UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

ANA LÍVIA FILETO MAZZONETTO

PROTEÍNAS DERIVADAS DA MATRIZ DO ESMALTE PARA O TRATAMENTO DE DEFEITOS INFRA-ÓSSEOS EM PACIENTES COM PERIODONTITE AGRESSIVA E CRÔNICA

ENAMEL MATRIX PROTEINS FOR THE TREATMENT OF INTRABONY DEFECTS IN PATIENTS WITH AGGRESSIVE AND CHRONIC PERIODONTITIS

Piracicaba, 2019

ANA LÍVIA FILETO MAZZONETTO

PROTEÍNAS DERIVADAS DA MATRIZ DO ESMALTE PARA O TRATAMENTO DE DEFEITOS INFRA-ÓSSEOS EM PACIENTES COM PERIODONTITE AGRESSIVA E CRÔNICA

ENAMEL MATRIX PROTEINS FOR THE TREATMENT OF INTRABONY DEFECTS IN PATIENTS WITH AGGRESSIVE AND CHRONIC PERIODONTITIS

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos para a obtenção de título de Doutora em Clínica Odontologica, na Área de Periodontia.

Orientador: PROF. DR. ANTONIO WILSON SALLUM

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA ANA LÍVIA FILETO MAZZONETTO, E ORIENTADA PELO PROF. DR. ANTONIO WILSON SALLUM

Piracicaba, 2019

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Odontologia de Piracicaba
Marilene Girello - CRB 8/6159

M459p

Mazzonetto, Ana Lívia Fileto, 1990-
Proteínas derivadas da matriz do esmalte para o tratamento de defeitos infra-ósseos em pacientes com periodontite agressiva e crônica / Ana Lívia Fileto Mazzonetto. – Piracicaba, SP : [s.n.], 2019.

Orientador: Antônio Wilson Sallum.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Periodontite agressiva. 2. Periodontite crônica. 3. Perda do osso alveolar. 4. Avaliação de resultados da assistência ao paciente. 5. Proteínas do esmalte dentário. I. Sallum, Antônio Wilson, 1943-. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Enamel matrix proteins for the treatment of intrabony defects in patients with aggresive and chronic periodontitis
Palavras-chave em inglês:
Aggressive periodontitis
Chronic periodontitis
Alveolar bone loss
Patient outcome assessment
Dental enamel proteins
Área de concentração: Periodontia
Titulação: Doutora em Clínica Odontológica
Banca examinadora:
Antônio Wilson Sallum [Orientador]
Marcio Zaffalon Casati
Renato Corrêa Viana Casarin
João Batista César Neto
Daiane Cristina Peruzzo
Data de defesa: 19-02-2019
Programa de Pós-Graduação: Clínica Odontológica

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0001-6564-4374
- Currículo Lattes do autor: http://lattes.cnpq.br/9566617041406902

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 19 de Fevereiro de 2019, considerou a candidata ANA LÍVIA FILETO MAZZONETTO aprovada.

PROF. DR. ANTONIO WILSON SALLUM

PROF. DR. JOÃO BATISTA CÉSAR NETO

PROFª. DRª. DAIANE CRISTINA PERUZZO

PROF. DR. MARCIO ZAFFALON CASATI

PROF. DR. RENATO CORRÊA VIANA CASARIN

A Ata da defesa, assinada pelos membros da Comissão Examinadora, consta no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade.

DEDICATÓRIA

Aos meus grandes amores nesta vida, meu filho **Antonio**, meu marido **Marcos**, minha mãe **Maria**, meu pai **Carlos** e meu irmão **Arthur**. Amor e gratidão.

AGRADECIMENTOS

À DEUS

À **Deus**, que em sua infinita bondade, traçou os meus caminhos e me permitiu estar aqui hoje. Agradeço ainda pelo dom da vida que por Ele me foi concedido, pelos obstáculos superados, objetivos alcançados e pela concretização de mais este sonho.

À **Maria**, Sua mãe, que sempre esteve a minha frente, desatando meus nós, e de todos aqueles que me rodeiam.

À FACULDADE DE ODONTOLOGIA DE PIRACICABA

À **Faculdade de Odontologia de Piracacicaba**, da Universidade Estadual de Campinas, na pessoa do seu diretor, Prof. Dr. Francisco Haiter Neto.

À **Coordenadoria Geral de Pós Graduação da FOP/UNICAMP**, em nome da Prof. Dra. Karina Gonzales Silvério Ruiz, por toda a atenção dispensada.

Ao **Programa de Pós Graduação em Clínica Odontológica,** em nome do coordenador Prof. Dr. Valentim Adelino Ricardo Barão.

Às funcionárias da área de Periodontia, **Regina Caetano, Mariana e Janaina**, que com muito carinho, atenção e paciência, estiveram sempre prontas para ajudar no que fosse preciso.

À **Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)**, órgão de fomento que concedeu auxílio à pesquisa, em nome do Prof. Dr. Antonio Wilson Sallum, processo nº 2015/1973-1.

O presente trabalho foi realizado com apoio da **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)**, código de financiamento 001.

AO EXÉRCITO BRASILEIRO

Ao **Exército Brasileiro**, em nome do Exmo. General de Exército Edson Leal Pujol.

Ao **Hospital Militar de Área de São Paulo**, em nome do Exmo. General de Brigada Médico Sergio dos Santos Szelbracikowski, diretor desta organização militar de saúde, pelos excelentes anos de vida militar na caserna experimentados desde 2016, quando novos desafios na minha carreira profissional foram lançados, com a oportunidade de servir à pátria por meio da força terrestre como oficial dentista temporária. HMASP! Saúde, Brasil.

AOS MESTRES

Ao **Prof, Dr. Antonio Wilson Sallum**, por acreditar nos meus sonhos e permitir que eu fosse sua orientada desde a graduação, pelos ensinamentos, pelas experiências compartilhadas, pela integral confiança em mim depositada, pelos cuidados e pela atenção, por nunca me deixar desistir e sempre me incentivar a chegar mais alto e, principalmente, por ser o nosso mais ilustre exemplo. Registro aqui que o professor Sallum é o grande segredo da odontologia.

Ao **Prof, Dr. Renato Corrêa Viana Casarin,** por delinear este estudo tão singular e confiá-lo a mim para sua execução, por estar onipresente durante toda a jornada, pela atenção integral e por incentivar continuamente meu crescimento profissional.

Aos professores da área de Periodontia desta universidade, **Prof. Dr. Enilson Antonio Sallum, Prof. Dr. Francisco Humberto Nociti Junior, Prof. Dr. Márcio Zafalon Casati, Prof. Dra. Karina Gonzales Silvério Ruiz, Prof. Dr. Renato Corrêa Viana Casarin,** pelos grandes ensinamentos e pelo conhecimento compartilhado.

Aos membros da minha banca de qualificação, **Prof. Dra. Karina Gonzales Silvério Ruiz, Prof. Dr. Renato Corrêa Viana Casarin, Prof. Dr. Rafael Ortega Lopes e Prof. Dra. Cristiane Ribeiro Salmon,** pela atenção e conhecimento dispensados nessa etapa tão importante de aprimoramento deste trabalho.

Aos professores titulares internos **Prof. Dr. Márcio Zafalon Casati** e **Prof. Dr. Renato Corrêa Viana Casarin** e aos professores titulares externos **Prof. Dra. Daiane Cristina Peruzzo** e **Prof. Dr. João Batista Cesar Neto,** pela prontidão em aceitar fazer parte desta banca de defesa e pelas contribuições singulares para o aprimoramento deste estudo.

Aos professores suplentes **Prof. Dra. Karina Gonzales Silvério Ruiz, Prof. Dra. Fernanda Vieira Ribeiro e Prof. Dr. Tiago Taiete**, pela prontidão em aceitar fazer parte desta banca de defesa.

