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CENTRO DE CIÊNCIAS DA SAÚDE  
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Juliana Maier

**EFEITO DA FREQUÊNCIA DO AUTOCONTROLE MECÂNICO DE  
PLACA NAS CONDIÇÕES GENGIVAIS DE PACIENTES COM  
HISTÓRICO DE PERIODONTITE E EM MANUTENÇÃO  
PERIODONTAL**

Santa Maria, RS  
2018

**Juliana Maier**

**EFEITO DA FREQUÊNCIA DO AUTOCONTROLE MECÂNICO DE PLACA NAS  
CONDIÇÕES GENGIVAIS DE PACIENTES COM HISTÓRICO DE PERIODONTITE  
E EM MANUTENÇÃO PERIODONTAL**

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, ênfase em Periodontia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutor em Ciências Odontológicas**.

Orientador: Prof. Dr. Carlos Heitor Cunha Moreira

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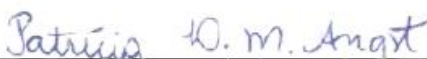
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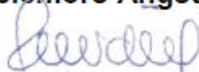
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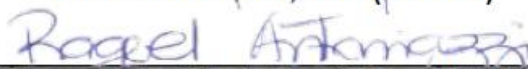
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**Raquel Pippi Antoniazzi, Dra. (UFSM)**



**Ticiane de Góes Mário, Dra. (UFN)**

Santa Maria, RS  
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## DEDICATÓRIA

Dedico este trabalho...

À Deus e meu anjo da guarda,  
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Gisele Maier Malgarin e Giovanna Maier Malgarin.**

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*Na vida, não vale tanto o que temos, nem  
tanto importa o que somos. Vale o que  
realizamos com aquilo que possuímos e, acima  
de tudo, importa o que fazemos de nós!*

*Chico Xavier*

## RESUMO

### EFEITO DA FREQUÊNCIA DO AUTOCONTROLE MECÂNICO DA PLACA NAS CONDIÇÕES GENGIVAIS DE PACIENTES COM HISTÓRICO DE PERIODONTITE E EM MANUTENÇÃO PERIODONTAL

AUTORA: Juliana Maier  
ORIENTADOR: Carlos Heitor Cunha Moreira

A presente tese foi estruturada em dois artigos científicos que investigaram o efeito da frequência do autocontrole mecânico da placa bacteriana em parâmetros supra e subgingivais de pacientes com histórico de periodontite e em manutenção periodontal. O primeiro artigo objetivou avaliar o efeito de diferentes frequências no autocontrole mecânico de placa (ACMP) na saúde gengival de indivíduos com histórico de periodontite, tratados e participantes de um programa de manutenção periodontal. Quarenta e dois indivíduos com histórico de periodontite e participantes de manutenção periodontal (máximo 7,5% dos sítios com sangramento gengival) foram randomizados para diferentes frequências de ACMP: 12, 24 e 48 horas. Índice de placa (IP), índice gengival (IG) foram avaliados no baseline, 15, 30 e 90 dias. Profundidade de sondagem, nível de inserção clínica e sangramento à sondagem foram avaliados no baseline, 30 e 90 dias. Modelos mistos foram utilizados para análises e comparações entre os grupos experimentais. A média de IG entre o baseline e 90 dias permaneceu inalterada apenas no grupo de 12 horas ( $0,74 \pm 0,057$  versus  $0,85 \pm 0,059$ ). Somente no grupo de 48 horas houve um aumento estatisticamente significativo nas médias de IG ao longo do período experimental, já apresentando diferenças significativas entre baseline e 15 dias ( $P < 0,05$ ). No final do período experimental, as médias do IG foram significativamente maiores no grupo de 48 horas do que nos grupos de 12 e 24 horas ( $P < 0,05$ ). Quando considerados percentuais de IG=2, apenas o grupo de 48 horas não conseguiu manter a saúde gengival ao final do período experimental (18,83%) ( $P < 0,05$ ). O ACMP realizado a cada 12 ou 24 horas foi compatível com a manutenção da saúde gengival em indivíduos com histórico de periodontite, tratados e participantes de um programa periódico de manutenção periodontal. O segundo artigo objetivou elucidar como as diferentes frequências de ACMP interferem nos parâmetros inflamatórios subgingivais, através da avaliação da correlação entre sangramento gengival (IG=2) e sangramento à sondagem (SS) em indivíduos com histórico de periodontite. Quarenta e dois indivíduos com histórico de periodontite e participantes de manutenção periodontal (máximo 7,5% dos sítios com sangramento gengival) foram randomizados para diferentes frequências de ACMP: 12, 24 e 48 horas. Índice gengival e sangramento à sondagem foram avaliados no baseline, 30 e 90 dias. As diferenças intragrupos foram determinadas usando ANOVA de medidas repetidas. Diferenças intergrupos no baseline foram verificadas usando um teste qui-quadrado e teste t independente. O coeficiente de correlação de Spearman entre IG e SS para os diferentes grupos foi calculado no baseline, 30 e 90 dias. Maiores mudanças na magnitude da correlação foram observadas entre baseline e 90 dias para o grupo de 48 horas. Correlações positivas estatisticamente significantes foram observadas entre as médias de IG e SS em todos os grupos e aumentaram ao longo do período experimental, mas apenas o grupo 48h apresentou correlação moderada entre IG e SS aos 90 dias ( $r=0,34$ ) ( $P < 0,01$ ). Quando a correlação entre IG e SS foi avaliada em bolsas rasas ( $PS \leq 3$  mm), aos 30 dias todos os grupos apresentaram correlação positiva estatisticamente significativa e apenas no grupo 48h a correlação foi moderada ( $r=0,31$ ) ( $P < 0,01$ ). Ao final de 90 dias, houve declínio na correlação no grupo de 12h, e aumento nos outros grupos, sendo uma correlação moderada no grupo de 48h ( $r=0,36$ ) e fraca nos demais grupos experimentais ( $P < 0,01$ ). A realização do ACMP a cada 48 horas resultou em um aumento significativo das médias de IG=2 em comparação aos demais grupos. Essas alterações inflamatórias marginais tiveram repercussões no ambiente subgingival através de um aumento no sangramento à sondagem (SS), demonstradas através da moderada correlação estatisticamente significativa entre IG=2 e SS nesse grupo experimental.

## ABSTRACT

### EFFECT OF SELF-PERFORMED MECHANICAL PLAQUE CONTROL FREQUENCIES IN MAINTENANCE OF GINGIVAL HEALTH IN PATIENTS WITH A HISTORY OF PERIODONTITIS AND PERIODONTAL MAINTENANCE: A RANDOMIZED CLINICAL TRIAL

AUTHOR: Juliana Maier  
ADVISOR: Carlos Heitor Cunha Moreira

The present thesis was structured in two scientific articles that investigated the effect of self-performed mechanical plaque control frequencies on supra and subgingival parameters of patients with a history of periodontitis and periodontal maintenance. The first article aimed to evaluate the effect of different frequencies on self-performed mechanical plaque control (SPC) on the gingival health of individuals with a history of periodontitis, treated and participants in a periodontal maintenance program. Forty-two individuals with a history of periodontitis and periodontal maintenance participants (maximum 7.5% of sites with gingival bleeding) were randomized to different frequencies of SPC: 12, 24 and 48 hours. Plaque index (PII) and gingival index (GI) were evaluated at the baseline, 15, 30 and 90 days. Probing depth, attachment level and bleeding on probing were assessed at the baseline, 30 and 90 days. Mixed linear models were used for the analysis and comparison of experimental groups. The mean GI between baseline and 90 days remained unchanged only in the 12h group ( $0.74 \pm 0.057$  versus  $0.85 \pm 0.059$ ). Just in the 48h group was there an increase in the GI means throughout the experimental period, already showing significant differences between baseline and 15 days ( $P < 0.05$ ). At the end of the trial period, the GI means were significantly higher in the 48-hour group than in the 12 and 24-hour groups ( $P < 0.05$ ). When the percentage of GI=2 were considered, only the 48h group could not maintain gingival health at the end of the experimental period (18.83%) ( $P < 0.05$ ). SPC performed every 12 to 24 hours was compatible with the maintenance of gingival health in individuals with a history of periodontitis, treated, and participants in a periodontal maintenance program. The second article aimed to elucidate how the different frequencies of SPC interfere in the subgingival inflammatory parameters by evaluating the correlation between gingival bleeding (GI=2) and bleeding on probing (BoP) in the different groups of SPC in individuals with a history of periodontitis. Forty-two individuals with a history of periodontitis and periodontal maintenance participants (maximum 7.5% of sites with gingival bleeding) were randomized to different frequencies of SPC: 12, 24 and 48 hours. Gingival index and bleeding on probing were evaluated at baseline, 30 and 90 days. Intragroup differences were determined using repeated measures ANOVA. Intergroup differences in the baseline were verified using a chi-square test and independent t-test. The Spearman correlation coefficient between GI and BoP for the different groups was calculated at the baseline, 30 and 90 days. Significant changes in correlation were observed between baseline and 90 days for the 48h group. Statistically significant positive correlations were found between the GI means and BoP in all groups and increased throughout the experimental period, but only 48h group presented a moderate correlation between GI and BoP at 90 days ( $r=0.34$ ) ( $P < 0.01$ ). When the correlation between GI and SS was evaluated in shallow pockets ( $PD \leq 3$  mm), at 30 days all groups had a statistically significant positive correlation and only in the 48h group the correlation was moderate ( $r=0.31$ ) ( $P < 0.01$ ). At the end of 90 days, there was a decline in correlation in the 12h group, and an increase in the other groups, a moderate correlation in the 48h group ( $r=0.36$ ) and weak in the different experimental groups ( $P < 0.01$ ). The performance of the SPC every 48 hours resulted in a significant increase in the GI=2 mean compared to the other groups. These marginal inflammatory changes had repercussions on the subgingival environment through an increase in bleeding on probing, demonstrated by the moderate statistically significant correlation between GI=2 and BoP in this experimental group.

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## 1 INTRODUÇÃO

Gengivite induzida por placa bacteriana é definida como uma inflamação resultante de interações entre a placa e a resposta imuno-inflamatória do hospedeiro, que permanece confinada à gengiva não se estendendo além da junção mucogengival e ao periodonto de suporte (cimento, ligamento periodontal e osso alveolar). Apresenta caráter reversível, através da redução nos níveis de placa bacteriana dentária. Dependendo de onde a inflamação gengival ocorre, em um periodonto intacto ou reduzido, ou em um paciente diagnosticado com periodontite, pode ser classificada como: gengivite em um periodonto intacto, gengivite em um periodonto reduzido em um paciente sem periodontite ou gengivite em um periodonto reduzido em um paciente com histórico de periodontite e tratado com sucesso (CHAPPLE et al., 2018). É a forma mais comum de doença periodontal, altamente prevalente em diferentes populações (GJERMO et al., 2002; SUSIN et al., 2004, TONETTI et al., 2017) e trata-se de um processo inflamatório causado por uma infecção endógena e oportunista (PAGE; BAAB, 1985; RUBY; BARBEAU, 2002; SOCRANSKY; HAFFAJEE, 1994). Løe et al. (1965) estabeleceram a sua etiologia através de um estudo clássico de gengivite experimental. Os resultados desse estudo demonstraram que a presença de inflamação gengival está relacionada ao acúmulo de microrganismos e sua persistência ao longo da margem gengival, por um período mínimo de 10 a 21 dias, de acordo com variações individuais. Após o retorno de medidas de higiene bucal, e consequente desorganização da placa supragengival, a saúde gengival foi restabelecida entre 7 a 10 dias (LÖE; THEILADE; JENSEN, 1965).

Edema, mudanças no contorno e consistência do tecido, alteração de cor, sangramento na margem gengival e aumento do fluido crevicular gengival são características clínicas da gengivite (AMERICAN ACADEMY OF PERIODONTOLOGY, 2000). Histologicamente, apresenta-se através da proliferação de células basais no epitélio juncional, tecido conjuntivo com degradação de fibras colágenas, aumento da vascularização e permeabilidade vascular adjacentes ao epitélio juncional, além da presença de intenso infiltrado inflamatório (PAGE; SCHROEDER, 1976). A gravidade dos seus sinais e sintomas pode ser influenciada por diversos fatores (MARIOTTI, 1999). Diabetes mellitus (CARNEIRO et al., 2015), alterações hormonais presentes na puberdade (CHAITRA et al., 2012), período

menstrual (BECERIK et al., 2010) e gravidez (CARRILLO-DE-ALBORNOZ et al., 2012) têm sido relacionadas a um aumento na gravidade da inflamação. Fumo tem sido relatado como redutor dos sinais inflamatórios (PERUZZO et al., 2016). Algumas doenças sistêmicas, como a leucemia (FRANCISCONI et al., 2016), têm sido associadas a episódios de sangramento e edema gengival; e alguns medicamentos (nifedipina, fenitoína e ciclosporina) associados ao aumento de volume gengival (GANESH, 2016). Dentre as morbidades geradas pela gengivite destacam-se a presença de halitose e sangramento marginal, os quais podem ter repercussão negativa no convívio social e na qualidade de vida dos indivíduos portadores dessa patologia (BUSET et al., 2016; ZAITSU et al., 2011).

A gengivite é uma doença de caráter reversível através do controle de seu fator etiológico, sem prejuízo ao periodonto de suporte (MARIOTTI, 1999) e sua suscetibilidade pode diferir significativamente entre indivíduos (TATAKIS; TROMBELLI, 2004; TROMBELLI et al., 2008). Essas diferenças individuais na resposta ao acúmulo de placa são dependentes de fatores relacionados ao hospedeiro, sendo possivelmente de origem genética (SCAPOLLI; MAMOLINI; TROMBELLI, 2007). A epidemiologia e a história natural da gengivite e periodontite indicam que a inflamação gengival é um componente que precede a periodontite (LINDHE; HAMP; LÖE, 1975, LÖE et al., 1986). No entanto, nem todos os casos de gengivite irão progredir para periodontite (BROWN; LÖE, 1993; PRAYITNO; ADDY; WADE, 1993). Isto se deve ao fato de que a placa não é suficiente para o desenvolvimento da periodontite, sendo necessária também a presença de um hospedeiro suscetível (PAGE; SCHROEDER, 1981, TROMBELLI et al., 2004).

