não-fumantes respondam melhor a tratamento periodontal, existem evidências clínicas de melhoras significantes nos pacientes fumantes após tratamento não-cirúrgico da DPIC mostrando que o fumo irá comprometer ao invés de impedir a regeneração dos tecidos (Palmer, 1999). Em pacientes não-fumantes, o controle do biofilme supragengival resulta em redução do sangramento gengival, da PS e ganho de inserção clínica, principalmente em bolsas rasas e moderadas em sítios não tratados com RAR (Haber et al., 1994; Benowitz et al., 1996; Palmer, 1978; Powell, 1998), embora alguns estudos tenham mostrado também benefícios em bolsas mais profundas (Pitzer et al., 1996). Nos fumantes, Gomes e colaboradores (2007) realizaram um dos poucos estudos que avaliou as mudanças nos tecidos periodontais após o controle supragengival do biofilme, que consistia na remoção do cálculo supragengival com curetas, exodontias, tratamentos endodônticos, confecção de restaurações e próteses provisórias.

Resultados similares ao nosso estudo foram encontrados. Foi demonstrado que resultados clínicos comparáveis foram alcançados com fumantes e não-fumantes, independente da severidade da doença, levando a redução nos IP e SS, redução na PS e ganho de inserção clínica 30 dias após ao controle supragengival do biofilme. Esses resultados foram mantidos até os 180 dias avaliados no estudo. Além disso, no presente estudo a análise dos dados mostrou que os fumantes apresentavam menor redução da porcentagem de IP comparado aos não-fumantes (20% e 30%, respectivamente), apesar dos dois grupos terem apresentado redução em relação ao baseline. No caso das bolsas profundas, no presente estudo o controle supragengival levou a uma redução mais discreta dos IP e SS para ambos os grupos. Em resumo, os achados clínicos sugerem que os fumantes e os não-fumantes com DPIC severa podem ser beneficiados de forma similar pelo controle supragengival do biofilme.

Em relação ao perfil microbiológico não foi possível chegar à mesma conclusão. Frequentemente os estudos tem avaliado a relação dos pacientes fumantes e não-fumantes após a terapia periodontal com os peridontopatógenos mais comumente associados com a DPIC como *P. gingivalis*, *T. denticola*, and *T. Forsythia (*Beltrami et al., 1987; Katsanoulas et al., 1992;Hellström et al., 1996; Haffajee et al., 1997;Söder et al., 1999; van Winkelhoff et al., 2001; Van der Velden et al., 2003; Gomes et al., 2008). No entanto, Shchipkova e colaboradores (2010) realizaram um importante estudo demonstrando que o fumo poderia afetar os níveis de diferentes gêneros de microrganismo não comumente associados com a DPIC. No presente estudo, a avaliação da composição do biofilme subgengival de fumantes e não-fumantes no baseline mostrou uma alta prevalência de alguns gêneros como *Fusobacterium* and *Bacteroides* (gêneros associados com a DPIC); e baixos níveis de *Streptococcus, Synergistetes and Eubacterium*. Esses achados confirmam que a exposição ao fumo pode alterar a composição do

biofilme subgengival não apenas aumentando a proporção dos patógenos associados à DPIC, mas tambémalgumas espécies não comumente incluídas comopatógenos periodontais tais como o *Filifactor alocis,*quetem sido sugerido comoummarcador depotencial para a doençaativa(Kumaret al., 2006; Schlafer et al., 2010) Além disso, foi possível observar uma redução dos microrganismos associados à saúde, aumento daqueles associados com a DP e uma resposta menos favorável ao controle supragengival nos fumantes. O único microrganismo que sofreu uma redução significativa nos fumantes foi o *Filifactor alocis* e houve um aumento nos níveis de algumas espécies associadas à DP como o *P. Endodontalis,* que tem sido descrito como uma das importantes novas espécies com associadas à DP (Kumar et al., 2003). Delima e colaboradores (2010) demonstraram em seu estudo que*P. endodontali*s sofria uma redução nos seus níveis subgengivais em pacientes que paravam de fumar, evidenciando a influência desse microrganismo na patogênese da DP em fumantes.