AOS AMIGOS

Às eternas amigas **Erika Sana, Carolina Gimenes** e **Patrícia Vilas Boas**, por sempre estarem ao meu lado. Por superarem as distâncias e acreditarem na nossa amizade. Amor e gratidão.

Às minhas queridas contemporâneas de pós-graduação **Marcela Di Moura Barbosa, Mabelle de Freitas Monteiro, Isabela Lima França Grohmann** e **Manuela Rocha Bueno,** pelos maravilhosos momentos profissionais e pessoais compartilhados, por toda leveza e alegria contagiante que transformam os meus dias piracicabanos.

À **Rafaela Videira Clima da Silva**, minha parceira de pesquisa, mas acima de tudo, minha amiga. Obrigada por estar sempre ao meu lado durante esse árduo projeto, pelos ensinamentos, pela cumplicidade.

Aos meus colegas de pós graduação na área de Periodontia **Mabelle Monteiro, Marcela Di Moura, Isabela França, Elis Lira, Rafaela Videira, Amanda Bandeira, Rahyza Freire, Tiago Bueno, Aurélio Amorim, Catharina Sacramento, Javier Purisaca, Tamires Dutra, Thayane Businari** e **Thiago Rangel** pelo companheirismo nesta jornada.

Aos meus amigos **voluntários**, que aceitaram participar desta pesquisa, que desprenderam tempo para estarem presentes nas consultas, pela prontidão e paciência. Parte fundamental para os achados aqui apresentados.

À todos os meus **colegas e amigos da graduação e da pós graduação** pelos momentos já vividos e por todos aqueles que ainda estão por vim.

À querida cidade **de Piracicaba**, por ter me acolhido há 10 anos, sempre proporcionado muitos dos momentos mais importantes da minha vida desde então, e por hoje ser meu lar.

À FAMÍLIA

Ao meu filho, **Antonio Fileto Mazzonetto**, que, mesmo ainda dentro do meu ventre, já muito me ensina. Faz com que vida tenha um novo sentido todos os dias, supremo a tudo que já vivi até então. Amor e gratidão incondicionais.

Ao marido e pai, **Marcos Mazzonetto**, por todos os sentimentos e momentos, bons e ruins, que compartilhamos, por estar ao meu lado sempre, pelas ajudas diretas e indiretas a este trabalho. Gratidão por me fazer acreditar que, sim, é possível amar e ser amado.

À minha mãe, **Maria Aparecida Fileto Santana**, pelo amor incondicional, pela dedicação, carinho e zelo. Por sempre acreditar, ter fé em mim e poder tornar todos os meus sonhos possíveis. Por ser meu maior exemplo, meu porto seguro, meu escudo. Por me fazer acreditar na presença de Deus.

Ao meu pai, **Carlos Roberto Santana**, meu maior exemplo de honestidade, de amor, dedicação e perseverança. Por me emprestar um pouco da sua personalidade, o que com certeza fez com que eu chegasse até aqui hoje.

Ao meu irmão, **Arthur Fileto Santana**, com quem não saberia viver sem. Pelo companheirismo, pelo carinho, pelo amor! Por cuidar de mim tão bem, principalmente na nossa face paulistana.

À minha madrinha, **Leonice Benᡠ Cineis** e à minha tia **Maria Donizette Santana**, mulheres maravilhosas, grandes exemplos para mim, que nunca duvidaram do meu potencial, sempre me incentivaram e acreditaram em mim. Hoje também estou aqui por vocês.

Aos meus sogros**, Ivete Ducatti Mazzonetto** e **Antonio Fernando Mazonetto**, e a minha cunhada **Juliana Mazzonetto**, por me receberem sempre tão bem, sendo uma segunda família para mim. Minha família piracicabana.

A todos os **meus tios e primos** que contribuíram direta ou indiretamente para que esse sonho se concretizasse.

A todos àqueles que também fazem parte da minha família, **padrinhos, tios e primos do coração.**

“O importante não é a magnitude de nossas ações, mais sim a quantidade de amor que é colocada nelas.”

Madre Teresa de Calcutá

RESUMO

A grande perda óssea associada à idade jovem na periodontite agressiva (PAg) justifica o uso de estratégias bem fundamentada na intenção de estabilizar a progressão da doença, como o uso de terapias regenerativas nos estágios avançados de tratamento. Destaca-se a aplicação de proteínas derivadas da matriz de esmalte (EMD) em defeitos infra-ósseos de pacientes com periodontite crônica, mas há pouca evidência sobre os efeitos desse material na PAg. Assim, o objetivo deste estudo foi avaliar clinicamente, radiograficamente e no que concerne ao impacto na qualidade de vida dos sujeitos, o uso de EMD em defeitos infra-ósseos em pacientes com PAg, comparando-os com indivíduos com periodontite crônica (PC). Para isso, 40 defeitos infra-ósseos em pacientes com AgP e vinte defeitos em pacientes com PC com evidências radiográficas de ao menos 4 mm de profundidade e 2 mm horizontais, associados a profundidade de sondagem residual ≥ 6 mm (PS) foram incluídos no estudo e foram aleatoriamente atribuídos aos grupos: PAg + AC (n = 20), portadores de PAg que receberam debridamento por meio de acesso cirúrgico (AC); PAg + AC/EMD (n = 20), pacientes com PAg que receberam AC e aplicação de EMD; PC + AC/EMD (n = 20), pacientes com PC que receberam AC e aplicação de EMD. A PS, o nível de inserção clínica relativo (NICr) e a recessão gengival (RG) foram registrados no *baseline*, aos 6 e 12 meses. A resolução de defeitos e o preenchimento ósseo foram utilizados para análise radiográfica. A qualidade de vida foi registrada no *baseline* e 6 meses utilizando o questionário OHIP-14 e a escala de VAS para o período de pós-operatório inicial (15 dias). Após o tratamento, a PS e o NICr melhoraram para todos os grupos ao longo dos acompanhamentos comparando com o *baseline* ($p \leq 0,05$), e PAg + AC/EMD apresentou menor NICr aos 12 meses (7,8 ± 1,5 mm) em relação ao *baseline* quando em comparação com os pacientes com PAg que não receberam EMD (8,8 ± 1,5 mm) ($p \leq 0,05$). Os sítios tratados com PAg + AC/EMD apresentaram uma diminuição de 0,05 ± 0,9 mm em GR, enquanto que os sítios tratados com PC + AC/EMD apresentaram um aumento de 0,5 ± 0,9 mm, revelando diferença estatisticamente significante entre os grupos que receberam EMD para a estabilidade da margem gengival. Não foram observadas diferenças radiográficas entre os grupos em nenhum momento ($p > 0,05$). Os grupos PAg apresentaram maior sensibilidade radicular quando comparado ao grupo PC ($p \leq 0,05$). Para a avaliação da percepção tardia do paciente e global, não foram observadas diferenças entre os grupos ($p \leq 0,05$). EMD provou ser uma terapia viável para o tratamento de defeitos infra-ósseos de indivíduos com PAg, com uma taxa de regeneração similar dos pacientes com PC.