A periodontite é uma doença de natureza infecto-inflamatória que resulta na perda irreversível dos tecidos de suporte dentários (PAGE et al., 1997). Seu fator etiológico é a placa subgengival, porém sua suscetibilidade está relacionada a outros fatores locais e sistêmicos que apresentam importantes papéis modificadores na sua patogênese. Dentre eles, o tabagismo e diabetes mellitus destacam-se como fatores de risco importantes para a perda de tecido periodontal (ALBANDAR; RAMS, 2002). Na ausência de tratamento, seu desfecho final pode ser a perda dental, com consequências funcionais e psicológicas aos indivíduos (AL-HARTI et al., 2013; LORENTS et al., 2010). Embora nem todos os pacientes com gengivite evoluam para periodontite, o manejo da gengivite é tanto uma estratégia de prevenção primária, assim como uma estratégia de prevenção secundária para periodontite

recorrente (CHAPPLE et al., 2015). A literatura tem demonstrado que o insucesso de uma terapia subgingival bem realizada pode estar associado à falta de um adequado controle de placa supragengival (LINDHE; NYMAN, 1984) o qual estabelece a importância de adequado autocontrole mecânico de placa (ACMP), bem como a participação sistemática em um programa de manutenção periódica preventiva (TONETTI et al., 2015).

O principal mecanismo de prevenção da gengivite é o estabelecimento de hábitos adequados de higiene bucal, com meticulosa desorganização da placa supragengival, através de ACMP utilizando escovas multicerdas e dispositivos interdentais (SAMBUNJAK et al., 2011). Tais ferramentas são, conseqüentemente, fundamentais para a redução da presença de inflamação no periodonto e redução do risco de perda de inserção e perda dentária futura (HUGOSON; SJODIN; NORDERYD, 2008). Porém, a efetividade desse procedimento na prevenção e tratamento da gengivite depende de dois fatores: a capacidade do indivíduo em desorganizar placa de todas as superfícies dentárias e a frequência em que esta desorganização é executada (JEPSEN, 1998). Convencionalmente e conforme recomendações da American Dental Association (ADA), dentistas orientam seus pacientes a realizarem a escovação duas vezes ao dia para prevenir cárie e gengivite, embora exista evidência limitada dessa recomendação no que diz respeito à manutenção da saúde gengival (CHAPPLE et al., 2015).

Em pacientes com adequado controle de placa, Kelner et al. (1974) determinaram que a frequência de higiene bucal a cada 24 horas é compatível com saúde gengival, sendo que quando realizada a cada 72 horas os pacientes desenvolveram alterações nos sinais inflamatórios. Lang et al. (1973) observaram que uma frequência de higiene bucal a cada 48 horas foi compatível com saúde gengival, sendo que em frequências superiores ocorreu o desenvolvimento de gengivite. Entretanto, algumas limitações metodológicas desse estudo, tornam difícil a inferência desses achados para a população em geral. Os participantes eram estudantes de odontologia, com conhecimento da etiologia e patogênese da doença, além de a remoção de placa ser realizada com o uso de evidenciadores e ser supervisionada por higienistas. Isto é, a remoção completa e meticulosa da placa foi realizada a cada escovação, o que usualmente não ocorre na população em geral.

Neste tópico, De Freitas et al. (2016) realizaram um ensaio clínico randomizado (ECR) com estudantes não pertencentes a cursos da área da saúde.

Foram comparadas as condições gengivais entre indivíduos que executaram o ACMP não supervisionado em intervalos de 12, 24 e 48 horas. Frequências de 12 e 24 horas foram suficientes para manutenção dos níveis de saúde gengival em indivíduos com adequado controle de placa. O intervalo de 48 horas mostrou-se não ser compatível com a manutenção da saúde gengival. Do mesmo modo, Pinto et al. (2013), em um ECR com metodologia semelhante, porém com a utilização de dentifício com ação antimicrobiana (contendo fluoreto estanhoso), observaram resultados semelhantes. Além de não observarem qualquer benefício adicional associado ao uso de dentifício com fluoreto estanhoso. Contudo, nesses dois ECRs, somente indivíduos sem histórico de periodontite foram incluídos.

Dessa forma, não há evidências na literatura a respeito do efeito de diferentes frequências de ACMP e alterações inflamatórias gengivais em indivíduos com histórico de periodontite, que foram tratados, tiveram sua saúde periodontal restabelecida e que participam de um programa de manutenção periódica preventiva. Uma recente revisão sistemática recomenda que ensaios clínicos randomizados sejam realizados para responder esta questão (TONETTI et al., 2015).

É de suma importância avaliar qual a frequência de ACMP é compatível com saúde gengival nesses indivíduos, devido às condições anatômicas dentárias muito distintas daquelas encontradas em indivíduos sem histórico de periodontite. Como por exemplo, exposição de lesões de furca, ausências dentárias, apinhamentos dentários, presença de espiantagens, presença de espaçamento entre os dentes, ausência de papila nos espaços interdentais e concavidades radiculares expostas. Além disso, os auto cuidados são necessários para manter a estabilidade pós tratamento periodontal nessa população suscetível, pois sabe-se que sítios que mantêm constantemente sangramento gengival apresentam um risco aumentado para perda de inserção e perda dental futura (LANG; SCHÄTZLE; LÖE., 2009, SCHÄTZLE et al., 2003). Isto por que a presença da gengivite proporciona condições favoráveis ao estabelecimento e desenvolvimento da placa subgengival (WEIDLICH et al., 2001) e, em indivíduos suscetíveis, a presença dessa placa levará a consequente inflamação e maior probabilidade de atividade de doença (LANG et al., 1986).

Desta forma, o objetivo desse estudo foi avaliar o efeito de diferentes frequências de auto remoção mecânica da placa supragengival na manutenção da

saúde gengival e parâmetros subgengivais de indivíduos com histórico de periodontite, tratados e participantes da fase de manutenção periódica e preventiva.

**2 ARTIGO 1 – EFFECT OF SELF-PERFORMED MECHANICAL PLAQUE CONTROL FREQUENCIES IN MAINTENANCE OF GINGIVAL HEALTH IN PATIENTS WITH A HISTORY OF PERIODONTITIS AND PERIODONTAL MAINTENANCE: A RANDOMIZED CLINICAL TRIAL**

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**Juliana Maier<sup>1</sup>, Ana Paula Pereira Reiniger<sup>1</sup>, Karla Zanini Kantorski<sup>1</sup>, Carlos Heitor Cunha Moreira<sup>1</sup>**

<sup>1</sup>Divisão de Periodontia, Departamento de Estomatologia, Faculdade de Odontologia, Federal Universidade de Santa Maria, Santa Maria, Brasil.

Autor correspondente:

Carlos Heitor Cunha Moreira

Rua Serafim Valandro, 1514/302, 97015-630, Santa Maria /RS, Brasil.

Telefone: +55.55.3025.6538

Email: carlosheitor@rosuline.com.br

## ABSTRACT

**Aim:** This randomized clinical trial evaluated the effect of different self-performed mechanical plaque control (SPC) frequencies in the gingival health of individuals with a history of periodontitis, treated and participants of periodic preventive maintenance program (PMP).

**Materials and Methods:** Forty-two individuals with a history of periodontitis and participants of PMP (maximum 7,5% of sites with gingival bleeding) were randomized to different SPC frequencies: 12, 24 and 48 hours. Plaque index (PII) and gingival index (GI) were evaluated at baseline, 15, 30 and 90 days. Probing depth, clinical attachment level and bleeding on probing were assessed at baseline, 30 and 90 days. Mixed linear models were used for the analysis and comparison of experimental groups.

**Results:** The GI mean between baseline and 90 days statistically remained unchanged only in the 12 hours group ( $0.74 \pm 0.057$  versus  $0.85 \pm 0.059$ ). Just in the 48 hours group, an increase in the GI means was observed throughout the experimental period, since it already showed a significant difference between baseline and 15 days. At the end of trial period, the GI means were significantly higher in 48 hours group than 12 and 24h groups. When percentages of GI=2 were considered, only the 48 hours group was not able to maintain gingival health at the end of the experimental period (18,83%).

**Conclusion:** Self-performed mechanical plaque control every 12 or 24 hours is enough to maintain gingival health in individuals with a history of periodontitis, treated and participating in a PMP. (ClinicalTrials.gov: 50208115.9.0000.5346).

**Keywords:** dental plaque; gingivitis; oral hygiene; periodontal diseases.

## INTRODUCTION

Plaque-induced gingivitis is defined as an inflammation resulting from interactions between the dental plaque and the host's immune-inflammatory response, which remains confined within the gingiva and does not extend beyond the mucogingival junction and to the periodontal attachment (cementum, periodontal ligament and alveolar bone). It has reversible character by reducing levels of dental plaque. Depending on whether dental plaque-induced gingival inflammation occurs on an intact or reduced periodontium, or in a patient diagnosed with periodontitis, gingivitis can be further classified as: gingivitis on an intact periodontium, gingivitis on a reduced periodontium in a non-periodontitis patient or gingivitis on a reduced periodontium in a successfully treated periodontitis patient (Chapple et al., 2018). It can occur in teeth with or without attachment loss, being the most common form of periodontal disease, highly prevalent worldwide (Gjeramo et al., 2002; Susin et al., 2004, Tonetti et al., 2017). The epidemiology and natural history of gingivitis and periodontitis indicate that gingival inflammation precedes periodontitis (Lindhe; Hamp; Löe, 1975, Löe et al. 1986), but not all cases of gingivitis will progress to periodontitis (Brown; Löe, 1993; Prayitno; Addy; Wade, 1993). In this sense, management of gingivitis is both a primary prevention strategy for periodontitis and a secondary prevention strategy for recurrent periodontitis (Chapple et al., 2015).

The main mechanism of gingivitis prevention is the establishment of proper habits of oral hygiene, with meticulous disruption of supragingival plaque, through self-performed mechanical plaque control (SPC) using multi-bristle toothbrushes and interdental devices (Sambunjak et al., 2011). Different SPC frequencies have been proposed such as by the American Dental Association (ADA) which recommends brushing twice daily to prevent caries and gingivitis, although there is limited evidence of this recommendation regarding maintenance of gingival health (Chapple et al., 2015). Studies of the 1970s showed that frequencies between 24 (Kelner et al., 1974) and 48 hours (Lang et al., 1973) were compatible with the maintenance of gingival health. However, some methodological limitations make difficult to infer these findings for the general population.

Recently, Pinto et al. (2013) and De Freitas et al. (2016) demonstrated that frequencies of 12 and 24 hours were compatible with the maintenance of gingival health levels in individual with appropriate plaque control. Frequencies of 48 hours



did not maintain gingival health levels, even with the use of toothpaste containing antimicrobial (Pinto et al., 2013). However, in these two randomized controlled trials (RCTs), only subjects without a history of periodontitis were included. There is no evidence in the literature regarding the effect of different frequencies of SPC on inflammatory gingival changes in individuals with a history of periodontitis, treated, and participants in a periodic preventive maintenance program. A recent systematic review recommends that RCTs be undertaken to answer this issue (Tonetti et al., 2015).

It is important to assess what frequency of SPC is compatible with gingival health in these more susceptible individuals, due to several reasons: different anatomical conditions, such as exposure of furcation lesions, ridge associated with lost teeth, dental crowding, presence of splinting, spaces among teeth, lack of papilla in the interdental spaces and exposed root concavities. Besides, self-care is required to maintain post-treatment periodontal stability in this susceptible population, as it is known that sites that consistently maintain gingival bleeding presented an increased risk for attachment loss and future dental loss (Lang; Schätzle; Loe, 2009, Schätzle et al., 2003). This is because the presence of gingivitis provides conditions favoring the establishment and development of the subgingival plaque (Weidlich et al., 2001) and, in susceptible individuals, plaque presence will lead to the consequent inflammation and a higher probability of disease activity (Lang et al. al., 1986).

In this context, the aim of this study was to evaluate the effect of different SPC frequencies in the gingival health maintenance of individuals with a history of periodontitis.

## **MATERIALS AND METHODS**

### **Study design**

This study was a single-masked, parallel design, three arms randomized clinical trial.

### **Sample**

Individuals diagnosed with periodontitis (Tonetti & Claffey, 2005), treated at the Post-Graduation Clinic of the Federal University of Santa Maria (UFSM, Rio Grande do Sul, Brazil) and included in the periodontal maintenance program were eligible. Patients included in the maintenance program were regularly recalled every 4-6 months. Individuals should present 35 years or more of age and at least 12 teeth in the mouth, maximum 7.5% of sites with GI=2 and 25% of sites with bleeding on probing (BoP). Smokers, pregnant women, diabetics, individuals presenting xerostomia, psychomotor disorders, patients with a fixed orthodontic appliance, who needed antimicrobial prophylaxis to perform oral exams, users of any medication associated with gingival enlargement and having used antibiotic/anti-inflammatory drugs in the 3 months prior to baseline exam were not included.

The study was conducted between November 2015 and February 2018 at the Federal University of Santa Maria.

### **Sample size calculation**

A sample of 14 individuals per group was estimated based on difference in the gingival index (GI) mean of 0.25 and standard deviation of 0.21 (Pinto et al. 2013), considering a power of 80%; significance level of 0.05; and attrition rate of 15%.

### **Ethical considerations**

Eligible individuals provided informed consent. This study was conducted by following the Guidelines and Norms Regulating Research involving humans. The research protocol was submitted and approved by the Research Ethics Committee of the Federal University of Santa Maria, RS, Brazil (CAAE: 50208115.9.0000.5346) and ClinicalTrials.gov (50208115.9.0000.5346).

### **Pre-experimental period**

Before starting the experimental period, eligible participants with GI=2 superior to 7.5% received oral hygiene instruction until the reference value for inclusion in the study could be reached. We arbitrarily established a percentage of gingival health and gingivitis as 7,5% and 15% of gingival bleeding (GI=2), respectively. The period between the last maintenance session and the inclusion period was approximately three months.