De maneira geral, os resultados encontrados pelo primeiro capítulo do presente estudo conseguiram demonstrar a íntima relação que existe entre os ambientes supra e subgengivais tanto nos pacientes fumantes quanto nos não-fumantes.

Outro assunto de grande importância abordado pelo nosso estudo foi a questão do tipo de terapia periodontal disponível para melhorar a resposta dos fumantes frente à DPIC. Ao longo dos últimos anos, muitos ensaios clínicos foram realizados num esforço para determinar se seria vantajoso alterar a terapia considerada padrão ouro para o tratamento peridontal, ou seja, RAR dividida em sessões 4 a 6 sessões, para uma abordagem com tempo reduzido concentrada em uma única sessão (Koshy et al., 2005, Zanatta et al., 2006, Moreira et al., 2007, Del Peloso Ribeiro et al., 2008). Esta terapia mecânica periodontal não-cirúrgica realizada por quadrante conhecida como RAR é uma modalidade de tratamento eficaz para a doença periodontal, no

entanto, existe a preocupação em relação a recolonização microbiana entre as sessõese ao nível de instrumentação necessária para chegar a saúde periodontal (Kinane 2005, Del Peloso Ribeiro et al., 2008). Além disso, nosso estudo envolve pacientes fumantes, acrescentando um fator de risco muito importante para o desenvolvimento da doença periodontal e para uma resposta menos favorável ao tratamento (Ismail et al., 1983; Grossi et al., 1996; Palmer et al., 1999b). O tabagismo é provavelmente o mais importante, fator de risco ambiental, controlável na periodontite (Bergström e Preber, 1994).Grossi e colaboradores (1995) constataram que o fumo foi fortemente associado a perda óssea periodontal. Além disso, fumantes são mais suscetíveis do que os não-fumantes as formas avançadas e agressivas de periodontite (Haber et al. 1994, Ketabi & Hirsh 1997) e apresentam respostas menos favoráveis aos tratamentos periodontais, incluindo não-cirúrgicos, procedimentos cirúrgicos, regeneração e cirurgia mucogengival (al-Yahfoufi et al., 1995; Stavropoulos et al., 2004; Darby et al., 2005). Os mecanismos pelos quais o fumo afeta a resposta ao tratamento periodontal podem estar relacionados com a alteração na resposta inflamatória e imune que tem sido observada em fumantes e/ou pela persistência patógenos periodontais em fumantes após o tratamento (Boströmet al., 1999; Chambrone et al., 2009; Yilmaz et al., 2010). Neste contexto, o debridamento periodontal, modalidade de tratamento na qual a dentição inteira é tratada em sessão única de 45 minutos, poderia ser uma boa alternativa para o tratamento dos pacientes fumantes com a DPIC. Assim, o objetivo do presente estudo foi avaliar os efeitos clínicos do debridamento periodontal no tratamento da DPIC em fumantes comparado com a terapia de RAR convencional.

Nossos resultados estão de acordo com estudos anteriores que investigaram os efeitos clínicos da terapia periodontal não-cirúrgica em não fumantes (Knowles et al., 1979; Badersten et al., 1981; 1984; Hammerle et al., 1991; Haffajee et al., 1997; Del Peloso Ribeiro et al., 2008). Não houve