PALAVRAS-CHAVE Periodontite agressiva; periodontite crônica; perda de osso alveolar; proteínas do esmalte dentário, avaliação dos resultados da assitência ao paciente

ABSTRACT

The great bone loss associated with young age in aggressive periodontitis (AgP) justifies the use of well-founded strategies in the intention to stabilize the progression of the disease, such as the use of regenerative therapies in the advanced stages of treatment. It is noteworthy the application of proteins derived from the enamel matrix (EMD) in intrabony defects of patients with chronic periodontitis, but there is little evidence on the effects of this material on AgP. Thus, the aim of this study was to evaluate clinically, radiographically and in what concerns the impact on the quality of life of volunteers, the use of EMD in intrabony defects in patients with AgP, comparing them to individuals with chronic periodontitis (CP). Forty intrabony defects in AgP patients and twenty defects in CP patients of at least 4 mm depth and 2 mm horizontal, associated with ≥ 6 mm probing pocket depth (PPD) were included in the study and were randomly assigned to the groups: AgP+OFD (n=20), AgP patients which received open flap debridement (OFD); AgP+OFD/EMD (n=20), AgP patients which received OFD and application of EMD; CP+OFD/EMD (n=20), CP patients which received OFD and application of EMD. PPD, relative clinical attachment level (rCAL), and gingival recession (GR) were recorded at baseline, at 6 and 12 months. Defect resolution (DR) and bone filling (BF) were used for radiographic analysis. The quality of life was recorded at baseline and 6 months using the OHIP-14 questionnaire and VAS scale to early post-therapy period (15 days). After treatment, PPD and rCAL has improved for all groups along the follow-ups comparing to baseline ($p \leq 0.05$), and AgP+OFD/EMD presented a lower rCAL at 12 months (7.8 ± 1.5 mm) in relation to baseline when compared to AgP patients who did not receive (8.8 ± 1.5 mm) ($p \leq 0.05$). AgP+OFD/EMD-treated sites presented a discreet decrease of 0.05 ± 0.9 mm in GR while CP+OFD/EMD-treated sites had an increase of 0.5 ± 0.9 mm, revealing a statistically significant difference between the groups that received EMD for the stability of the gingival margin. No radiographic differences were observed among groups at any time points ($p > 0.05$). AgP patients presented a higher root sensitivity when compared CP group ($p \leq 0.05$). For the late and global patient's perception evaluation all groups had a positive impact on OHIP-14 total score ($p \leq 0.05$) and no differences were observed among them ($p > 0.05$). EMD has proven to be a viable therapy for the treatment of intrabony defects of individuals with AgP, with a similar rate of regeneration to that of patients with CP.

KEYWORDS Aggressive periodontitis; chronic periodontitis; alveolar bone loss; dental enamel proteins; patient outcome assessment.

LISTA DE ABREVIATURAS E SIGLAS

Aa	-	*Aggregatibacter actinomycecemtocomitans*
AAP	-	American Academy of Periodontolgy/Academia Americana de Periodontia
ACP	-	Alveolar crest position
AgP/PAg	-	Agressive periodontitis/Periodontite agressiva
BC	-	Bone crest
BD	-	Basis of defect
BF	-	Bone filling
BG	-	Bone graft
CAL/NIC	-	Clinical attachment level/Nível de inserção clínica
CEJ	-	Cememt-enamel junction
CM	-	Collagen membrane
CONSORT	-	Consolidated Standards of Reporting Trials
CP/PC	-	Chronic periodontitis/Periodontite crônica
DD	-	Defect depth
DR	-	Defect resolution
EMD	-	Enamel matrix proteins
FMBI	-	Full mouth bleeding index
FMPI	-	Full mouth plaque index
GR	-	Gingival recession
GTR/RTG	-	Guided tissue regeneration/Regeneração tecidual guiada
IC	-	Intraclass correlation
IL	-	Interleukin
MDM	-	Modified perforated collagen membrane
MIST	-	Minimally invasive surgical technique
OFD/AC	-	Open flap debridement/Acesso cirúrgico
OHIP-14	-	Oral Health Impact Profile-14
Pg	-	*Porphyromonas gingivalis*
PPD/PS	-	Pocket probing depth /Profundidade de sondagem

PRF	-	Platelet rich fibrin
RAR	-	Raspagem e alisamento radicular
rCAL/NICr	-	Relative clinical attachment level/Nível de inserção clínica relativo
RCT	-	Randomized clinical trial
RS	-	Root surface
SS	-	Sangramento à sondagem
ST	-	Stent
VAS	-	Visual analog scale

SUMÁRIO

1 INTRODUÇÃO

Periodontite agressiva

Várias podem ser as formas de doença periodontal que afetam crianças, adolescentes e jovens adultos. Embora o tipo mais comum reconhecido seja a gengivite, doenças relacionadas ao periodonto de inserção também podem ocorrer. As primeiras classificações sugeriram diferentes nomenclaturas para este quadro clínico, no qual formas destrutivas da doença periodontal podem ser observadas em indivíduos jovens, como periodontose, ou ainda periodontite juvenil, periodontite pré-puberal e periodontite de progressão rápida, considerando apenas o fator idade como fundamental para o diagnóstico. Com as mudanças significativas nestas classificações segundo o conceito proposto pela Academia Americana de Periodontologia (AAP), em um encontro internacional no ano de 1999 (1), uma classe de periodontite foi determinada, aglomerando classificações prévias em uma única denominada periodontite agressiva. Denominação esta que foi utilizada amplamente até 2018, quando a mais recente classificação foi proposta (2), e passou-se a reconhecer esta condição como periodontite de estágios III/IV (severa, com potencial de perda dental), com extensão e distribuição incisivo-molar, grau C (com rápida taxa de progressão).

A periodontite agressiva engloba um conjunto de diferentes subformas que revelam um fenótipo clínico comum: acometimento de indivíduos clinicamente saudáveis (exceto pela periodontite); ausência de condições sistêmicas adversas; rápida perda de inserção e de destruição óssea; agregação familiar. Algumas características secundárias comuns as periodontites agressivas, mas não necessariamente universais, são: quantidades de depósitos microbianos inconsistentes com a gravidade de destruição periodontal, anormalidades fagocitárias, um fenótipo hiper-responsivo de macrófago, incluindo níveis elevados de prostaglandinas E2 e interleucina (IL) 1-β (1,3). A periodontite agressiva em sua forma localizada comumente tem um início circumpubertal, com um dano periodontal confinado a região de primeiros molares e incisivos. Esta forma de periodontite agressiva está normalmente associada a presença de proporções elevadas de *Actinobacillus* (atualmente *Aggregatibacter*) actinomycecemtocomitans (Aa) e a anormalidades fagocitárias. Uma resposta exacerbada de anticorpos séricos aos agentes infectantes é frequentemente detectada. Já a periodontite agressiva generalizada normalmente afeta pessoas com idade inferior a 30 anos,

podendo os pacientes serem mais velhos. Nessa classe, existem perdas de inserção interproximal generalizadas, acometendo ao menos 3 dentes permanentes, além de incisivos e molares. A perda de inserção ocorre em períodos episódicos de pronunciada destruição e está normalmente associada a proporções elevadas de Aa e *Porphyromonas gingivalis* (Pg), além de anormalidades na função neutrofílica. Uma pobre resposta de anticorpos séricos aos agentes infectantes é frequentemente detectada (4).

A periodontite agressiva pode ser considerada relativamente rara, porém sua importância desponta em determinadas populações específicas, com fatores genéticos e ambientais predisponentes, nas quais este número pode alcançar a marca de 10% (5). No Brasil, estudos epidemiológicos mostraram uma prevalência de 5,5% da população jovem (até 29 anos) apresentando algum grau de destruição periodontal, caracterizando a ocorrência de periodontite agressiva, sendo essa porcentagem próxima de 10% na faixa de 25-29 anos (6). É possível afirmar ainda que a prevalência de periodontite agressiva varia significantemente entre diferentes localizações geográficas e entre diferentes raças/etnias (6,7). Apesar da frequência relativamente baixa, existe um importante interesse no manejo clínico destes indivíduos. Além de sua forma não usual de apresentação clínica, há ainda o maior risco de progressão desta com o passar da idade (8). Somado ao fato da pouca idade desses indivíduos, a determinação de terapias mais efetivas de tratamento passa a ser um importante passo. Outro fator importante é que a perda dental em idades mais jovens pode acarretar em mudanças de atitude e comportamento, podendo ainda culminar em depressão psicológica e afastamento do convívio social (9).