### **Randomization and experimental groups**

The randomization sequence was generated by a computer program (Random Allocation Software, version 2.0) and maintained confidential using serially numbered opaque envelopes. The randomization process was performed by a researcher (C.S) not involved in data collection.

Participants were randomized into three experimental groups according to the frequency of SPC:

- Group 12h: SPC every 12 hours;
- Group 24h: SPC every 24 hours;
- Group 48h: SPC every 48 hours.

In addition to brushing frequency orientations, at the randomization moment, subjects received a calendar containing the frequency and dates of SPC, and also a leaflet containing recommendations and the contact of researcher responsible for the randomization for elucidate possible query (Appendix A).

### **Experimental period**

The experimental period was of 90 days. At the baseline, participants were interviewed (Appendix B) and received prophylaxis with rubber cup (Microdont®, São Paulo, Brazil) and abrasive paste. Each individual received a kit containing: soft multi-bristle toothbrush (Colgate® Twister® Compact Head, New York, USA), dental floss (Colgate®, waxed, New York, USA) and/or interdental brush (Bitufo®, São Paulo, Brazil), dentifrice (Colgate® Maximum Protection Anticaries®, 90g, New York, USA) and a mouthwash containing 0.05% fluoride solution (Nova Derme, 1500ml, Santa Maria, Brazil).

Individuals were instructed to use the fluoride solution twice daily to provide oral freshness as a strategy to increase adhesion of individuals to SPC frequencies. The fact that individuals started the study with maximum 7.5% of sites with GI=2 was

indicative of an adequate method of SPC, regardless of the brushing technique or the type of interproximal device used.

At the time of oral hygiene, individuals were instructed to cover the width of the toothbrush bristles transversely at a single point with the dentifrice. Thus, approximately 0.5g of toothpaste would be used at each SPC frequency. After the end of the study, the dentifrice tubes were weighed (Digital Balance Scale Professional-Mini, model 1480, Tanita Corporation, Tokyo, Japan) to verify the experimental groups' adherence at the randomized frequency. Also, as a measure of adherence, questions relating to frequency compliance and possible reasons for non-compliance were applied at each session (Appendix C). At the end of the experimental period, subjects were instructed to return to their usual SPC frequencies. Maintenance periodontal procedures did not done during the experimental period; they were reestablished after final exam.

### **Early Stopping Guideline**

Individuals who reached at least 30% of sites (LANG et al., 1990) with gingival bleeding (GI=2) during the experimental period were removed from the study. These individuals were instructed for return their SPC and reexamined every seven days until the restoration of gingival health (maximum 7.5% of sites with gingival bleeding).

### **Clinical Parameters**

The clinical parameters evaluated were Plaque Index (PII) (Silness & Loe 1964 - Annex B), Gingival Index (GI) (Loe & Silness 1963, modified by Loe 1967 - Annex C), probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BoP). PII, GI were evaluated at the baseline, 15, 30 and 90 days. PD, CAL and BoP were performed at baseline, 30 and 90 days (Appendix D). PD was measured as the distance from the gingival margin to the most apical bottom of the sulcus/pocket. CAL was considered as the distance from the cemento-enamel junction the most apical sounding portion of the sulcus/pocket. PD and CAL were measured in millimeters and rounded to the nearest whole millimeter. The interproximal measurements were performed as close as possible to the contact point. BoP was recorded up to 15 seconds after PD measurement and classified into scores: 0 (absence) and 1 (presence).

All clinical parameters were evaluated in six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual) using a millimeter periodontal probe (CP 15 UNC, Neumar / Brazil), in all erupted teeth except third molars.

Clinical examinations (at days 15, 30 and 90), were performed before the patient performed their SPC. In this way, the maximum plaque scores for each frequency can be evaluated. All examinations were conducted by two blind examiners for the experimental groups. The first examiner (A.P.R) evaluated PII. The second examiner (J.M), after the patient performed their SPC, evaluated GI, PD, CAL, and BoP.

### **Training and calibration of examiners**

The examiners underwent a period of training by an experienced examiner. The training consisted of a theoretical evaluation of periodontal parameters, discussion about each score or category and possible disagreements, and it was concluded at the moment that a reasonable level of agreement and understanding about the parameters were reached.

After, an examiner (J.M) was calibrated before the start of the study for PD evaluation (Weighted Kappa=0.98) and CAL (Weighted Kappa=0.96). Intra-examiner reproducibility was evaluated in a one thousand sites, through duplicate exams with interval of one hour. Flat mouth mirror and periodontal probe (CP 15 UNC, Neumar / Brazil) were used in all examinations.

### **Adverse effects**

A questionnaire about reports of possible changes in oral tissues was applied to the participants at 30 and 90 days (Appendix E).

### **Statistical Analysis**

Data were processed and analyzed using the Statistical Package for Social Science (SPSS for Windows, version 21.0, SPSS Inc., Chicago, IL, USA). PII, GI, PD, CAL, percentage of sites with BoP and the percentage of sites with different GI scores were presented as a mean and standard error. Mixed linear models were used for the analysis and comparison of experimental groups. The best covariance structure (component symmetry) was tested. The parameters of the model were estimated through maximum probability. The level of significance was set at 5% and

the protocol strategy was used for dropouts. Patients who reached the stopping rule had their data counted in subsequent examinations.

## RESULTS

Forty-two individuals were randomized between the experimental groups; however four did not complete the study (Figure 1). Only one participant in the 48-hour group reached the early stopping guideline, this happened at 30 days of follow-up reaching 30% of the sites with GI=2. The patient was instructed to return their SPC and was followed up for two weeks, when reached again gingival health (maximum of 7.5% of the sites with GI=2).

Table 1 describes the baseline sociodemographic, behavioral and clinical characteristics. No significant differences in PD ( $P=0.94$ ), BoP ( $P=0.83$ ) and CAL ( $P=0.37$ ) were observed among the experimental groups.

Table 2 presents the mean and standard error of GI in the three experimental groups at baseline, 15, 30 and 90 days. Only the 12h group remained with stable GI means throughout the trial period, the 24h group demonstrated significant differences in GI values of 30 and 90 days to the baseline. In the 48h group, an increase in the means of GI was observed throughout the experimental period, since there was a significant difference between baseline and 15 days. Significant differences in the means of GI in the 90-day period can also be observed between 12 and 48h and 24 and 48h groups.

Figure 2 describes the percentage of GI=2 in the different groups throughout the experimental period. The 48h group showed a higher percentage of GI=2 in 90 days in comparison to the other experimental groups, indicating a higher amount of sites with gingival bleeding than 12/24h groups ( $P<0.05$ ). When percentages of GI=2 at proximal and free sites were analyzed (Figure 3 and 4), the same behavior pattern was observed.

Concerning the dental plaque (Table 3), baseline PII data showed no significant differences between groups. However, at the end of the experimental period, the 48h group were significant differences ( $P<0.05$ ) in the PII mean in relation to the other groups. Intra-group data revealed that in the 12 and 24h groups, a significant change in plaque means occurred only in 15 days, which remained stable throughout the 90 days of the study. In subjects who performed SPC at 48h, significant changes in PII means occurred in 15 and again in 90 days.

The total amount of dentifrice used during the study was 92g, 50g and 55g for 12, 24 and 48h groups, respectively. Table 4 presents the adverse effects mentioned during the periods of 30 and 90 days, with similar number of observations between the groups during the study.



## DISCUSSION

This randomized clinical trial demonstrated that subjects with a history of periodontitis previously treated and participating in a periodic preventive maintenance program that performed SPC every 12 or 24 hours maintained levels of gingival health for until 90 days. In contrast, subjects who performed SPC every 48 hours demonstrated a significant increase in gingival inflammation.

Recent evidence observed the relationship of different frequencies of SPC with the maintenance of gingival health in healthy individuals with no history of periodontitis (De Freitas et al., 2016; Pinto et al., 2013). However, up to the present time, there was no evidence about the effect of these different frequencies on individuals with a history of periodontitis, which are individuals known to be more susceptible to periodontitis. In this context, our study provides clinical evidence that SPC performed at 12 and 24h were also compatible with the maintenance of gingival health (maximum 15% of sites with GI=2) for individuals with a history of periodontitis.

This study corroborated with the results found in Pinto et al. (2013) and De Freitas et al. (2016), where the 12 and 24h SPC intervals were compatible with gingival health, although the studies samples had distinct characteristics and differences in susceptibility to periodontitis. However, in the findings of Lang et al. (1973), 48h interval was compatible with the maintenance of gingival health, and in our study, this interval showed a significant increase in gingival inflammation. This discrepancy can be explained by the methodological differences of Lang et al. (1973), where the tooth brushing was performed under the supervision, and the plaque was stained and entirely removed at each brushing interval; also the sample was composed of dental students.

When changes in the GI means were analyzed over the experimental period, the 48h group was the only one that presented a significant increase already in 15-day follow-up and it was maintained during period experimental, of 90 days differing significantly of the other experimental groups. In the 24h group only after 30 days, GI means differed from the baseline and 15 days. Just in the 12h group, no significant differences were found in GI means throughout the experimental period.

There is no consensus in the literature to which cutoff points in the inflammatory parameters should be considered for determining clinically significant

gingivitis. We arbitrarily established a percentage of gingival health and gingivitis as 7.5% and 15% of gingival bleeding (GI=2), respectively. Within this standard, the 12 and 24h groups maintained gingival health (percentage of sites with GI=2 of 10.71% and 8.09%, respectively) at the end of the experimental period. However, the 48h group completed the experiment with a percentage of sites with GI=2 of 18.83%, showing twice more gingival inflammation in comparison with the other. The same behavior pattern was demonstrated when changes in the percentage of GI=2 were analyzed in free and proximal sites.

Although the 12 and 24h groups did not reach the percentage of gingivitis and did not present a significant difference in the GI means between them at the end of 90 days, both groups had an increase in these and the 12h group presented higher means compared to the 24h group. This can be explained by the significant difference observed between the groups in the GI means at the baseline. The 12h group started the experimental period with the highest GI mean (highest percentage of sites with GI=1 for the calculation of mean) and maintained their means stable throughout the experiment while in the 24h group an increase was observed in the GI means. Already at 15 days and over the 90 days, there was no significant difference in the GI means between groups 12 and 24h. The same explanation can be considered for the absence of significant differences between the GI means in the 12 and 48h groups in the periods of 15 and 30 days.

The PII means differed significantly at the end of 90 days between subjects who performed SPC at 48h and who performed at 12/24h, with higher mean values in the 48h group. Intra-group analyses showed an increase in PII means over 15 days in the 12 and 24h groups, however over 90 days plaque means remained stable in these groups. In the 48h group there was a significant increase in PII averages at 15 days and again at the end of 90 days, differing significantly from all experimental periods. Probably at 48h group higher plaque levels were associated with increase on gingival bleeding. De David et al. (2018) evaluated the correlation between plaque accumulation (PII) and gingival inflammation (GI) in subjects with gingival health and no history of periodontitis who performed effective oral hygiene at different frequencies for 30 days. The correlation between PII and GI during this period was maintained only in the groups of 48 and 72 hours, in which the increase in the plaque means increased the gingival inflammation mean. Corroborating with our findings and reinforcing that the 48h interval may have been sufficient for allowing microbial

succession and plaque formation containing more pathogenic bacteria, resulting in the presence of clinically significant gingivitis in this group.

Strengths of the study include the experimental design since a recent systematic review recommended that RCTs be performed to answer this question (Tonetti et al., 2015). The 90 day experimental period may also be considered a strength of the present study, since other studies on the subject are restricted to a period of 30-45 days of follow-up (De Freitas et al., 2016; Lang et al., 1973; Kelner et al., 1974; Pinto et al., 2013).

The limitations of our study include the impossibility of effectively evaluating the actual frequency at which individuals performed the SPC procedures. As a measure to assess adherence to the randomized frequencies, at the end of the experiment the tubes of dentifrice were weighed and revealed that only in the 48h group the use of the dentifrice was superior to that recommended. This indicates that the individuals in this group used, at each brushing, more dentifrice than the one oriented or they performed the brushing at a higher frequency than the randomized one. However, this difference was not able to interfere in the results, as we observed the distinction between the 12/24h groups and the 48h group, both in the plaque and in the supragingival inflammatory parameters. An additional measure of adherence used was the questioning, at each session, regarding compliance with frequencies and what possible reasons for non-compliance. There was no report of noncompliance that could interfere in the different frequencies randomized. Another important limitation is the strict criteria of maximum 7.5% of sites with GI=2 used to include participants. Due to this, there is a reduction in the external validity of our findings, restricting the inference of results for individuals with a history of periodontitis with an adequate standard of oral hygiene.

In conclusion, SPC performed every 12 or 24 hours is enough to maintain gingival health in individuals with a history of periodontitis, treated and participating in a periodic preventive maintenance program.

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## TABLES

Table 1 – Sociodemographic, behavioral and clinical parameters of experimental groups at baseline

Parameter		12h group (n=14)	24h group (n=14)	48h group (n=14)	P
<b>Age (<math>\bar{x} \pm sd</math>)* years</b>		56.35±7.22	56.42±7.76	59.92±8.09	0.38
<b>Gender n(%)#</b>					0.31
Male		8(57.14)	4(28.57)	6(42.86)	
Female		6(42.96)	10(71.53)	8(57.14)	
<b>Socioeconomic level n(%)#</b>					
Family monthly income	≤1 Brazilian minimum wage	3(21.43)	2(14.28)	4(28.57)	0.65
	≥2 Brazilian minimum wage	11(78.57)	12(85.72)	10(71.53)	
Educational level (years of study)	≤ 8 years	6 (42.96)	5(35.71)	7(50.00)	0.74
	> 8 years	8 (57.14)	9(64.29)	7(50.00)	
<b>Behavioral n(%)#</b>					
Daily frequency of toothbrushing	Up to two	2(14.28)	5(35.71)	3(21.43)	0.39
	≥ 3 times	12(85.72)	9(64.29)	11(78.57)	
Interdental device frequency (*weekly)	Once*	-	1(7.15)	1(7.15)	0.68
	2-3 times*	3(21.43)	1(7.15)	2(14.28)	
	4-5 times*	1(7.15)	1(7.15)	-	
	Daily	7(50.00)	8(57.14)	5(35.71)	
	2 times a day	2(14.28)	1(7.15)	1(7.15)	
	≥ 3 times a day	1(7.15)	2(14.28)	5(35.71)	
Interdental device type	Dental floss	2(14.28)	-	6(42.96)	0.03
	Interdental brush	3(21.43)	2(14.28)	-	
	More than one device	9(64.29)	12(85.72)	8(57.14)	
<b>Clinics (<math>\bar{x} \pm sd</math>)*</b>					
GI=2 (%)		4.99±1.89	4.24±2.26	5.50±2.28	0.30
PD (mm)		2.36±0.20	2.33±0.25	2.34±0.26	0.94
BoP (%)		13.52±3.45	14.00±4.52	13.05±4.27	0.83
CAL (mm)		2.93±0.82	3.14±0.81	3.44±1.19	0.37

GI = Gingival Index; PD= Probing depth; BoP = Bleeding on probing; CAL = Clinical attachment level.