diferença nos níveis de biofilme encontrados entre fumantes e não fumantes, o que é consistente com outros relatos (Kinane & Radvar 1997, Kamma et al. De 1999, Darby et al. De 2000, Haffajee & Socransky 2001a, Apatzidou et al., 2005), o que implica que os efeitos nocivos do tabagismo sobre a saúde periodontal não podem ser associados somente ao acúmulo de biofilme e má higiene oral (Bergström e Eliasson, 1987). O volume de fluido gengival crevicular significativamente menor (GCF) foi encontrado para fumantes comparado aos não-fumantes, em pacientes com periodontite (Kinane & Radvar 1997). Zambon e colaboradores (1996) demonstraram por imunofluorescência que os fumantes abrigavam proporções mais elevadas de espécies como *Tanerella Forsythia, Porphyromonas gingivalis e Actinobacillus Aggregatibacter,* alguns dos periodontopatógenos mais importantes para o estabelecimento e progressão da DPIC. Do mesmo modo, Kamma e colaboradores (1999) compararam os perfis microbianos de fumantes e não fumantes em um grupo de pacientes com periodontite agressiva utilizando técnicas de cultura. A análise do biofilmesubgengival revelou que uma variedade de patógenos periodontais, incluindo *T. forsythia e P. gingivalis*, foi encontrada em números significativamente maiores e mais frequentemente nos fumantes. Apatzidou e colaboradores (2004) avaliaram a terapia de RAR por quadrantes e a RAR de boca toda em sessão única em relação à freqüência de cinco supostos patógenos periodontais *(Porphyromonas gingivalis, Actinobacillus Aggregatibacter, Prevotella intermedia, Treponema denticola e Tanerella forsythus*), e mostrou que estas duas terapias foram capazes de reduzir a frequência destes periodontopatógenos com nenhuma diferença significativa entre elas. Del Peloso Ribeiro e colaboradores (2008) compararam a terapia de RAR por quadrante com desbridamento periodontal com ultrasom em sessão única de 45 minutos para tratamento periodontite crônica severa e mostraram uma redução significativa nos níveis de bactérias após tratamento em ambas

às terapias. A análise microbiológica não foi capaz de demonstrar diferenças significativas entre as diferentes terapias. Além disso, ambos os grupos apresentaram médias semelhantes de redução na PS e ganho de inserção clínica ao longo do tempo. Finalmente, nenhuma diferença foi observada entre os grupos com relação aos níveis de mediadores inflamatórios em fluído gengival crevicular. Com este conhecimento e os nossos dados em mente, podemos sugerir que ambas as terapias são capazes de alterar a microbiota de uma forma positiva e que a diferença encontrada entre os fumantes e não fumantes não depende do tratamento utilizado, mas depende do hábito do fumo. Houve reduções significativas no SS e PS com ganho no nível de inserção em todos os grupos, independente da terapia. O presente estudo demonstrou que, em fumantes com periodontite crônica severa, o debridamento periodontal (G3) resultou em melhora clínica de todos os parâmetros avaliados comparando com os dados do grupo tratado com a abordagem tradicional, ou seja, RAR por quadrantes (G2). No entanto, quando comparamos a resposta dos fumantes e dos não- fumantes, independente de modalidade terapêutica empregada, os não-fumantes apresentaram uma melhora significativamente maior do que os fumantes. Grossi e colaboradores (1994,1995) demonstraram que a severidade da perda de inserção peridontal estava altamente relacionada com o número de cigarros consumidos por dia e com a duração do hábito de fumar, portanto a gravidade da perda óssea foi positivamente relacionada com a experiência de fumar. Neste trabalho, todos os pacientes tinham o hábito de fumar há pelo menos 10 anos e fumavam no mínimo 20 cigarros por dia. Outro fator relevante sobre os fumantes é a supressão de sinais clínicos de inflamação gengival, como indicado pelo baixo índice de SS (Preber & Bergström 1985). Além disso, Darbye colaboradores (2000) analisaram a condição periodontal em pacientes com periodontite crônica e pacientes com periodontite agressiva generalizadas, e descobriram que os fumantes, em ambos os grupos de doenças exibiam índices