As abordagens e objetivos relacionados ao tratamento de pacientes com periodontite agressiva não são marcadamente distintos quando comparados a pacientes portadores da forma crônica da doença. Portanto, as diferentes fases de tratamento, incluindo as etapas sistêmicas, inicial, reavaliação, cirúrgica, manutenção e restauradora, são comuns a ambos os tipos de periodontite. Todavia, a quantidade considerável de perda óssea relativa a pouca idade, bem como a elevada taxa de perda óssea justificam uma estratégia bem fundamentada e mais agressiva para a abordagem durante o tratamento, na intenção de uma maior estabilização da progressão da doença, por meio da detenção da destruição periodontal e da recuperação da maior quantidade de inserção periodontal possível (8). Assim, o objetivo principal fundamentado pela literatura compreende no controle da doença, por meio da criação de uma condição clínica aceitável, conduzindo a manutenção da maior quantidade de dentes for possível pela maior quantidade de tempo.

Durante a fase ativa do tratamento a resposta destes indivíduos a terapia não cirúrgica é mais extensivamente documentada na literatura, em comparação as demais etapas. Mesmo assim, quando comparada a periodontite crônica, muitas lacunas ainda existem como consequência da escassez de estudos com amostras representativas e maiores tempos de acompanhamento. Efeitos da raspagem e alisamento radicular (RAR) positivos em relação à redução da profundidade de sondagem (PS), sangramento à sondagem (SS) e ganho no nível de inserção clínica (NIC) foram confirmados por diversos estudos comparativos os quais investigaram o efeito adjunto de antimicrobianos onde o grupo controle era composto somente pelo tratamento com RAR (10–18). Assim, é possível inferir que a periodontite agressiva generalizada responde bem a RAR em curto prazo, porém após 6 meses, recidivas e progressão da doença foram relatados, mesmo com manutenções frequentes e reforço no padrão de higiene oral (8).

Sabendo então que o tratamento da periodontite agressiva é fatidicamente um desafio, por responder menos previsivelmente a terapia mecânica convencional quando comparada a periodontite crônica (19,20), pesquisadores tem explorado tratamentos coadjuvantes a fim de alcançar estabilidade e previsibilidade para a terapia mecânica convencional. Diante da natureza microbiológica específica para as periodontites agressivas, o uso de antimicrobianos, locais e/ou sistêmicos, tem sido ostensivamente avaliado como auxiliar a RAR, despontando com resultados satisfatórios (11,21–24).

Embora haja atualmente uma relativa padronização para o tratamento da doença periodontal agressiva, raros são os estudos que abordam os tratamentos dos defeitos ósseos nessa condição. Um importante ponto a ser observado é o padrão de perda óssea presente nesses indivíduos. É possível detectar em pacientes com periodontite agressiva uma avançada perda de inserção comumente acompanhada de uma perda óssea alveolar de forma angulada, formando defeitos infra-ósseos. Além disso, é possível afirmar que estes defeitos são achados comuns em pacientes com periodontite agressiva. Contudo, até o presente momento, o tratamento desse tipo de lesão é realizado de forma empírica e tem sido pouco explorado em estudos clínicos.

Tratamento regenerativo em periodontite agressiva

O tratamento de defeitos infra-ósseos tem se baseado na regeneração tecidual dos elementos perdidos durante a progressão da doença periodontal, isto é, na indução a nova formação

dos tecidos ósseo, cementário e do ligamento periodontal (25). Nesse intuito, diferentes técnicas têm sido propostas como uso de substitutos ósseos, membranas para regeneração tecidual guiada, modificadores biológicos e combinações. Estas diferentes abordagens foram avaliadas e sugeriu-se que a efetividade depende diretamente da morfologia do defeito infra-ósseo, mobilidade dental e envolvimento de furca (8), isto é, resultados falhos são esperados no tratamento de defeitos horizontais, regiões de furca grau III e elevado grau de mobilidade (26).

Apesar de dada importância, até o presente momento, poucos trabalhos científicos avaliando terapias regenerativas em pacientes com periodontite agressiva foram realizados, sendo os existentes de baixa evidência científica, sem amostras representativas. Porém mesmo considerando o nível relativamente baixo de evidências, a cirurgia regenerativa periodontal pode ser realizada em pacientes acometidos por periodontite agressiva (27).

Alguns destes relatos tem mostrado efetividade na utilização de enxertos alógenos, xenógenos e aloplásticos no manejo de pacientes com periodontite agressiva (28–31). Somente poucos estudos controlados têm sido conduzidos utilizando um adequado número de pacientes, ou ainda comparando tratamentos, porém resultados favoráveis à utilização de algumas técnicas regenerativas têm se destacado (32–36).

O uso de membranas na intenção de influenciar o crescimento diferenciado dos tecidos em defeitos infra-ósseos por meio da regeneração tecidual guiada em pacientes portadores de periodontite agressiva também tem sido proposto, sendo que as não-absorvíveis compostas por polifluoretano expandido apresentaram melhores resultados nestes indivíduos (37–41). Sirinat et al. (39) supostamente encontraram dificuldades em obter resultados significantes ao avaliar o uso de membranas de polifluoretano com cirurgia ressectiva, após um ano de acompanhamento, possivelmente pelo baixo tamanho da amostra de pacientes portadores de periodontite agressiva, apenas 6 indivíduos. Zucchelli et al. (40) provavelmente enfrentaram o mesmo problema em relação a amostra, falhando ao tentar demostrar diferenças entre os potenciais regenerativos frente ao uso de membrana reforçada por titânio em defeitos infra-ósseos em 10 pacientes com periodontite crônica e 10 com a forma agressiva, agravado ainda pela necessidade de uma segunda intervenção cirúrgica. Análises clínicas e radiográficas foram realizadas para testar uma membrana perfurada modificada em comparação as membranas padrão de colágeno (42) e foi encontrado para o grupo teste um maior preenchimento linear do defeito aos 12 meses, comparando aos 6 meses, o

que pode indicar uma maior área de nova formação óssea; assim este estudo apoia o tratamento regenerativos de defeitos infra-ósseos em pacientes com PAg.

Outros materiais, tais como fatores de crescimento e diferenciação têm despontado na literatura como opções viáveis para regeneração. Mediadores como o plasma derivado de fator de crescimento, fator de crescimento insulínico, fator de crescimento fibroblástico, proteína óssea morfogênica e fator de crescimento transformador-β têm mostrado resultados promissores in vitro (43–45). O plasma rico em plaquetas mostrou melhorar parâmetros clínicos e radiográficos em dentes periodontalmente comprometidos (46,47). O plasma rico em fibrina demonstrou uma maior redução na PS, melhor ganho médio no NIC e boa quantidade de preenchimento ósseo em relação ao grupo controle, exclusivamente para defeitos de 3 paredes, mais previsíveis (48). Suas aparentes desvantagens são a baixa especificidade do tecido, bem como a ausência de conhecimento relacionado aos efeitos sistêmicos (49,50). Outra alternativa regenerativa é o uso das proteínas derivadas da matriz do esmalte (EMD).

Proteínas derivadas da matriz do esmalte

No final da década de 1990, o uso de EMD foi introduzido como uma abordagem biológica em regeneração. O EMD é um ácido purificado extraído do esmalte embrionário em desenvolvimento derivado de leitões com, em média, 6 meses de idade (51).

O EMD é composto de diferentes proteínas, 90% das quais são amelogeninas, uma família de proteínas hidrofóbicas, que tem a capacidade de induzir os processos envolvidos na regeneração dos tecidos periodontais de maneira semelhante ao desenvolvimento normal dos tecidos, estimulando as células epiteliais da Bainha de Hertwig Bainha da raiz (52). Sugere-se que o EMD estimule crescimento celular mesenquimal e provoque uma efeito sobre as células epiteliais. O EMD permanecerá no local tratado durante pelo menos 4 semanas, proporcionando tempo suficiente para que as células do ligamento periodontal recolonizem a região. Além disso, o EMD tem um efeito inibidor de placa, não permitindo o crescimento de bactérias gram-negativas, mas permitindo a colonização de gram-positivas (51).