\* ANOVA (post hoc test Tukey)

# chi-square statistics

Table 2 – Gingival Index mean (standard error) during the experimental period, for each experimental group

		<b>12h group (n=13)</b>	<b>24h group (n=13)</b>	<b>48h group (n=12)</b>
<b>GI</b>	<i>Baseline</i>	0.74(0.05) <sup>A,a</sup>	0.54(0.05) <sup>B,a</sup>	0.60(0.05) <sup>AB,a</sup>
	15 days	0.79(0.05) <sup>AB,a</sup>	0.65(0.05) <sup>A,ab</sup>	0.86(0.06) <sup>B,b</sup>
	30 days	0.82(0.05) <sup>AB,a</sup>	0.69(0.05) <sup>A,b</sup>	0.97(0.06) <sup>B,bc</sup>
	90 days	0.85(0.05) <sup>A,a</sup>	0.77(0.05) <sup>A,b</sup>	1.09(0.06) <sup>B,c</sup>

Mixed linear models analysis,

Different uppercase letters demonstrate intergroup differences (P<0.05)

Different lowercase letters show intragroup differences (P<0.05)

Table 3 – Plaque Index means (standard error) during the experimental period, for each experimental group

		<b>12h group (n=13)</b>	<b>24h group (n=13)</b>	<b>48h group (n=12)</b>
<b><i>PII</i></b>	<i>Baseline</i>	0.29(0.07) <sup>A,a</sup>	0.16(0.07) <sup>A,a</sup>	0.28(0.07) <sup>A,a</sup>
	15 days	0.51(0.07) <sup>A,b</sup>	0.42(0.07) <sup>A,b</sup>	0.79(0.08) <sup>B,b</sup>
	30 days	0.49(0.07) <sup>AB,b</sup>	0.36(0.07) <sup>A,b</sup>	0.69(0.08) <sup>B,b</sup>
	90 days	0.53(0.07) <sup>A,b</sup>	0.46(0.07) <sup>A,b</sup>	1.09(0.08) <sup>B,c</sup>

Mixed linear models analysis

Different uppercase letters demonstrate intergroup differences (P <0.05)

Different lowercase letters show intragroup differences (P <0.05)

Table 4 – Number of self-reported adverse effects during the experimental period

<b>Experimental period</b>	<b>Adverse effects</b>	<b>12h group (n=13)</b>	<b>24h group (n=13)</b>	<b>48h group (n=12)</b>
<b>30 days</b>	“Rough” teeth	1	3	5
	Dentinal sensitivity	-	1	1
	Painful sensation in the gums	-	1	-
	Gingival edema	-	-	1
	Gingival bleeding	2	-	1
	Bad taste in the mouth	2	4	4
	Mouth ulcers	-	1	-
	“Rough” teeth	-	1	3
<b>90 days</b>	Dentinal sensitivity	1	-	1
	Painful sensation in the gums	1	1	1
	Gingival edema	-	1	-
	Gingival bleeding	1	1	4
	Gingival redness	1	-	-
	Bad taste in the mouth	3	4	5
	Sensation of "tight teeth"	-	-	1

**FIGURE LEGENDS**

Figure 1: Study Flowchart.

Figure 2: Changes in the percentage of total sites with GI=2 in the different groups throughout the experimental period.

Figure 3: Changes in the percentage of free sites with GI=2 in the different groups throughout the experimental period.

Figure 4: Changes in the percentage of proximal sites with GI=2 in the different groups throughout the experimental period.

## FIGURES

Figure 1

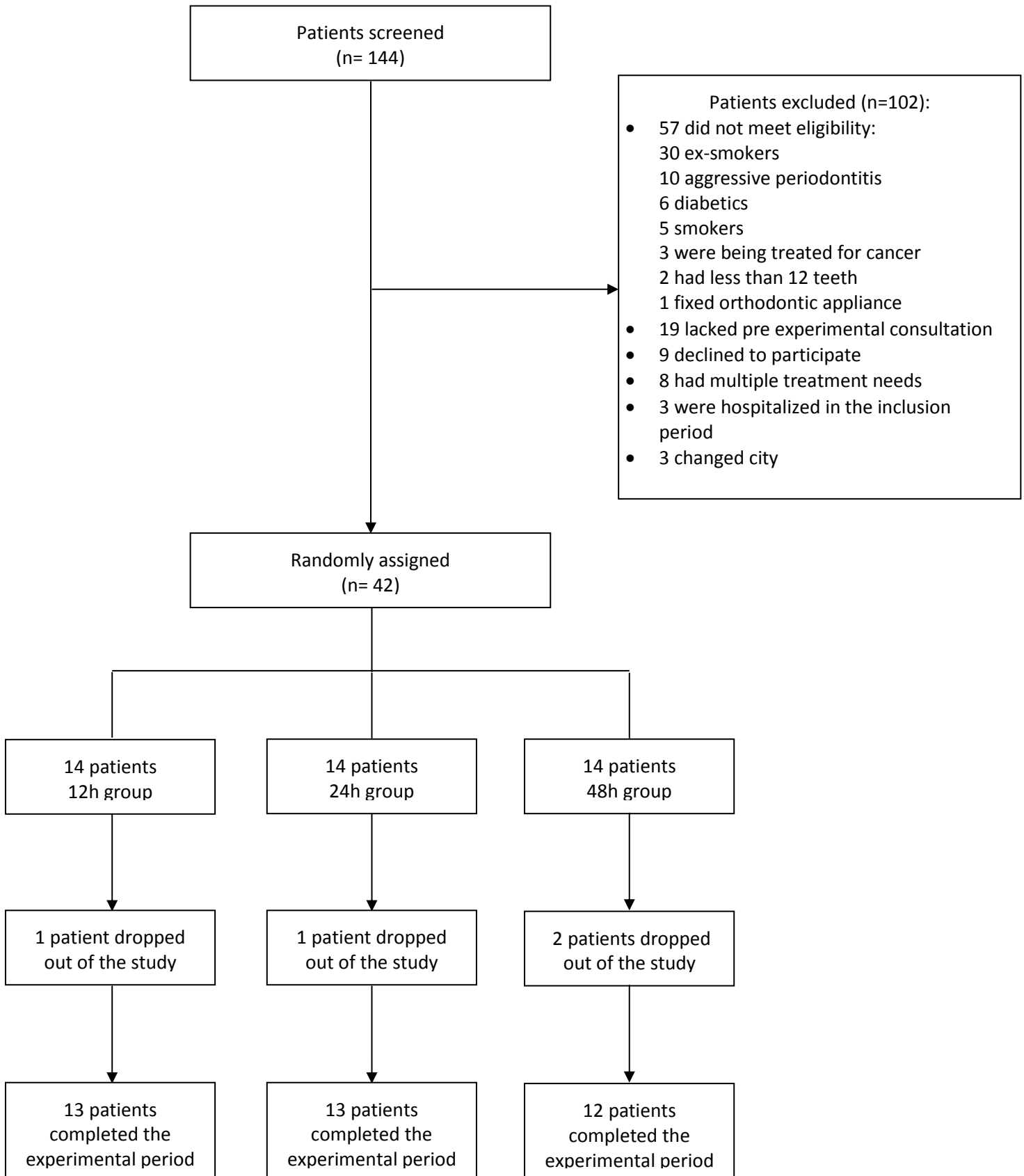


Figure 2

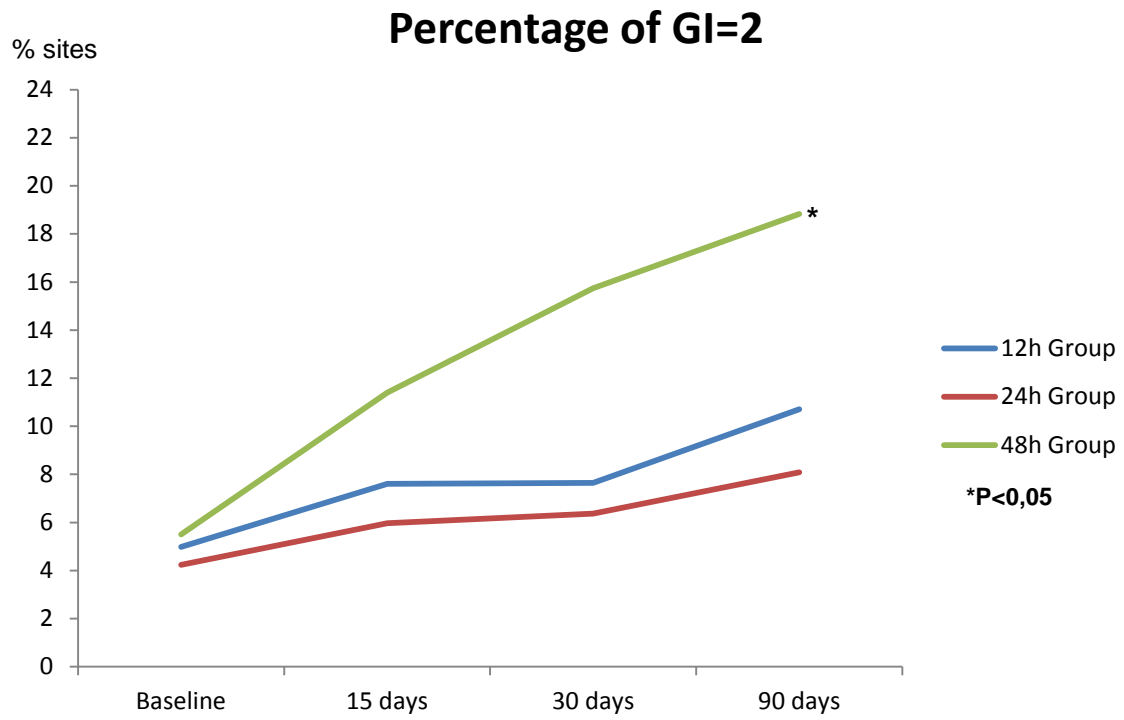


Figure 3

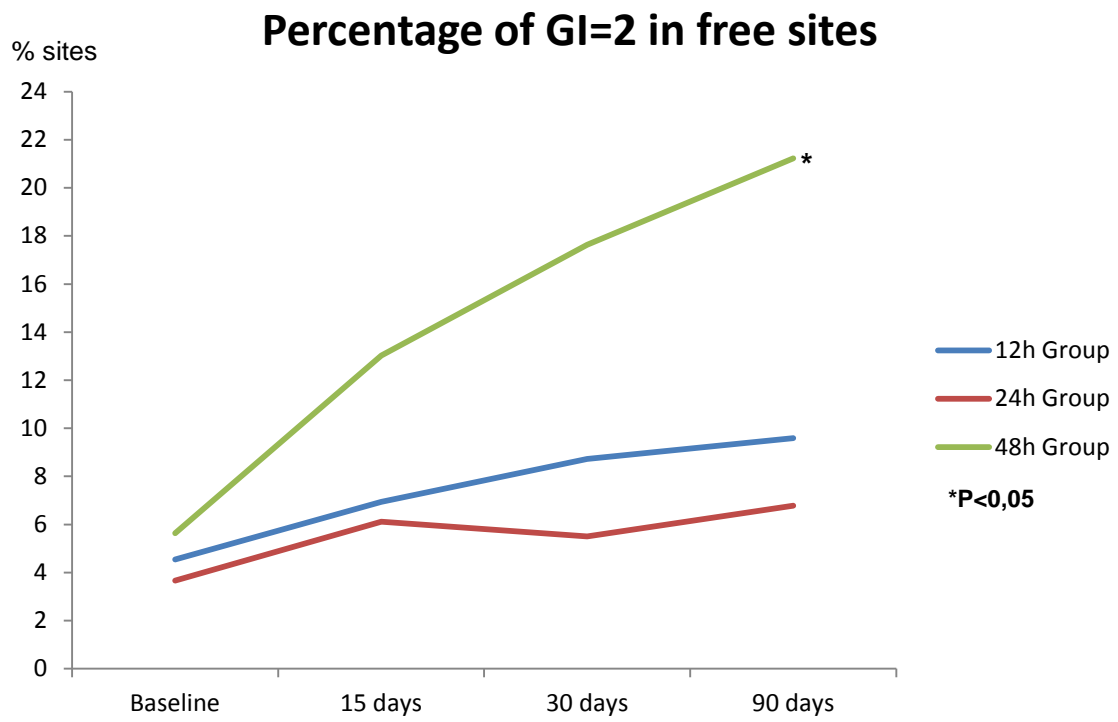
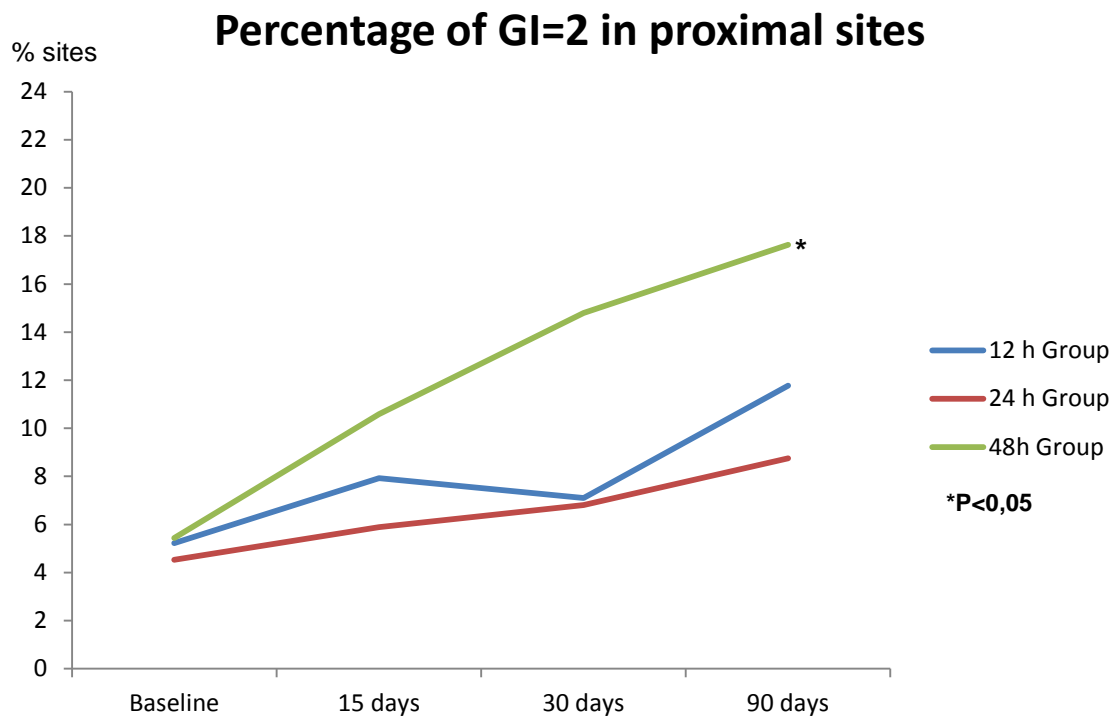