de sangramento significativamente mais baixos do que os não fumantes. Em nossos dados, esses índices em fumantes foram semelhantes aos não-fumantes, indo contra as obras anteriores. Uma hipótese possível para estes dados é que neste trabalho foi utilizado o sangramento à sondagem (sangramento do fundo da bolsa periodontal) apenas para análise ao invés do índice gengival, que indica o sangramento da margem de gengival apenas. Portanto, a partir dos resultados do presente estudo podemos sugerir que ambas as terapias foram capazes de alterar de maneira positiva os parâmetros clínicos nos pacientes fumantes e nos não-fumantes alcançando o objetivo maior da terapia periodontal que é restabelecer a saúde periodontal. No entanto, os fumantes apresentaram resultados menos favoráveis do que os não-fumantes, provavelmente, devido a influênciado fumo na resposta inflamatória e imune e no perfil microbiológico dos pacientes. Nesse estudo, foram avaliados apenas os achados clínicos, mais estudos imunológicos e microbiológicos são necessários para tentar esclarecer melhor o mecanismo de influência do fumo na doença periodontal.

CONCLUSÃO

1) Terapia supragengival apresentou resultados clínicos similares para fumantes e não-fumantes.
2) Perfil microbiológico subgengival dos fumantes é diferente dos não-fumantes.
3) Independente da terapia subgengival empregada os fumantes resposdem de forma inferior comparado aos não-fumantes.

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ANEXO 1

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Impact of supragingival therapy on subgingival microbial profile in smokers versus non-smokers with severe chronic periodontitis.

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Abstract

BACKGROUND:

The aim of this study was to assess subgingival microbiological changes in smokers versus non-smokers presenting severe chronic periodontitis after supragingival periodontal therapy (ST).

METHODS:

Non-smokers (n=10) and smokers (n=10) presenting at least nine teeth with probing pocket depth (PPD) (≥5 mm), bleeding on probing (BoP), and no history of periodontal treatment in the last 6 months were selected. Clinical parameters assessed were plaque index (PI), BoP, PPD, relative gingival margin position (rGMP) and relative clinical attachment level (rCAL). Subgingival biofilm was collected before and 21 days after ST. DNA was extracted and the 16S rRNA gene was amplified with the universal primer pair, 27F and 1492R. Amplified genes were cloned, sequenced, and identified by comparison with known 16S rRNA sequences. Statistical analysis was performed by Student's t and Chi-Square tests (α=5%).

RESULTS:

Clinically, ST promoted a significant reduction in PI and PPD, and gain of rCAL for both groups, with no significant intergroup difference. Microbiologically, at baseline, data analysis demonstrated that smokers harbored a higher proportion of Porphyromonas endodontalis, Bacteroidetes sp., Fusobacterium sp. and Tannerella forsythia and a lower number of cultivated phylotypes ($p<0.05$). Furthermore, non-smokers featured significant reductions in key phylotypes associated with periodontitis, whereas smokers presented more modest changes.

CONCLUSION:

Within the limits of the present study, ST promoted comparable clinical improvements in smokers and non-smokers with severe chronic periodontitis. However, in smokers, ST only slightly affected the subgingival biofilm biodiversity, as compared with non-smokers.

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ANEXO 2

COMITÊ DE ÉTICA EM PESQUISA
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UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Debridamento periodontal versus tratamento periodontal convencional em fumantes, com periodontite crônica avançada. Estudo clínico e imunológico**", protocolo nº **121/2008**, dos pesquisadores **TATIANA MEULMAN LEITE DA SILVA, ANA PAULA OLIVEIRA GIORGETTI, DAIANE CRISTINA PERUZZO, FRANCISCO HUMBERTO NOCITI JUNIOR e RENATO CORRÊA VIANA CASARIN**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 04/11/2008.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project "**Periodontal debridement versus basic periodontal therapy in smokers. A clinical and immunological study**", register number **121/2008**, of **TATIANA MEULMAN LEITE DA SILVA, ANA PAULA OLIVEIRA GIORGETTI, DAIANE CRISTINA PERUZZO, FRANCISCO HUMBERTO NOCITI JUNIOR and RENATO CORRÊA VIANA CASARIN**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 04/11/2008.

Prof. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.