Diversos estudos clínicos e em modelo animal têm demonstrado a utilização das proteínas derivadas da matriz do esmalte em procedimentos periodontais regenerativos para a formação de um novo periodonto (52–54). Estudos clínicos controlados em defeitos infra-ósseos

em pacientes portadores de periodontite crônica, têm demonstrado resultados favoráveis a utilização de EMD, com uma presente diminuição na PS, além de ganho no NIC, de forma semelhante as terapias com membranas (55–58). Uma recente revisão sistemática e meta-análise, a qual avaliou o uso de EMD em defeitos desta conformação, demonstrou que o uso de EMD resultou em uma redução média de PS igual a 4 mm, e um ganho médio no NIC de 3,4 mm (59). Adicionalmente, algumas análises histológicas tem demonstrado a presença de novo cemento, novas inserções conjuntivas e formação óssea em dentes tratados com EMD (60–63).

É possível ainda inferir vantagens do uso de EMD em relação a regeneração tecidual guiada (RTG), procedimento regenerativo com maiores evidências registradas na literatura. Com resultados clínicos semelhantes (25), a facilidade técnica é superior quando utiliza-se EMD, considerando-se que com a utilização de RTG a morbidade e as complicações pós operatórios, como exposição de membrana, são significativamente mais impactantes.

Todavia, mesmo com os resultados positivos alcançados com este biomaterial para o tratamento da forma crônica da doença, poucas são as evidências da utilização deste em periodontite agressiva. Apenas relatos de caso foram publicados utilizando EMD em pacientes portadores de periodontite agressiva (64–67).

A respeito disto, Vandana et al. (68) publicaram uma série de casos envolvendo 4 pacientes diagnosticados com periodontite crônica e 4 com periodontite agressiva. Um total de 16 defeitos infra-ósseos foram cirurgicamente tratados com EMD ou debridamento por meio de acesso cirúrgico somente, através de um desenho experimental de boca dividida. As médias de redução de PS e quantidade de preenchimento de defeito foram significativas para ambas as abordagens após 9 meses de acompanhamento. Não foram detectadas diferenças estatísticas nas médias de redução de PS, ganho no NIC, quantidade de preenchimento do defeito ou resolução do defeito entre os dois tratamentos, para ambos os grupos de pacientes. Este estudo então falhou em mostrar diferenças entre os tratamentos, bem como entre os grupos de periodontites.

Já um estudo retrospectivo (69) não mostrou diferenças estatísticas quando comparando a execução de RTG com a utilização de xenoenxerto ósseo desproteinizado e EMD juntos.

É possível afirmar que pouco se sabe sobre as diferenças no perfil da doença periodontal agressiva. Sabe-se que no que diz respeito a origem da doença, a periodontite agressiva

é distinta, porém, sobre o potencial regenerativo destes indivíduos, pouco ou nada se sabe. É possível ainda ressaltar que, caso haja diferenças entre os potenciais regenerativos em comparação a periodontite crônica, estratégias individualizadas deverão ser determinadas para cada tipo de paciente, respeitando essas diferenças. Assim, torna-se importante o desenvolvimento de estudos com amostras representativas e com maiores tempos de acompanhamento que comparem os potenciais regenerativos e o perfil de resposta entre indivíduos portadores de periodontite agressiva e periodontite crônica.

Frente a isso, o presente estudo clínico randomizado controlado foi realizado com intuito de avaliar clinicamente, radiograficamente e no que diz que respeito ao impacto na qualidade de vida dos voluntários, o uso de EMD em defeitos infra-ósseos em pacientes com periodontite agressiva, comparando-os a indivíduos com periodontite crônica.

2 ARTIGO

Ana Lívia Fileto Mazzonetto[1]; Renato Correa Viana Casarin[1]; Mauro Pedrine Santamaria[2]; Cássia Fernandes Araújo[2]; Rafaela Videira Clima da Silva[1]; Javier Eduardo Vivanco Purisaca[1]; Antonio Wilson Sallum[1].

CLINICAL, RADIOGRAPHIC AND PATIENT-CENTERED OUTCOMES AFTER USE OF ENAMEL MATRIX PROTEINS FOR THE TREATMENT OF INTRABONY DEFECTS IN PATIENTS WITH AGGRESSIVE PERIODONTITIS: A 12-MONTHS MULTICENTER CLINICAL TRIAL

Ana Lívia Fileto Mazzonetto
anafileto@hotmail.com
+55 19 21065301

[1] Department of Prosthesis and Periodontology.
State University of Campinas, Limeira Ave 901, 13414-903, Piracicaba, Brazil

[2] Department of Diagnosis and Surgery.
State University of São Paulo, Eng. Francisco José Longo Ave 777, 12245-000, São José dos Campos, Brazil

ABSTRACT

Objectives: The aim of this study was to evaluate clinically, radiographically and in what concerns about patient-centered outcomes, the use of EMD in intrabony defects in patients with aggressive periodontitis (AgP), comparing them to individuals with chronic periodontitis (CP).

Materials and Methods: Forty intrabony defects in AgP patients and twenty defects in CP patients of at least 4 mm depth and 2 mm horizontal, associated with ≥ 6 mm residual probing pocket depth (PPD) were included in the study and were randomly assigned to the groups: AgP+OFD (n=20), AgP patients which received open flap debridement (OFD); AgP+OFD/EMD (n=20), AgP patients which received OFD and EMD; CP+OFD/EMD (n=20), CP patients which received OFD and EMD. PPD, relative clinical attachment level (rCAL), and gingival recession (GR) were measured at baseline, at 6 and 12 months. Defect resolution (DR) and bone filling (BF) were used for radiographic analysis. The quality of life was recorded at baseline and 6 months using the OHIP-14 questionnaire and VAS scale to early post-therapy period (15 days).

Results: PPD and rCAL has improved for all groups along the follow-ups with statistically significant difference over time, comparing to baseline, and AgP+OFD/EMD presented a lower rCAL at 12 months (7.8 ± 1.5 mm) in relation to baseline when compared to AgP patients who did not receive (8.8 ± 1.5 mm). AgP+OFD/EMD-treated sites also presented a discreet decrease of 0.05 ± 0.9 mm in GR while CP+OFD/EMD-treated sites had an increase of 0.5 ± 0.9 mm, revealing a statistically significant difference between the groups that received EMD for the stability of the gingival margin. No radiographic differences were observed among groups at any time points. AgP patients presented a higher root sensitivity when compared CP group. All the groups reported a positive impact on OHIP-14 total score and without differences among them.

Conclusions: EMD has proven to be a viable therapy for the treatment of individuals with AgP, with a rate of regeneration similar to that of patients with CP.

KEYWORDS Aggressive periodontitis; chronic periodontitis; intrabony defects; periodontal bone loss; periodontal regeneration; enamel matrix proteins.

INTRODUCTION

Aggressive periodontitis (AgP) is characterized by rapid progression, in individuals who are systematically healthy, with relatively low amounts of biofilm, including significant destructions of the tooth-supporting structures, mainly in the proximal surfaces, resulting in the formation of intrabony defects (1). This type of defect, usually associated with periodontal pockets, are site-specific risk factors for periodontal disease progression and tooth loss (1).

Periodontal regeneration has the ability to improve the short- and long-term prognosis of periodontally affected teeth (2). Periodontal regenerative procedures, that aim to restore root cementum, alveolar bone and periodontal ligament fibers, in association with adequate integration among all regenerated tissues, can be safely performed in AgP (3) and also chronic periodontitis (CP) (4,5) patients, with recognized improvement in clinical parameters. Additionally, the results of these regenerative procedures in intrabony defects are influenced by the morphology of the defect, including, the number of remaining bone walls, the dimensions and the angle. (2).