Figure 4



**3 ARTIGO 2 – CORRELATION BETWEEN GINGIVAL INDEX AND BLEEDING ON PROBING IN PATIENTS WITH A HISTORY OF PERIODONTITIS IN DIFFERENT SELF-PERFORMED MECHANICAL PLAQUE CONTROL FREQUENCIES.**

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**Juliana Maier<sup>1</sup>, Ana Paula Pereira Reiniger<sup>1</sup>, Karla Zanini Kantorski<sup>1</sup>, Carlos Heitor Cunha Moreira<sup>1</sup>**

<sup>1</sup>Divisão de Periodontia, Departamento de Estomatologia, Faculdade de Odontologia, Federal Universidade de Santa Maria, Santa Maria, Brasil.

Autor correspondente:

Carlos Heitor Cunha Moreira

Rua Serafim Valandro, 1514/302, 97015-630, Santa Maria /RS, Brasil.

Telefone: +55.55.3025.6538

Email: carlosheitor@rosuline.com.br

## ABSTRACT

**Aim:** To elucidate how different self-performed mechanical plaque control frequencies intervene with subgingival inflammatory parameters this study aims to evaluate the correlation between gingival bleeding and bleeding on probing (BoP) in the different groups of SPC in individuals with history of periodontitis.

**Materials and Methods:** Forty-two individuals with history of periodontitis and participants of periodontal maintenance (maximum 7.5% of sites with gingival bleeding) were randomized to different frequencies of SPC: 12, 24 and 48 hours. Gingival index (GI) and BoP were evaluated at baseline, 30 and 90 days. Intragroup differences were determined using repeated measures ANOVA. Intergroup differences at baseline were verified using a chi-square test and independent t-test. The Spearman correlation coefficient between GI and BoP for different groups was calculated at baseline, 30 and 90 days.

**Results:** Greater changes in correlation were observed between baseline and 90 days for the 48-hour group. Statistically significant positive correlations were found between GI and BoP means in all groups and increased throughout the experimental period, but only 48h group presented a moderate correlation between GI and BoP at 90 days ( $r=0.34$ ). When the correlation between GI and BoP in shallow pockets ( $PD \leq 3$  mm) were evaluated, at 30 days all groups showed a statistically significant positive correlation and only in group 48h the correlation was moderate ( $r=0.31$ ). At the end of 90 days, there was a decline in correlation in the 12h group, and an increase in the other groups, being a moderate correlation in the 48h group ( $r=0.36$ ) and weak in the different experimental groups.

**Conclusion:** Performing the SPC every 48 hours resulted in a significant increase in the means of GI=2 compared to other groups. These marginal inflammatory changes had repercussions on the subgingival environment through an increase in BoP, demonstrated by the moderate statistically significant correlation between GI=2 and BoP in this experimental group.

**Keywords:** oral hygiene; gingivitis; gingival index; bleeding on probing; correlation

## INTRODUCTION

Periodontitis are diseases with high prevalence in different populations (Murray et al., 2012; Eke et al., 2012; Susin et al., 2004). They are caused by the accumulation of subgingival plaque that elicits an immunoinflammatory response which can activate the mechanisms of tissue destruction. Clinically, the presence of inflammation resulting from subgingival plaque is detected through the presence of bleeding at the bottom of the periodontal pocket (Löe et al., 1986; Page et al., 1997). Through the natural history of gingivitis and periodontitis, gingival inflammation precedes periodontitis (Lindhe, Hamp, Löe, 1975, Löe et al., 1986). The events sequence occurs through sequential supragingival colonization eliciting marginal inflammation and later, subgingival colonization with the consequent spread of inflammation to more apical periodontal regions (Page & Schroeder, 1976). Therefore, controlling gingivitis is a strategy of primary prevention of periodontitis and secondary prevention for its recurrence (Chapple et al, 2015).

Randomized clinical trials (RCTs) demonstrated that self-performed mechanical plaque control (SPC) at 12 and 24 hours were compatible with the maintenance of gingival health (Pinto et al., 2013, De Freitas et al., 2016). Similar results were found in a recent RCT using a sample of individuals with a history of periodontitis, treated and included in a periodic preventive maintenance program (Maier et al., 2018). Higher frequencies led to changes in gingival inflammatory parameters, but the effect of these alterations on the subgingival environment is not known. Sites with presence of gingival inflammation present a significantly higher risk of attachment loss over time when compared to healthy sites (Schätzle et al., 2003). In the presence of a gingival inflammatory response, other mechanisms may intervene, such as nutritional and ecological factors, favoring the establishment and development of the subgingival plaque (Weidlich et al., 2001) and, in susceptible individuals, this plaque will lead to the consequent inflammation, detected through of bleeding on probing, and higher probability of disease activity (Lang et al., 1986).

In assessing the correlation between plaque and gingival bleeding at different SPC frequencies, De David et al. (2018) demonstrated that gingival health was maintained in the groups with 12 and 24h SPC intervals despite increase plaque scores. Groups that performed SPC every 48 and 72 hours demonstrated that prolonged intervals of oral hygiene allowed qualitative and quantitative changes in

the plaque, favoring the establishment of pathogenic species beginning a clinical gingival inflammatory response. However, there is no evidence in the literature regarding the reflex of these different SPC frequencies in the subgingival environment. In other words, how different frequencies of oral hygiene interfere with changes in gingival inflammatory status and how these may be correlated with changes in subgingival inflammatory status.

Therefore, to elucidate how different SPC frequencies can cause changes on subgingival inflammatory parameters, this study aimed to evaluate the correlation between gingival bleeding (GI=2) and bleeding on probing (BoP) in the different SPC frequencies in individuals with a history of periodontitis.

## **MATERIALS AND METHODS**

### **Study design**

This study represents a secondary analysis of a randomized clinical trial performed between November 2015 and February 2018 at the Post Graduate Periodontics Clinic, the Federal University of Santa Maria (Maier et al., 2018). The methodology of the original article was briefly described.

### **Sample**

Individuals diagnosed with periodontitis (Tonetti & Claffey, 2005), treated at the Post-Graduation Clinic of the Federal University of Santa Maria (UFSM, Rio Grande do Sul, Brazil) and included in the periodontal maintenance program were eligible. Patients included in the maintenance program were regularly recalled every 4-6 months. Individuals should present 35 years or more of age and at least 12 teeth in the mouth, maximum 7.5% of sites with GI=2 and 25% of sites with bleeding on probing (BoP). Smokers, pregnant women, diabetics, individuals presenting xerostomia, psychomotor disorders, patients with a fixed orthodontic appliance, who needed antimicrobial prophylaxis to perform oral exams, users of any medication associated with gingival enlargement and having used antibiotic/anti-inflammatory drugs in the 3 months prior to baseline exam were not included.

### **Ethical considerations**

After an explanation of the purposes of the study, eligible individuals signed their informed consent. This study was conducted by following the Guidelines and Norms Regulating Research involving humans. The research protocol was submitted and approved by the Ethics Committee in Research of the Federal University of Santa Maria, RS, Brazil (CAAE: 50208115.9.0000.5346) and ClinicalTrials.gov (50208115.9.0000.5346).

### **Pre-experimental period, randomization and experimental period**

Before starting the experimental period, eligible patients with more than 7.5% of sites with GI=2 received oral hygiene instruction until the reference value for inclusion in the study was reached. When patients met these criteria, they received coronal polishing and were randomized to perform mechanical plaque control (SPC)

at 12-, 24- or 48-h interval. The randomization of patients was generated by a computer program (Random Allocation Software, version 2.0) and maintained confidential using opaque envelopes. The experimental period was 90 days. At baseline, all subjects received a soft multi-bristle toothbrush (Colgate® Twister® Compact Head, New York, USA), dental floss (Colgate®, tarpaulin, New York, USA) and/or interdental brush (Bitufo®, São Paulo, Brazil) fluoride dentifrice (Colgate® Anticaries Protection, 90g, New York, USA) and mouthwash containing fluoride solution (Nova Derme, 1500ml, Santa Maria, Brazil). Dentifrice quantity was standardized (a single point across the brush, approximately 0.5 g). Subjects received a schedule containing the days they should not perform SPC and with the days of the return visits. At the end of the study, patients were instructed to return their habitual SPC and dentifrice tubes were collected and weighed to assess compliance (Digital Balance Scale Professional-Mini, model 1480, Tania Corp, Japan). Subjects who reached a percentage of 30% (Lang et al., 1990) of the sites with gingival bleeding (GI score 2) during the experimental period were removed from the study. These individuals were instructed for return their SPC and examined every seven days until returned of gingival health (maximum of 7.5% of sites with gingival bleeding).

### **Clinical parameters**

Plaque index (PII) (Silness & Løe, 1964), gingival index (GI) (Løe & Silness 1963, modified by Løe 1967), probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BoP) were assessed at six sites per tooth excluding third molars. PII, GI, PD, CAL, and BoP were assessed at the baseline, 30 and 90 days. The data collection was performed by two previously trained and calibrated examiners, as well as blinded to the experimental groups to which the participants were allocated.

### **Statistical analysis**

For the statistical analysis, SPC intervals of 12, 24 and 48h were analyzed individually. Mean and standard deviation (SD) of GI and BoP was calculated to verify clinical parameters relative to SPC intervals. Intragroup differences were determined using repeated measures ANOVA. Intergroup differences at baseline were verified using ANOVA (post hoc Tukey) and chi-square test. The Spearman

correlation coefficient between GI and BoP for both groups was calculated at baseline, 30 and 90 days. Normal distribution was confirmed using Kolmogorov-Smirnov test. Statistical analysis was performed using statistical software (SPSS, version 21.0, Chicago, IL, USA). The significance level was set at 5%. The primary outcome of the study was correlation between GI=2 and BoP.



## RESULTS

Demographic and clinical characteristics of the participants at baseline are shown in Table 1. Groups did not differ statistically in age, gender, and periodontal clinical parameters. A significant increase in the GI=2 means in the 48h group in relation to the other groups can be observed at the 30 days of the experiment. This difference was maintained at the end of 90 days (Table 2). The same pattern was demonstrated between the groups when evaluating only sites with PD≤3mm. (Table 3). Regarding BoP, the groups did not differ between them during the experimental period (Table 2). The same absence of difference was observed when analyzes were made for PD≤3mm (Table 3).

When changes in GI intra-group were evaluated, all groups showed a significant difference of GI=2 at the end of 90 days compared to the baseline. The same pattern occurred when were evaluated pockets with PD≤3mm. Regarding BoP, 12 and 48h groups presented significant differences between baseline and 90 days. Only the 24h group maintained their similar BoP means over the experimental period (Table 2 and 3).

In the evaluation of the correlation between GI and BoP for each group, higher changes were observed between baseline and 90 days for the 48h group (Table 4). At 30 days, 12 and 48h groups had the most considerable variations regarding the baseline, with an increasing in the correlation in both groups, however still showing a weak correlation between GI and BoP. Statistically significant positive correlations were observed between GI and BoP means in all groups and increased throughout the experimental period, but only group 48h presented a moderate correlation ( $r=0.34$ ) between GI and BoP at 90 days (Table 4).

Concerning the correlation between GI and BoP in shallow pockets (PD≤3mm), at 30 days the 12 and 48h groups showed the greatest changes about the baseline (Table 5). However, all groups showed a statistically significant positive correlation and only in group 48h the observed correlation was moderate ( $r=0.31$ ). At the end of 90 days, there was a decline in correlation in the 12h group and an increase in the other groups. A moderate correlation in the 48h group ( $r=0.36$ ) and weak correlations in the 12 and 24h groups were found (Table 5).

## DISCUSSION

This study evaluated the correlation between gingival bleeding (GI=2) and bleeding on probing in individuals with history of periodontitis performing different SPC frequencies. Our study demonstrated that the correlation between GI=2 and BoP is influenced by different SPC frequencies. Higher correlation changes were found between baseline and 90 days in the 48h group. This result can be explained by the significant increase in the means of GI=2 in the 48h group at the end of the experimental period, that is, the increase in gingival bleeding was accompanied by an increase in bleeding on probing. Sites with gingival bleeding may have created greater conditions for the establishment of a subgingival plaque, and this allow an inflammatory response of the host, observed through bleeding on probing. In shallow pockets ( $PD \leq 3$  mm), an even higher correlation was found in the 48h group at the end of 90 days. In groups 12 and 24 hours, a lower correlation was found throughout the experimental period at same time that, in both groups, minor changes in the means of GI=2 were observed. So, in shallow pockets, the same pattern can be observed.

Although there is biological plausibility for the relationship between gingival bleeding and bleeding on probing, there are still doubts about the exact relationship between the two parameters and how different SPC frequencies may interfere in this correlation by facilitating colonization of the subgingival environment and the subsequent inflammatory response to the presence of subgingival plaque. Studies suggest that the marginal inflammation results in false-positive determinations of bleeding when probing in shallow pockets, or even that shallow pockets may be traumatized during probing (Caton et al. 1981; Karaiyannis et al., 1992). In our study, when we verified the percentage of shallow pockets that had gingival bleeding throughout the experimental period, the 48h group presented significant differences about the 12/24h groups. This factor may be one of the possible explanations for the highest correlation found at the end of 90 days in the 48h group. However, Chaves et al. (1993) evaluating individuals with gingivitis found different percentages of agreement between gingival bleeding and BoP, where these percentages varied depending on the depth of probing at the individual sites. They reported that lower percentages of an agreement were found in shallow ( $PD < 2$ mm) sites and concluded

that, even in shallow pockets, stimulation of the probe in the gingiva is not able to touch the apical part of the pocket and that GI and BoP are not interchangeable measurements bleeding tendency.

Following this line of thinking, the other possibility for the highest correlation found in our study at the end of 90 days in the 48h group is that the increase in supragingival plaque, evidenced by the increase in the presence of sites with GI=2, was associated with an increase in bleeding on probing probably due to the presence of the subgingival plaque. Corroborating with our findings, De David et al. (2018) showed that the correlation between plaque formation and gingival health is affected by oral hygiene frequency. Individuals who performed high standards of oral hygiene at 12 and 24-hour intervals maintained gingival health different for subjects who performed longer hygiene intervals. These results reinforce our findings and allow us to understand that mature microbial dental plaques that persist for long periods of time without disruption to their development are not compatible with gingival health and, consequently, may allow colonization of the subgingival environment, leading to the development of a subgingival inflammatory response expressed through BoP.

One of the limitations of our study was the inclusion of individuals with maximum 7.5% of GI=2, reducing the external validity since the results can be inferred only for individuals with a history of periodontitis and a high standard of oral hygiene. Another limitation is the sample number because it is a secondary analysis of a RCT and the sample calculation was not performed for the aim of this study, besides the monitoring period that may have been small and insufficient to verify the real expression of different frequencies of SPC in the subgingival environment.

In summary, in the evaluation of different frequencies of SPC in the subgingival environment, we concluded that performing the SPC every 48 hours in patients with history of periodontitis and in periodontal maintenance resulted in a significant increase in the means of GI=2 compared to other groups. These marginal inflammatory changes had repercussions on the subgingival environment measured by an increase in bleeding on probing (BoP), demonstrated by the moderate and statistically significant correlation between GI=2 and BoP in this experimental group.

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## TABLES

Table 1 – Group demographics and clinical parameters at baseline.

<b>Parameter</b>	<b>12h Group (n=14)</b>	<b>24h Group (n=14)</b>	<b>48h Group (n=14)</b>	<b>P</b>
<b>Age (<math>\bar{x} \pm sd</math>)* years</b>	56.35±7.22	56.42±7.76	59.92±8.09	0.38
<b>Gender n(%)<sup>#</sup></b>				0.31
Male	8(57.14)	4(28.57)	6(42.28)	
Female	6(42.86)	10(71.43)	8(57.14)	
<b>Periodontal parameters (<math>\bar{x} \pm sd</math>)*</b>				
PD (mm)	2.36±0.20	2.33±0.25	2.34±0.26	0.94
BoP (%)	13.52±3.45	14.00±4.52	13.05±4.27	0.83
CAL (mm)	2.93±0.82	3.14±0.81	3.44±1.19	0.37

PD= probing depth; BoP= Bleeding on probing; CAL= Clinical attachment level

\* ANOVA (post hoc test Tukey)

<sup>#</sup> chi-square statistics

Table 2 – GI=2 and BoP mean  $\pm$  SD, for each experimental group at baseline, 30 and 90 days.

	GI=2 (% sites)			BoP (% sites)		
	Baseline	30	90	Baseline	30	90
<b>G12</b>	4.79 $\pm$ 1.81 <sup>A,a</sup>	7.64 $\pm$ 4.47 <sup>A,b</sup>	10.71 $\pm$ 5.93 <sup>A,c</sup>	13.60 $\pm$ 3.58 <sup>A,a</sup>	16.13 $\pm$ 5.82 <sup>A,ab</sup>	20.41 $\pm$ 8.72 <sup>A,b</sup>
<b>G24</b>	4.42 $\pm$ 2.24 <sup>A,a</sup>	6.37 $\pm$ 4.76 <sup>A,ab</sup>	8.09 $\pm$ 5.65 <sup>A,b</sup>	14.30 $\pm$ 4.57 <sup>A,a</sup>	15.46 $\pm$ 5.85 <sup>A,a</sup>	16.86 $\pm$ 6.76 <sup>A,a</sup>
<b>G48</b>	5.49 $\pm$ 2.32 <sup>A,a</sup>	15.74 $\pm$ 10.12 <sup>B,b</sup>	18.83 $\pm$ 10.32 <sup>B,b</sup>	13.30 $\pm$ 4.60 <sup>A,a</sup>	22.15 $\pm$ 9.83 <sup>A,b</sup>	25.32 $\pm$ 10.99 <sup>A,b</sup>

\* ANOVA (post hoc Tukey test)

Different uppercase letters demonstrate intergroup differences (P <0.05)

Different lowercase letters show intragroup differences (P <0.05)

Table 3 – GI=2 and BoP mean ( $\pm$ SD) in sites with PD $\leq$ 3mm according to individual group at baseline, 30 and 90 days.

	GI=2 (%)			BoP (%)		
	Baseline	30	90	Baseline	30	90
<b>G12</b>	4.07 $\pm$ 2.01 <sup>A,a</sup>	7.59 $\pm$ 5.39 <sup>A,b</sup>	9.50 $\pm$ 6.47 <sup>A,b</sup>	10.37 $\pm$ 4.10 <sup>A,a</sup>	12.98 $\pm$ 6.55 <sup>A,ab</sup>	16.76 $\pm$ 8.72 <sup>A,b</sup>
<b>G24</b>	4.27 $\pm$ 2.49 <sup>A,a</sup>	6.32 $\pm$ 4.87 <sup>A,ab</sup>	7.60 $\pm$ 5.40 <sup>A,b</sup>	10.87 $\pm$ 4.21 <sup>A,a</sup>	12.57 $\pm$ 5.94 <sup>A,a</sup>	14.03 $\pm$ 6.97 <sup>A,a</sup>
<b>G48</b>	4.99 $\pm$ 2.33 <sup>A,a</sup>	15.23 $\pm$ 9.91 <sup>B,b</sup>	18.01 $\pm$ 10.18 <sup>B,b</sup>	10.92 $\pm$ 3.75 <sup>A,a</sup>	19.25 $\pm$ 9.66 <sup>A,ab</sup>	22.84 $\pm$ 10.84 <sup>A,b</sup>

\* ANOVA (post hoc Tukey test)

Different uppercase letters demonstrate intergroup differences (P <0.05)

Different lowercase letters show intragroup differences (P <0.05)



Table 4 - Correlation between GI=2 and BoP according to individual group at baseline, 30 and 90 days.

	<b>Baseline</b>	<b>30</b>	<b>90</b>
<b>G12</b>	0.16*	0.26*	0.27*
<b>G24</b>	0.18*	0.20*	0.28*
<b>G48</b>	0.20*	0.29*	0.34*

\*Spearman correlation coefficient ( $p < 0.01$ )

Table 5 – Correlation between GI=2 and BoP in sites with PD≤3mm according to individual group at baseline, 30 and 90 days.

	<b>Baseline</b>	<b>30</b>	<b>90</b>
<b>G12</b>	0.14*	0.28*	0.26*
<b>G24</b>	0.18*	0.22*	0.29*
<b>G48</b>	0.17*	0.31*	0.36*

\*Spearman correlation coefficient ( $p < 0.01$ )

## 4 DISCUSSÃO

A presente tese se propôs, por meio de dois artigos científicos, responder questões a respeito do efeito da frequência do autocontrole mecânico da placa em parâmetros supra e subgingivais de pacientes com histórico de periodontite, tratados e em manutenção periodontal (MPP). Uma recente revisão sistemática recomendou o desenvolvimento de ensaios clínicos randomizados para responder esta questão (TONETTI et al., 2015). Até o presente momento, havia uma carência na literatura sobre o efeito de diferentes frequências de autocontrole mecânico de placa em indivíduos com condições anatômicas dentárias distintas, alta suscetibilidade a periodontite e necessidade de uma estabilidade pós tratamento periodontal. Os estudos anteriormente realizados sobre essa temática utilizaram pacientes sem histórico de periodontite.

Em relação aos resultados encontrados, o primeiro artigo demonstrou que indivíduos, com histórico de periodontite, previamente tratados e participando de um programa de MPP que realizaram o autocontrole de placa a cada 12 ou 24 horas mantiveram níveis de saúde gengival durante um período de 90 dias. Em contrapartida, indivíduos que realizaram o autocontrole de placa a cada 48 horas demonstraram um aumento significativo na inflamação gengival.

Convencionalmente e conforme recomendações da American Dental Association (ADA), dentistas orientam seus pacientes a realizarem a escovação duas vezes ao dia para prevenir cárie e gengivite, embora exista evidência limitada dessa recomendação no que diz respeito à manutenção da saúde gengival (CHAPPLE et al., 2015). Estudos anteriores também demonstraram que intervalos de 12 e 24 horas de ACMP foram compatíveis com saúde gengival, embora as amostras utilizadas tenham características e suscetibilidade distintas da amostra utilizada no presente estudo (PINTO et al., 2013; DE FREITAS et al., 2016). Entretanto, nos achados de Lang et al. (1973) o intervalo de 48 horas mostrou-se compatível com a manutenção de saúde gengival, e no nosso estudo esse intervalo mostrou um aumento significativo de inflamação gengival. Isso pode ser explicado pela diferença metodológica do estudo de Lang et al (1973), onde o ACMP era realizado sob supervisão e a placa corada e removida a cada intervalo de escovação, além da amostra ser composta por estudantes de odontologia.

Através do resultado obtido nesse estudo podemos, baseados em evidências, orientar indivíduos com histórico de periodontite, tratados e em MPP a controlarem a placa a cada 12 ou 24 horas sem que ocorram alterações significantes nos níveis de saúde gengival. Outro fator que deve ser levado em consideração é a possibilidade de maior aderência dos pacientes à realização de autocontrole mecânico de placa para manutenção dos resultados pós tratamento, uma vez que todos os dispositivos de limpeza dental e interdental possam ser utilizados a cada 24 horas.

No segundo artigo, ao avaliarmos o efeito das diferentes frequências de ACMP nos parâmetros subgengivais, os resultados demonstraram que a correlação entre sangramento gengival e sangramento à sondagem é influenciada por diferentes intervalos de ACMP. Maiores mudanças na magnitude da correlação foram encontradas entre baseline e 90 dias no grupo 48 horas.

Como explicação para a maior correlação no grupo 48 horas temos que sítios com sangramento gengival podem ter criado maiores condições para o estabelecimento de placa subgengival e este uma resposta inflamatória do hospedeiro. Isto é, o aumento de placa supragengival, evidenciado através do aumento na presença de sítios com IG=2, esteve associado a um aumento do sangramento à sondagem provavelmente decorrente da presença de p. Corroborando com os nossos achados, De David et al (2018) mostrou que a correlação entre formação de placa e saúde gengival é afetada pela frequência de higiene oral. Os indivíduos que executaram altos padrões de higiene oral em intervalos de 12 e 24 horas mantiveram a saúde gengival em contraposição aos sujeitos que realizavam intervalos de higiene maiores. Esses resultados reforçam nossos achados e nos permitem compreender que placas dentárias microbianas maduras que persistem por longos períodos de tempo sem desorganização ao seu desenvolvimento não são compatíveis com a saúde gengival e, conseqüentemente, podem permitir a colonização do ambiente subgengival, levando ao desenvolvimento de uma resposta inflamatória subgengival expressa através de sangramento à sondagem.

## 5 CONCLUSÃO

Autocontrole mecânico da placa realizado a cada 12 ou 24 horas é suficiente para manutenção da saúde gengival em indivíduos com histórico de periodontite, tratados e participantes de um programa de manutenção periódica preventiva. Realizar o autocontrole mecânico da placa a cada 48 horas resultou em um aumento significativo nas médias de IG=2. Além disso, essas alterações inflamatórias marginais tiveram repercussões no ambiente subgengival através de um aumento no sangramento à sondagem, demonstradas através da correlação moderada estatisticamente significativa entre IG=2 e SS nesse grupo experimental.

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## APÊNDICE A- FOLHETO DE RECOMENDAÇÕES AOS PACIENTES

### RECOMENDAÇÕES:

- REALIZAR A SUA FREQUÊNCIA RECOMENDADA: \_\_\_\_\_;
- LEMBRE-SE, A MANEIRA COMO ESCOVA SEUS DENTES NÃO IRÁ MUDAR, O QUE IRÁ MUDAR É A FREQUÊNCIA DE SUA ESCOVAÇÃO (DE QUANTO EM QUANTO TEMPO IRÁ ESCOVAR)
- DEVERÁ UTILIZAR UMA QUANTIDADE DE PASTA QUE CUBRA TRANSVERSALMENTE AS CERDAS DA SUA ESCOVA EM UM PONTO (COMO DEMOSTRADO PELA C.D CAMILA)
- A SOLUÇÃO DE FLÚOR DEVERÁ SER UTILIZADA DA SEGUINTE FORMA: 15ml (1 TAMPINHA) DE SOLUÇÃO 2 VEZES AO DIA. BOCHECHAR DURANTE 1 MINUTO E CUSPIR
- **VOCÊ NÃO DEVE ESCOVAR COM OUTRA ESCOVA OU CREME DENTAL E NEM TROCÁ-LOS OU JOGÁ-LOS FORA, ELES SERÃO RECOLHIDOS AO FINAL DO ESTUDO;**
- VOCÊ SERÁ RECHAMADO PARA ACOMPANHAMENTO EM 15,30, E 90 DIAS APÓS O INÍCIO DO ESTUDO;
- **A CADA RECHAMADA VOCÊ DEVERÁ TRAZER A SUA ESCOVA DENTAL E SEU FIO E/OU ESCOVAS INTERDENTAIS. VOCÊ REALIZARÁ SUA ESCOVAÇÃO NA FACULDADE, ISTO É, SOMENTE PODERÁ ESCOVAR NO DIA DA CONSULTA APÓS A REALIZAÇÃO DOS EXAMES;**
- **VOCÊ NÃO DEVE FALAR AO DENTISTA QUE IRÁ LHE EXAMINAR QUAL A SUA FREQUÊNCIA DE ESCOVAÇÃO;**
- CASO VOCÊ NOTE SANGRAMENTO EM SUA GENGIVA **NÃO SE PREOCUPE E CONTINUE SUA FREQUÊNCIA DE ESCOVAÇÃO ATÉ A PRÓXIMA CONSULTA. O DENTISTA IRÁ EXAMINÁ-LO E IRÁ RESTABELECEER A SUA SAÚDE GENGIVAL.**
- EM CASO DE DÚVIDA OU EVENTUALIDADES: (055) 9159-XXXX – CIRURGIÃ-DENTISTA CAMILA.