About AgP and CP diagnoses should be cited that according to the results found in the World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions in 2017, there is insufficient evidence to support AgP as pathophysiologically distinct disease; grouping the chronic and aggressive forms into a single category, periodontitis, that are currently characterized based on a multi-dimensional staging and classification system (6). As the present study was carried out before the disclosure and recognition of this new classification, it was adopted the classification of 1999, also proposed by the American Academy of Periodontology in that opportunity, which separates AgP and CP in distinct groups (1).

The proteins derived from the enamel matrix (EMD) are a compound of distinct proteins, of which 90% are amelogenins, that has the ability to conduct events related to the regeneration of periodontal tissues comparably to that observed during the normal development of these. (7). Randomized clinical trials (RCT) with reconstructive periodontal therapy in CP patients have shown favorable results in the use of EMD, with a decrease in PPD, besides gain in the CAL, similarly to therapies with membranes (8–11). A recent systematic review and meta-analysis, which evaluated the use of EMD in intrabony defects, demonstrated that the use of this compound resulted in an average reduction of PPD equal to 4 mm, and an average gain in the CAL of 3.4mm (5).

The clinical gains achieved in the literature may be associated with the positive impact that regenerative therapies, as EMD, had on patients' quality of life. Starting in the 1970, with social scientists, through the 90's decade with the accession of medical researchers spread the reasoning about the importance of evaluating indicators of hybridism of well-being and quality of life and understanding of how the disease and your treatment affect the quality of life of patients. More recently it has been noted that concern in the context of oral conditions, but this qualitative data are still scarce (12).

Even with the positive results achieved with this biomaterial for the treatment of the chronic form of the disease, there are few evidences of the use of this in AgP, only case reports and a retrospective study were published (3).

It is known that with regard to the origin of the disease AgP is distinct, however, about the regenerative potential of these individuals, little or nothing is known. It is also possible to emphasize that, if there are differences between regenerative potentials compared to CP,

individualized strategies should be determined for each type of patient, respecting these differences. Thus, the aim of this multicenter study was to evaluate clinically, radiographically and in what concerns about patient-centered outcomes, the use of EMD in intrabony defects in patients with AgP, comparing them to individuals with CP.

MATERIALS AND METHODS

Study design

This study is designed as a double-blind, prospective clinical trial enrolled in the Unites States National Institutes of Health Clinical Trials Registry (NCT3025204). Two different surgical approach were compared clinically, radiographically and in what concerns about patient-centered outcomes for the treatment of intrabony defects in patients with AgP and CP: AgP+OFD (n=20), AgP patients which received open flap debridement (OFD); AgP+OFD/EMD (n=20), AgP patients which received OFD and application of EMD (Emdogain, Straumann, Basel, Switzerland); CP+OFD/EMD (n=20), CP patients which received OFD and application of EMD. The investigators who assessed all the outcomes were unaware of the identity of the treatment protocol applied.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (13). Additionally, this study was approved by the Ethics Committee of Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil (CAAE 48351115.9.0000.5418). For all selected individuals, the informed consent term was applied.

Sample-size calculation

The sample size required for each group was calculated considering clinical attachment level (CAL) as the primary outcome. To detect a difference of 1 mm with α=5%, considering a standard deviation of the error of 1 mm and power=80% (10), 12 patients was required in each group.

Population screening

The subject population was screened from July 2015 through February 2017 among those who sought care or were referred to the Periodontology Clinic of the Piracicaba Dental School (State University of Campinas, Piracicaba, Brazil) or to Periodontology Clinic of São José dos Campos Dental School (State University of São Paulo, São José dos Campos, Brazil).

Inclusion criteria

Patients included in this study were diagnosed with AgP or CP according to 1999 American Academy of Periodontology classification (1). The inclusion criteria adopted were: (a) presence of at least 4 mm depth and 2 mm horizontal, as detected in diagnostic periapical radiographs, associated with ≥ 6 mm residual PPD (10); (b) full mouth plaque index (FMPI) and full mouth bleeding index (FMBI) ≤ 20%; (c) the selected tooth should be vital or endodontically treated appropriately; (d) signed informed consent form. All subjects were adequately clarified about the study objectives, as well as possible risks and benefits offered by the adopted treatment protocol.

Exclusion criteria

The exclusion criteria adopted were: (a) systematic medical compromising conditions; (b) use of any medication, punctual or continuously, that may interfere with the periodontal condition; (c) current or previous smoking; (d) pregnant and lactating women; (e) furcation involvement or mobility in the referred tooth; (f) extruded or malpositioned teeth; (g) record of periodontal surgery in the region of interest.

Initial therapy

All patients underwent non-surgical scaling and root planing motivation sessions and periodontal maintenance therapy. After 3 months, patients who fulfilled the inclusion criteria were definitively incorporated into the surgical phase.

Randomization and allocation

The allocation of AgP patients was performed with the help of a randomization table generated in an automatic way by computer, organized by a single individual (RVCS) unrelated to the stages of recruitment, treatment or evaluation of patients. In sequence, opaque and sealed envelopes were organized with the treatment codes for randomization of the patients. The revelation of the treatments happened to the surgeon only after the conclusion of the OFD. Both patients and examiners were not aware of the treatments performed at any time of the study. The randomization parameters and blinding were performed according to the CONSORT (Consolidated Standards of Reporting Trials) 2010 statement (14).

Treatment protocol

Teeth that presented residual PPD ≥ 6mm associated with radiographic evidence of intrabony defects, according to the aforementioned inclusion criteria, were included in the surgical phase. The regenerative procedures were only scheduled when all the necessary non-surgical procedures were performed.

The surgical procedures were performed by two previously calibrated surgeons (RCVC and MPS), one in each center of the study, taking into account the principles proposed in the modified minimally invasive papilla preservation technique (15). Anti-inflammatory drugs were administered 1 hour before the procedure, 4 mg dexamethasone (Aché Pharmaceutical Laboratories SA, Guarulhos, SP, Brazil). An open flap debridement (OFD) was performed with the same flap technique (15) using periodontal curettes for all the groups and the root was carefully planned.

After the OFD was when the allotted treatment was presented to the surgeon, with the opening of the sealed envelope. On dry surface, the EMD was used and left in contact for at least 2 minutes. The application started in the most apical portion of the bone level until the total coverage of the defect and the root surface. Finally a single modified internal mattress suture (VICRYL 5/0, Johnson & Johnson, New Brunswick, NJ, USA) in the inter-dental areas and simple passing sutures in vertical incisions (Figure 1).