## APÊNDICE B – ENTREVISTA

**Nome:** \_\_\_\_\_

**Gênero:** ( )M ( )F **Profissão:** \_\_\_\_\_

**Endereço:** \_\_\_\_\_

**Telefone(s) para contato:** \_\_\_\_\_

**Idade:** \_\_\_\_\_ anos. **Data de nascimento:** \_\_\_\_\_

**Estado civil:** ( ) casada ( ) separada ( ) divorciada ( ) viúva ( ) solteira

**Cor/ raça:** ( ) branca ( ) preta ( ) parda ( ) indígena ( ) amarela

### Dados Odontológicos

Qual a frequência com que você realiza escovação dos dentes?

\_\_\_\_\_

Que tipo de escova usa? ( ) Macia ( ) Média ( ) Dura

Faz uso de dispositivo interdental? \_\_\_\_\_

Qual? ( ) Fio ( ) Escova interdental ( ) Escova unitufo ( ) Outro \_\_\_\_\_

Com que frequência (vezes por semana)? \_\_\_\_\_

Faz uso de dentífrico? Qual? \_\_\_\_\_

Faz uso de alguma solução pra bochecho? Qual? \_\_\_\_\_

Você usa o bochecho com que objetivo? \_\_\_\_\_

Tem sangramento gengival? ( ) Sim ( ) Não. Se sim, quando ele ocorre? \_\_\_\_\_

Tem sensibilidade nos dentes? ( ) Sim ( ) Não. Sente mau gosto na boca? ( ) Sim ( ) Não.

Sente mau hálito? ( ) Sim ( ) Não. Alguém já comentou a respeito do seu hálito? ( ) Sim ( ) Não.

### Dados Médicos

Está em tratamento médico atualmente? ( ) Sim ( ) Não. Qual? \_\_\_\_\_

Esteve em tratamento nos últimos 3 meses? ( ) Sim ( ) Não. Qual? \_\_\_\_\_

Apresenta alguma doença sistêmica? \_\_\_\_\_

Está tomando alguma medicação? \_\_\_\_\_

### Nível socioeconômico e Escolaridade

Qual é a renda da sua família?

\_\_\_\_\_ salários mínimos. ( ) Não respondeu ( ) Não recebe salário

Qual é seu grau de escolaridade? \_\_\_\_\_

Qual o grau de escolaridade do chefe da sua família? \_\_\_\_\_

## APÊNDICE C – ADERÊNCIA A FREQUÊNCIA RANDOMIZADA

Paciente: \_\_\_\_\_

### EXAME 15 DIAS

Por algum motivo, durante os 15 dias anteriores, você não realizou a frequência de escovação recomendada? ( ) SIM ( ) NÃO ( ) NÃO LEMBRA

Se sim, quantos dias não realizou a frequência correta? \_\_\_\_\_

Se sim, qual o motivo que o fez não realizar a frequência correta?

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### EXAME 30 DIAS

Por algum motivo, durante os 15 dias anteriores, você não realizou a frequência de escovação recomendada? ( ) SIM ( ) NÃO ( ) NÃO LEMBRA

Se sim, quantos dias não realizou a frequência correta? \_\_\_\_\_

Se sim, qual o motivo que o fez não realizar a frequência correta?

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### EXAME 90 DIAS

Por algum motivo, durante os 15 dias anteriores, você não realizou a frequência de escovação recomendada? ( ) SIM ( ) NÃO ( ) NÃO LEMBRA

Se sim, quantos dias não realizou a frequência correta? \_\_\_\_\_

Se sim, qual o motivo que o fez não realizar a frequência correta?

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APÊNDICE D – FICHAS PARA EXAMES PERIODONTAIS

Fichas de exame clínico periodontal - IPI, IG E FCG

Período do exame: ( ) Baseline ( ) 15 dias ( ) 30 dias ( ) 90 dias

Data: \_\_\_\_\_ Paciente: \_\_\_\_\_ Examinador: \_\_\_\_\_

	17	16	15	14	13	12	11	21	22	23	24	25	26	27
	D	V	M	D	V	M	D	V	M	D	V	M	D	V
IPI														
IG														
	D	P	M	D	P	M	D	P	M	D	P	M	D	P
IPI														
IG														
	47	46	45	44	43	42	41	31	32	33	34	35	36	37
	D	V	M	D	V	M	D	V	M	D	V	M	D	V
IPI														
IG														
	D	L	M	D	L	M	D	L	M	L	D	M	L	D
IPI														
IG														

Número total de dentes:			
FCG 1	DENTE	SÍTIO	VALOR
FCG 2			
FCG 3			
FCG 4			
FCG 5			
FCG 6			
FCG 7			
FCG 8			



## APÊNDICE E – QUESTIONÁRIO SOBRE EFEITOS ADVERSOS

### Questionário

1. Você percebeu alguma alteração sobre as superfícies de seus dentes a partir da última avaliação que fizemos?

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2. Apresentou alguma alteração de sua gengiva durante a higiene bucal?

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3. Você tem sentido gosto ruim na boca?

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4. Você percebe outras alterações além das expostas acima?

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## ANEXO A- NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *JOURNAL OF CLINICAL PERIODONTOLOGY*

### *Author Guidelines*

The journal to which you are submitting your manuscript employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

#### **1.GENERAL**

*Journal of Clinical Periodontology* publishes original contributions of high scientific merit in the fields of periodontology and implant dentistry. Its scope encompasses the physiology and pathology of the periodontium, the tissue integration of dental implants, the biology and the modulation of periodontal and alveolar bone healing and regeneration, diagnosis, epidemiology, prevention and therapy of periodontal disease, the clinical aspects of tooth replacement with dental implants, and the comprehensive rehabilitation of the periodontal patient. Review articles by experts on new developments in basic and applied periodontal science and associated dental disciplines, advances in periodontal or implant techniques and procedures, and case reports which illustrate important new information are also welcome.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in *Journal of Clinical Periodontology*. Authors are encouraged to visit [Wiley-Blackwell's Author Services](#) for further information on the preparation and submission of articles and figures.

#### **2. ETHICAL GUIDELINES**

*Journal of Clinical Periodontology* adheres to the below ethical guidelines for publication and research.

##### **2.1.Authorship and Acknowledgements**

Authors submitting a paper do so on the understanding that the manuscript have been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal.

*Journal of Clinical Periodontology* adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements. Please note that it is a requirement to include email addresses for all co-authors at submission. If any of the email-addresses supplied are incorrect the corresponding author will be contacted by the journal administrator.

**Acknowledgements:** Under acknowledgements please specify contributors to the article other than the authors accredited.

##### **2.2.Ethical Approvals**

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association [Declaration of Helsinki](#) (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

### 2.3 Clinical Trials

Clinical trials should be reported using the CONSORT guidelines available at [A](#). A should also be included in the submission material.

*Journal of Clinical Periodontology* encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries: [. .](#) The clinical trial registration number and name of the trial register will then be published with the paper.

### 2.4 DNA Sequences and Crystallographic Structure Determinations

Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly.

### 2.5 Conflict of Interest and Source of Funding

*Journal of Clinical Periodontology* requires that all authors (both the corresponding author and co-authors) disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author's objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If authors are unsure whether a past or present affiliation or relationship should be disclosed in the manuscript, please contact the editorial office at [. .](#) The existence of a conflict of interest does not preclude publication in this journal.

The above policies are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the International Committee of Medical Journal Editors. It is the responsibility of the corresponding author to have all authors of a manuscript fill out a conflict of interest disclosure form, and to upload all forms together with the manuscript on submission. The disclosure statement should be included under Acknowledgements. Please find the form below: [Conflict of Interest Disclosure Form](#).

### 2.6 Appeal of Decision

Under exception circumstances, authors may appeal the editorial decision. Authors who wish to appeal the decision on their submitted paper may do so by e-mailing the editorial office at [. .](#) with a detailed explanation for why they find reasons to appeal the decision.

*Please note that all revisions and resubmissions of papers should also include a separate rebuttal and a tracked changes document to assist in peer review.*

### 2.7 Permissions

If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

## 3. MANUSCRIPT SUBMISSION PROCEDURE

Manuscripts should be submitted electronically via the online submission site <http://mc.manuscriptcentral.com/jcpe>. The use of an online submission and peer review site enables immediate distribution of manuscripts and consequentially speeds up the review process. It also allows authors to track the status of their own manuscripts. Complete instructions for submitting a paper is available on the submission site. Further assistance can be obtained from the Senior Editorial Office Assistant, Kim Harris, at [cpeedoffice@wiley.com](mailto:cpeedoffice@wiley.com).

Please note that all revisions and resubmissions of papers should also include a separate rebuttal and a tracked changes document to assist in peer review.

### 3.1. Manuscript Files Accepted

Main manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected). The text file must contain the entire manuscript including title page, abstract, clinical reference, main text, references, acknowledgement, statement of source of funding and any potential conflict of interest, tables, and figure legends, but no embedded figures. In the text, please reference any figures as for instance 'Figure 1', 'Figure 2' etc. to match the tag name you choose for the individual figure files uploaded.

Figure files should be uploaded separately to the main text. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing.

Manuscripts should be formatted as described in the Author Guidelines below.

Please ensure that ALL items (figures and tables) are cited in the main text.

### 3.2. Blinded Review

All manuscripts submitted to *Journal of Clinical Periodontology* will be reviewed by two or more experts in the field. Papers that do not conform to the general aims and scope of the journal will, however, be returned immediately without review. *Journal of Clinical Periodontology* uses single blinded review. The names of the reviewers will thus not be disclosed to the author submitting a paper.

### 3.3. Suggest a Reviewer

*Journal of Clinical Periodontology* attempts to keep the review process as short as possible to enable rapid publication of new scientific data. In order to facilitate this process, please suggest the name and current email address of one potential international reviewer whom you consider capable of reviewing your manuscript. In addition to your choice the editor will choose one or two reviewers as well.

### 3.4. Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

### 3.5. E-mail Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

### 3.6 Resubmissions

If your manuscript was given the decision of reject and resubmit, you might choose to submit an amended version of your manuscript. This should be submitted as a new submission following the guidelines above under 3.2. In addition you should upload comments to the previous review as “supplementary files for review”.

## 4. MANUSCRIPT TYPES ACCEPTED

*Journal of Clinical Periodontology* publishes **original research articles, reviews, clinical innovation reports and case reports**. The latter will be published only if they provide new fundamental knowledge and if they use language understandable to the clinician. It is expected that any manuscript submitted represents unpublished original research.

**Original Research Articles** must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original articles will be published under the heading of clinical periodontology, implant dentistry or pre-clinical sciences and must conform to the highest international standards in the field.

**Clinical Innovation Reports** are suited to describe significant improvements in clinical practice such as the report of a novel surgical technique, a breakthrough in technology or practical approaches to recognized clinical challenges. They should conform to the highest scientific and clinical practice standards. **Case Reports** illustrating unusual and clinically relevant observations are acceptable but their merit needs to provide high priority for publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered.

**Reviews** are selected for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should take a broad view of the field rather than merely summarizing the authors' own previous work, so extensive citation of the authors' own publications is discouraged. The use of state-of-the-art evidence-based systematic approaches is expected. Reviews are frequently commissioned by the editors and, as such, authors are encouraged to submit a proposal to the Journal. Review proposals should include a full-page summary of the proposed contents with key references.

## 5. MANUSCRIPT FORMAT AND STRUCTURE

**5.1. Format Language:** The language of publication is English. Authors for whom English is a second language may choose to have their manuscript professionally edited before submission to improve the English. It is preferred that manuscript is professionally edited. Please refer to English Language Editing Services offered by Wiley at <http://wileyeditingservices.com/en/>.

Japanese authors can also find a list of local English improvement services at <http://www.wiley.co.jp/journals/editcontribute.html>. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

**Abbreviations, Symbols and Nomenclature:** *Journal of Clinical Periodontology* adheres to the conventions outlined in *Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors*. Abbreviations should be kept to a minimum, particularly those that are not standard. Non-standard abbreviations must be used three or more times and written out completely in the text when first used.

## 5.2. Structure

All articles submitted to *Journal of Clinical Periodontology* should include:

- Title Page
- Conflict of Interest and Source of Funding
- Clinical Relevance
- Abstract
- Introduction
- Materials and Methods
- Results
- Discussion
- References
- Tables (where appropriate)
- Figure Legends (where appropriate)
- Figures (where appropriate and uploaded as separate files)

All manuscripts should emphasize clarity and brevity. Authors should pay special attention to the presentation of their findings so that they may be communicated clearly. Technical jargon should be avoided as much as possible and be clearly explained where its use is unavoidable.

**Title Page:** The title must be concise and contain no more than 100 characters including spaces. The title page should include a running title of no more than 40 characters; 5-10 key words, complete names of institutions for each author, and the name, address, telephone number, fax number and e-mail address for the corresponding author.

**Conflict of Interest and Source of Funding:** Authors are required to disclose all sources of institutional, private and corporate financial support for their study. Suppliers of materials (for free or at a discount from current rates) should be named in the source of funding and their location (town, state/county, country) included. Other suppliers will be identified in the text. If no funding has been available other than that of the author's institution, this should be specified upon submission. Authors are also required to disclose any potential conflict of interest. These include financial interests (for example patent, ownership, stock ownership, consultancies, speaker's fee,) or provision of study materials by their manufacturer for free or at a discount from current rates. Author's conflict of interest (or information specifying the absence of conflicts of interest) and the sources of funding for the research will be published under a separate heading entitled "Conflict of Interest and Source of Funding Statement".

See Editor-in-Chief Maurizio Tonetti's [Editorial on Conflict of Interest and Source of Funding](#) and [www.icmje.org/#conflicts](http://www.icmje.org/#conflicts) for generally accepted definitions.

**Abstract:** is limited to 200 words in length and should not contain abbreviations or references. The abstract should be organized according to the content of the paper.

For Original Research Articles the abstract should be organized with **aim, materials and methods, results and conclusions**.

For clinical trials, it is encouraged that the abstract finish with the clinical trial registration number on a free public database such as [clinicaltrials.gov](http://clinicaltrials.gov).

**Clinical Relevance:** This section is aimed at giving clinicians a reading light to put the present research in perspective. It should be no more than 100 words and should not be a repetition of the abstract. It should provide a clear and concise explanation of the rationale for the study, of what was known before and of how the present results advance knowledge of this field. If appropriate, it may also contain suggestions for clinical practice.

It should be structured with the following headings: **scientific rationale for study, principal findings, and practical implications**.

Authors should pay particular attention to this text as it will be published in a highlighted box within their manuscript; ideally, reading this section should leave clinicians wishing to learn more about the topic and encourage them to read the full article.

**Acknowledgements:** Under acknowledgements please specify contributors to the article other than the authors  
accredited.

### 5.3. Original Research Articles

These must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original articles will be published under the heading of clinical periodontology, implant dentistry or pre-clinical sciences and must conform to the highest international standards in the field.

The word limit for original research articles is 3500 words, and up to 7 items (figures and tables) may be included. Additional items can be included as supplementary files online (please see 5.9 below). Main Text of **Original Research Articles** should be organized with

- Introduction,
- Materials and Methods,
- Results and Discussion.
- References (Harvard, see section 5.7)

The background and hypotheses underlying the study, as well as its main conclusions, should be clearly explained. Please see Sample Manuscript.

**Introduction:** should be focused, outlining the historical or logical origins of the study and not summarize the results; exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation.

**Material and Methods:** must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced. As a condition of publication, authors are required to make materials and methods used freely available to academic researchers for their own use. This includes antibodies and the constructs used to make transgenic animals, although not the animals themselves.

(a) *Clinical trials* should be reported using the CONSORT guidelines available at [www.consort-statement.org](http://www.consort-statement.org). A [CONSORT checklist](#) should also be included in the submission material. If your study is a randomized clinical trial, you will need to fill in all sections of the CONSORT Checklist. If your study is not a randomized trial, not all sections of the checklist might apply to your manuscript, in which case you simply fill in N/A.

*Journal of Clinical Periodontology* encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries: [www.clinicaltrials.gov](http://www.clinicaltrials.gov), <http://clinicaltrials.ifpma.org/clinicaltrials/>. The clinical trial registration number and name of the trial register will then be published with the paper.

(b) *Statistical Analysis:* As papers frequently provide insufficient detail as to the performed statistical analyses, please describe with adequate detail. For clinical trials intention to treat analyses are encouraged (the reasons for choosing other types of analysis should be highlighted in the submission letter and clarified in the manuscript).

(c) *DNA Sequences and Crystallographic Structure Determinations:* Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly

(d) *Experimental Subjects:* Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association [Declaration of Helsinki](#) (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors



reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used. **Results:** should present the observations with minimal reference to earlier literature or to possible interpretations.

**Discussion:** may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The discussion section should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.

The discussion may usefully be structured with the following points in mind (modified from the proposal by [Richard Horton \(2002\), The Hidden Research Paper, The Journal of the American Medical Association, 287, 2775-2778](#)). Not all points will apply to all studies and its use is optional, but we believe it will improve the discussion section to keep these points in mind.

#### Summary of key finding

- \* Primary outcome measure(s)
- \* Secondary outcome measure(s)
- \* Results as they relate to a prior hypothesis

#### Strengths and Limitations of the Study

- \* Study Question
- \* Study Design
- \* Data Collection
- \* Analysis
- \* Interpretation
- \* Possible effects of bias on outcomes

#### Interpretation and Implications in the Context of the Totality of Evidence

- \* Is there a systematic review to refer to?
- \* If not, could one be reasonably done here and now?
- \* What this study adds to the available evidence
- \* Effects on patient care and health policy
- \* Possible mechanisms

#### Controversies Raised by This Study Future Research Directions

- \* For this particular research collaboration
- \* Underlying mechanisms
- \* Clinical research

### **5.4. Clinical Innovation Reports**

These are suited to describe significant improvements in clinical practice such as the report of a novel surgical technique, a breakthrough in technology or practical approaches to recognized clinical challenges. They should conform to the highest scientific and clinical practice standards. The word limit for clinical innovation reports is 3000 words, and up to 12 items (figures and tables) may be included. Additional items can be included as supplementary files online (please see 5.9 below).

The main text of Clinical Innovation Reports should be organized with

- Introduction,
- Clinical Innovation Report,
- Discussion and Conclusion
- References (see section 5.7)

### **5.5. Case Reports**

Case reports illustrating unusual and clinically relevant observations are acceptable but their merit needs to provide high priority for publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered.

The main text of Case Reports should be organized with

- Introduction,

- Case report,
- Discussion and Conclusion
- References (see section 5.7)

### 5.6. Reviews

Reviews are selected for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should take a broad view of the field rather than merely summarizing the authors' own previous work, so extensive citation of the authors' own publications is discouraged. The use of state-of-the-art evidence-based systematic approaches is expected. Reviews are frequently commissioned by the editors and, as such, authors are encouraged to submit a proposal to the Journal. Review proposals should include a full-page summary of the proposed contents with key references.

The word limit for reviews is 4000 words.

The main text of Reviews should be organized with

- Introduction,
- Review of Current Literature,
- Discussion and Conclusion
- References (see section 5.7)

### 5.7. References

It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

References should be prepared according to the Publication Manual of the American Psychological Association (6th edition). This means in text citations should follow the author-date method whereby the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998). The complete reference list should appear alphabetically by name at the end of the paper.

A sample of the most common entries in reference lists appears below. Please note that a DOI should be provided for all references where available. For more information about APA referencing style, please refer to the [APA FAQ](#). Please note that for journal articles, issue numbers are not included unless each issue in the volume begins with page one.

#### *Journal article*

Beers, S. R. , & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. *The American Journal of Psychiatry*, 159, 483–486. doi:[10.1176/appi.ajp.159.3.483](https://doi.org/10.1176/appi.ajp.159.3.483)

#### *Book*

Bradley-Johnson, S. (1994). *Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school* (2nd ed.). Austin, TX: Pro-ed.

#### *Chapter in an Edited Book*

Borström, I., & Elbro, C. (1997). Prevention of dyslexia in kindergarten: Effects of phoneme awareness training with children of dyslexic parents. In C. Hulme & M. Snowling (Eds.), *Dyslexia: Biology, cognition and intervention* (pp. 235–253). London: Whurr.

#### *Internet Document*

Norton, R. (2006, November 4). How to train a cat to operate a light switch [Video file]. Retrieved from <http://www.youtube.com/watch?v=Vja83KLOXZs>

Please note that all unpublished papers (submitted or in press) included in the reference list should be provided in a digital version at submission. The unpublished paper should be uploaded as a supplementary file for review.

### 5.8. Tables, Figures and Figure Legends

**Tables:** should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title. **Figures:** All figures should be planned to fit within either 1 column width (8.0 cm), 1.5 column widths (13.0 cm) or 2 column widths (17.0 cm), and must be suitable for photocopy reproduction from the printed version of the manuscript. Lettering on figures should be in a clear, sans serif typeface (e.g. Helvetica); if possible, the same typeface should be used for all figures in a paper. After reduction for publication, upper-case text and numbers should be at least 1.5-2.0 mm high (10 point Helvetica). After reduction symbols should be at least 2.0-3.0 mm high (10 point). All half-tone photographs should be submitted at final

reproduction size. In general, multi-part figures should be arranged as they would appear in the final version. Each copy should be marked with the figure number and the corresponding author's name. Reduction to the scale that will be used on the page is not necessary, but any special requirements (such as the separation distance of stereo pairs) should be clearly specified.

Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible.

Figures should be on a white background, and should avoid excessive boxing, unnecessary colour, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms should not be truncated to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size. Figures divided into parts should be labelled with a lower-case, boldface, roman letter, a, b, and so on, in the same typesize as used elsewhere in the figure. Lettering in figures should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature or the nomenclature common to a particular field. Thousands should be separated by thin spaces (1 000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general, visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc.)

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**Permissions:** If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

**Figure Legends:** should be a separate section of the manuscript, and should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.

## 5.9. Supplementary Material

Supplementary material, such as data sets or additional figures or tables that will not be published in the print edition of the Journal but which will be viewable in the online edition, can be uploaded as 'Supporting information for review and online publication only'.

Please see <http://authorservices.wiley.com/bauthor/suppmat.asp> for further information on the submission of Supplementary Materials.

## 6. AFTER ACCEPTANCE

Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

### 6.1 Proof Corrections

The corresponding author will receive an email alert containing a link to a web site. A working email address must therefore be provided for the corresponding author. The proof can be downloaded as a PDF (portable document format) file from this site. Acrobat Reader will be required in order to read this file. This software can be downloaded (free of charge) from the following Web site: [www.adobe.com/products/acrobat/readstep2.html](http://www.adobe.com/products/acrobat/readstep2.html). This will enable the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof. Hard copy proofs will be posted if no e-mail address is available; in your absence, please arrange for a colleague to access your e-mail to retrieve the proofs. Proofs must be returned to the Production Editor within three days of receipt. As changes to proofs are costly, we ask that you only correct typesetting errors. Excessive changes made by the author in the proofs, excluding typesetting errors, will be charged separately. Other than in exceptional circumstances, all illustrations are retained by the publisher. Please note that the author is responsible for all statements made in his work, including changes made by the copy editor.

### 6.2 Early View (Publication Prior to Print)



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### 6.3 Production Tracking

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### 6.4 Accepted Articles

'Accepted Articles' have been accepted for publication and undergone full peer review but have not been through the copyediting, typesetting, pagination and proofreading process. Accepted Articles are published online a few days after final acceptance, appear in PDF format only (without the accompanying full-text HTML) and are given a Digital Object Identifier (DOI), which allows them to be cited and tracked. The DOI remains unique to a given article in perpetuity. More information about DOIs can be found online at <http://www.doi.org/faq.html>. Given that Accepted Articles are not considered to be final, please note that changes will be made to an article after Accepted Article online publication, which may lead to differences between this version and the Version of Record. The Accepted Articles service has been designed to ensure the earliest possible circulation of research papers after acceptance. Given that copyright licensing is a condition of publication, a completed copyright form is required before a manuscript can be processed as an Accepted Article.

Accepted articles will be indexed by PubMed; therefore the submitting author must carefully check the names and affiliations of all authors provided in the cover page of the manuscript, as it will not be possible to alter these once a paper is made available online in Accepted Article format.

### 6.5 Video Abstracts

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## ANEXO B – ÍNDICE DE PLACA: PROPOSTO POR SILNESS & LÖE, 1964

Pelo Índice de Placa (IPI) cada sítio do dente receberá escores, variando de 0 a 3. O valor do IPI para o dente é dado pela soma dos escores dos sítios, dividido pelo número de sítios. Valor de IPI para o indivíduo é dado pela soma dos escores dos sítios, em todos os dentes, dividido pelo número de sítios.

Critérios para definição dos escores:

Escore 0: a área cervical, próxima a margem gengival, é literalmente livre de placa. A superfície é testada a procura de placa, passando-se uma sonda com ponta romba próxima a margem gengival, após secagem adequada da superfície.

Escore 1: Placa não é observada *in situ* por meio de inspeção visual, mas placa é visível na ponta romba da sonda uma vez que essa tenha sido passada próxima a margem gengival, após secagem adequada da superfície.

Escore 2: A área cervical é coberta com uma fina a moderadamente espessa camada de placa, visível a olho nu.

Escore 3: Pesado acúmulo de material macio, ultrapassando em espessura o nicho natural produzido pela margem gengival/superfície dental é encontrado. Superfície proximal é preenchida com leves debris.

### **ANEXO C – ÍNDICE GENGIVAL PROPOSTO POR SILNESS & LÖE, 1963, MODIFICADO POR LÖE, 1967**

Pelo Índice Gengival (IG) cada sítio do dente receberá escores, variando de 0 a 3. O valor do IG para o dente é dado pela soma dos escores dos sítios, dividido pelo número de sítios. Valor de IG para o indivíduo é dado pela soma dos escores dos sítios, em todos os dentes, dividido pelo número de sítios.

Critérios para definição dos escores:

Escore 0: gengiva normal. A gengiva deve ter cor variando de rosa pálido a rosa. Sob secagem, o tecido deve ser opaco. À palpação com a ponta romba de uma sonda, o tecido deve ser firme. Margem gengival pode estar localizada sobre o esmalte dentário, ou em vários níveis apicais a junção amelo-cementária.

Escore 1: leve inflamação. Ligeira mudança na cor do tecido gengival e leve edema da margem gengival. Não ocorre sangramento quando uma sonda com ponta romba é passada ao longo da margem gengival.

Escore 2: inflamação moderada. Tecido gengival está avermelhado, com edema da margem gengival e brilhoso. Sangramento ocorre quando a ponta romba de uma sonda é passada ao longo da margem gengival.

Escore 3: severa inflamação. Marcada vermelhidão do tecido e edema. Ulceração pode estar presente. Há tendência ao sangramento espontâneo.