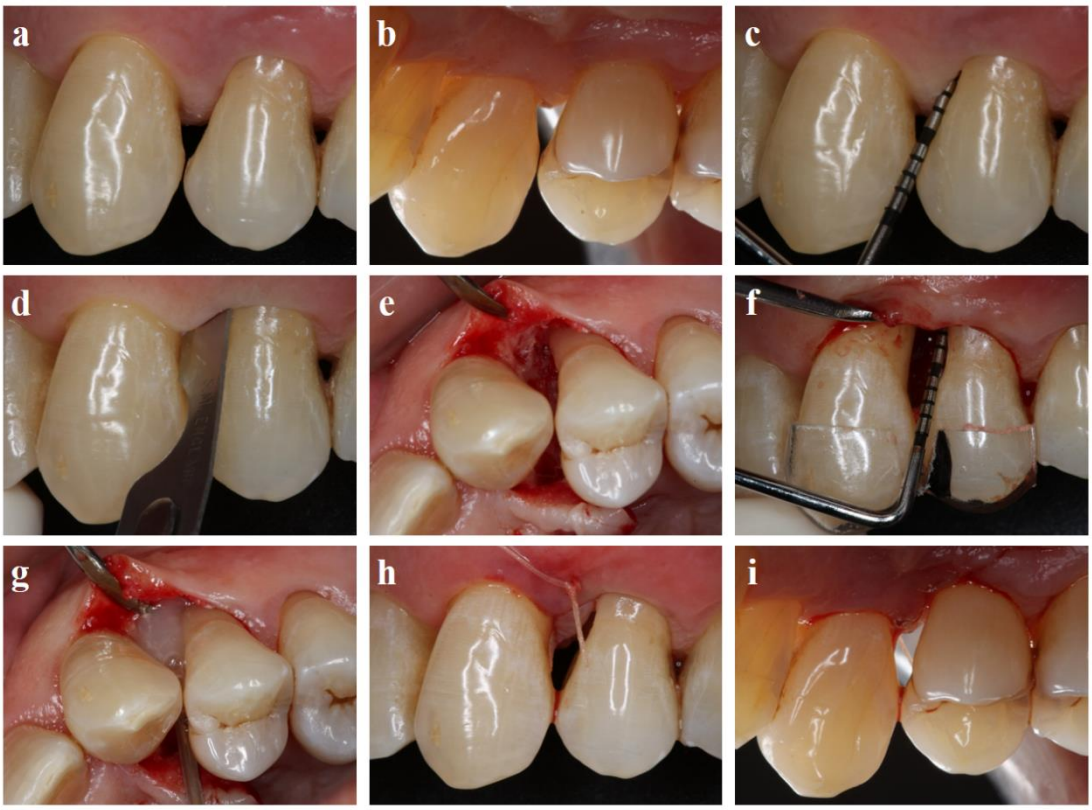


Figure 1. Surgical procedure EMD+OFD. **a-c)** Preoperative condition. **d)** Incisions perfomed by a modified papila preservation. **e)** 2-wall intrabony defect after debridement. **f)** Intrabony defect measurements. **g)** EMD application. **h-i)** Horizontal mattres suture.

As a post-operative protocol, for pain control, analgesic was prescribed, 500 mg dipyrone (Aché Pharmaceutical Laboratories SA, Guarulhos, SP, Brazil), every 4 hours, if it were necessary. The use of chlorhexidine (0.12%) rinse every 12 hours for 1 minute and discontinue tooth brushing of the sites were also recommended. In the postoperative return of 15 days, the dental brushing was released and the sutures were removed. After this, during the 12 consecutive months, the patients were included in the periodontal maintenance routine, including biofilm control and reinforcement in the hygiene performance, as well as other necessary procedures.

Calibration of examiners

All the measurements used in this study were performed by two calibrated periodontists (ALFM and CFA, one in each center). For this, 6 patients not belonging to the study sample, with

AgP or CP were selected. To determine the intra-examiner and inter-examiner reproducibility of the measurements, the intraclass correlation (IC) was used. The examiners measured two times full-mouth relative clinical attachment level (rCAL) with an interval of 24 h. ALFM reached IC = 0.82 intra-examiner agreement values and CFA, IC = 0.87. Inter-examiner agreement was IC = 0.85.

For the training of all researchers, three calibration meetings were held to standardize the objectives, protocols, cases selection, clinical measures approach method and surgical procedures (16).

Clinical parameters

Clinical evaluation was performed with the aid of a periodontal probe (NC probe 15 mm, Hu-Friedy, Jacarepaguá, RJ, Brazil), using an individually acrylic stent made from study models previously molded by the examiners to standardize the position of periodontal probe; at six points per tooth and rounded off to the nearest millimeter (1) FMPI was recorded as the percentage of total surfaces that revealed the presence of plaque, (2) FMBI was assessed dichotomously too, (3) PPD was defined as the distance from the gingival margin to the base of the periodontal pocket, (4) rCAL was determined as the distance from the stent to the deepest point of the pocket, and (5) gingival recession (GR) as the distance from the gingival margin to cemento-enamel junction (CEJ). FMPI and FMBI were measured at baseline and 12 months later to control the hygiene standard of the entire sample, and the other clinical parameters were recorded at baseline, 6, and 12 months after.

Clinical characterization of the intrabony defect.

During the trans-surgical moment, for all groups, the defects were characterized as to the morphology, with an individually acrylic stent, in terms of relative depth (distance measured from the bone crest to the stent – BC-ST); total depth (distance measured from bone crest to the deepest point in the defect – BC-BD) and width (distance between bone crest to the root surface – BC-RS). The defects were described as 1-, 2-, and 3-wall.

Radiographic evaluation

Periapical radiographics of all intrabony defects were performed at baseline and after 12 months, using the technique of parallelism. For the standardization of the images, occlusal stents were made in chemically activated acrylic resin (Jet, Artigos Odontológicos Clássico LTDA, São Paulo, SP, Brazil) together with radiographic positioners (Indusbello, Londrina, PR, Brazil). A known-sized standard orthodontic wire was inserted on the occlusal bracket, in a specific position, and the image of this wire was obtained on radiographs for correction of vertical and horizontal angulation during the analyses.

The radiographs were performed by a digital system, which uses flexible and reusable phosphorus storage plates (PSP; 31mm x 41mm) (Apixia phosphor plates, Masterlink, Glendale, AZ, USA). The selection of the exposure time occurred according to the tooth related to the defect, because the apparatus used (X-ray Sommo of movable column; tube: 70 KV; 7 MA; focal length: 20 cm; Specifications: 800-1200 VA, 50-60 Hz; Gnatus, Riberão Preto, SP, Brazil) had a semi-automatic system of selection of teeth, with exposure times already established, varying between 0.32 and 0.40 seconds. A specific scanner was used to read the images (Apixia PSP scanner, Masterlink, Glendale, AZ, USA). The images obtained were then stored in the computer until the time of the analysis. The default resolution is 96x96 DPI and 32 bits. All images were recorded in TIFF format (.tif).

Only one researcher (JEVP), using a specific software (ImageJ, National Institutes of Health, Bethesda, MD, USA), performed the analysis of the radiographic measurements obtained. For this, the following anatomical references of intrabony defect were identified in the radiographs:

1. Cement-enamel junction (CEJ). If restorations are present, the apical margin of restorations was used as the reference point of CEJ.

2. The most coronal position of the bone crest (BC), when reaches the root surface of the adjacent tooth before treatment.

3. The deepest point of the defect, where the space of the periodontal ligament still keeps your normal width before treatment. The basis of the defect (BD)

For proper evaluation, distances between BC and BD (BC-BD), CEJ and BD (CEJ-BD) were measured (Figure 2). Differences between the values found in the baseline and 1 year post-op indicated the bone filling (BF) and defect resolution (DR), respectively (17).

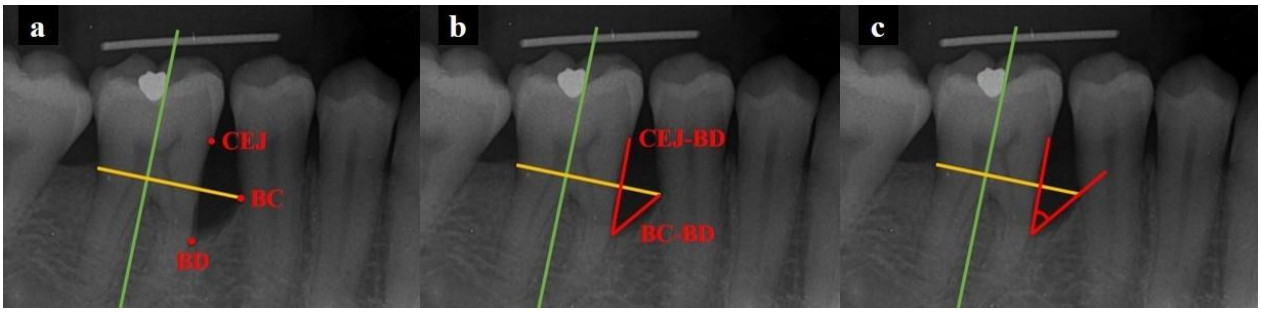


Figure 2. Radiographic evaluation of intrabony defect. **a)** Auxiliary lines and anatomical references, CEJ-cement-enamel junction, BC-bone crest, BD-basis of the defect. **b)** Anatomical distances, CEJ-BD and BC-BD. **c)** Defect angle.

Patient centered outcomes

Analyzing patient-centered perception about the performed therapies and post operative, patients received a questionnaire on day 15 after the procedure. The discomfort and/or pain reported during the procedure were recorded with a horizontal visual analogue scale of 100 mm (VAS) (18,19). The amount of analgesic ingested was also questioned. Moreover, the extent of discomfort, root hypersensitivity, edema, hematoma, high fever and interference in daily activities during the two initial postoperative weeks were recorded in the same way.

At baseline and 6 months after de surgical procedure, the Oral Health Impact Profile-14 (OHIP-14) was used in order to have access to the oral health-related quality of life and assess the patient's perception of the procedure performed in the late post-operative period. The impact dimensions are: functional limitation, physical pain, psychological discomfort, physical disability, psychological disability and disability to perform daily activities (20).

Statistical analyses

For the analysis of the socio-demographic and clinical characteristics of patients inter-group differences in metric data were observed from one-way ANOVA test and analysis of the qualitative distribution was used the Chi square test. PPD, rCAL and GR were analyzed using Ancova test for comparison between groups in the follow-up times, considering the baseline as covariate; to compare the baseline, a T test was performed; one-way repeated measures - *ANOVA* was used for intragroup differences within time. Additionally, for the radiographic parameter intragroup differences comparing to baseline was performed with T test. Inter-group differences using VAS scale were observed from analysis of variance and Tukey test. Finally, OHIP-14 inter-group differences were observed from one-way Kruskall-Wallis test and intra-group differences were observed from Wilcoxon test. An experimental level of significance was determined at 5% for all statistical analysis. All analyses were performed on SigmaPlot Scientific Data Software.

RESULTS

Briefly, 1093 individuals were assessed for eligibility. A total of 975 were excluded because of not meeting inclusion criteria and 6 for refusing to participate in the study. 112 patients were submitted to the initial therapy and maintained in periodontal supportive therapy. After 6 months, 72 intrabony defects were selected and randomized to the surgical phase. Of these defects, 68 received the intervention allocated and 8 were lost in the follow-ups. Finally, 60 defects, 20 for each group, were included in the statistical analyses (Figure 3).

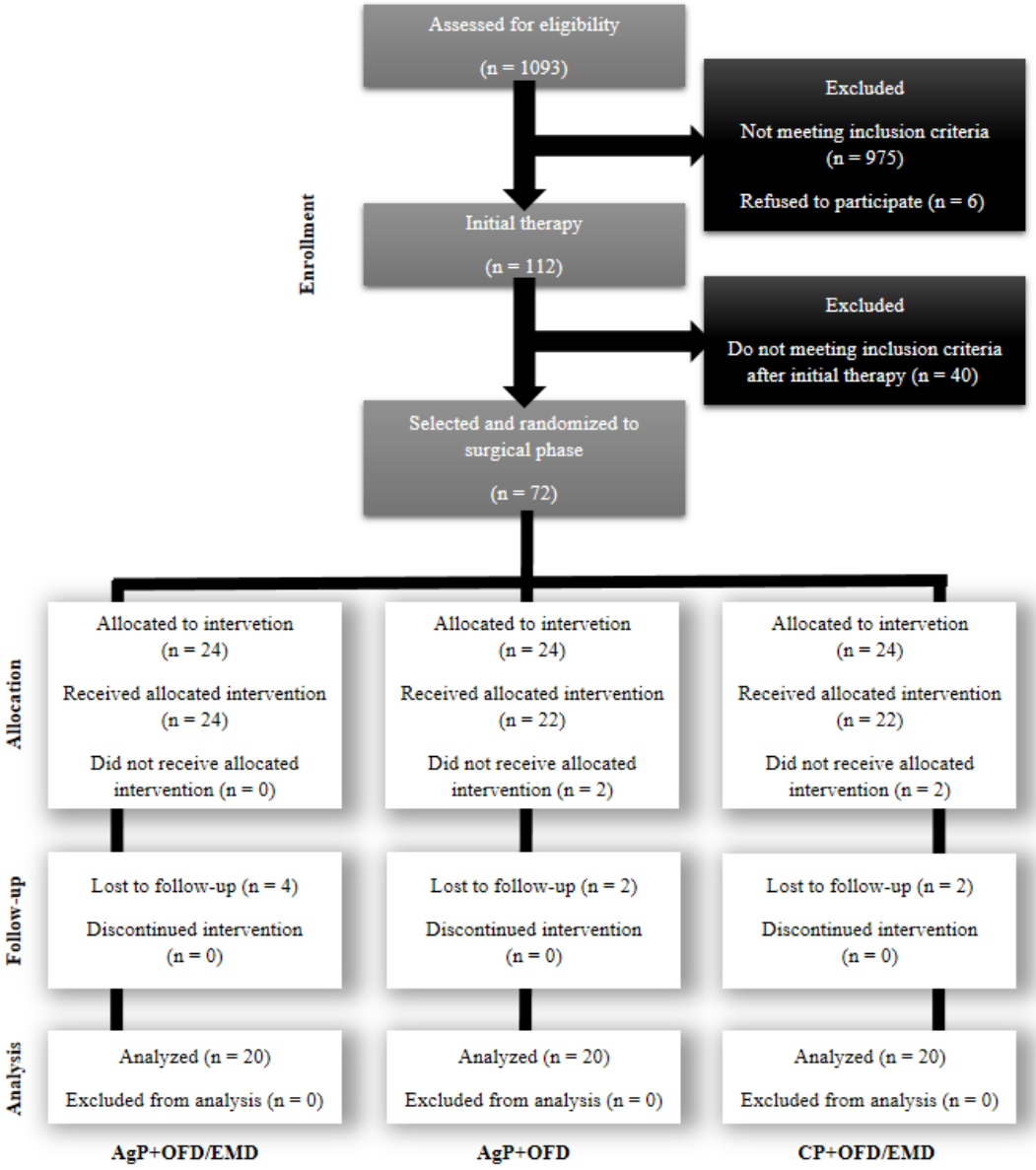


Figure 3. CONSORT flowchart of the RCT

The characteristics of the patients included in the baseline are shown in Table 1. This study included 60 patients, 68% were female, and 72% of teeth were posteriors. All studied groups were similar as the clinical and patient-related characteristics at baseline and trans-surgical ($p>0.05$), with the exception of patients AgP and CP, in which early age is one of the determinant factors for the diagnosis of AgP ($p\leq0.05$). FMPI and FMBI were maintained below 20% during the study period.

Table 1. Socio-demographic and clinical characteristics of patients

Variable	Category	AgP+OFD/EMD	AgP+OFD	CP+OFD/EMD
Age	(means ± SD), years	32.30 ± 5.08	32.00 ± 5.35	51.75 ± 6.36*
Gender	Male, n (%)	3 (15.00)	5 (25.00)	11(55.00)[#]
	Female, n (%)	17 (85.00)	15 (75.00)	9 (45.00)
Teeth	Anterior, n (%)	4 (20.00)	5 (25.00)	8 (40.00)
	Posterior, n (%)	16 (80.00)	15 (75.00)	12 (60.00)
FMPI	Baseline (means ± SD), %	19.94 ± 9.09	18.92 ± 8.32	16.24 ± 11.19
	12 months (means ± SD), %	9.40 ± 6.82[τ]	10.55 ± 9.00[τ]	13.13 ± 7.02
FMBI	Baseline (means ± SD), %	16.50 ± 6.99	14.26 ± 5.55	13.15 ± 13.79
	12 months (means ± SD), %	12.21 ± 4.87[#]	12.29 ± 5.68	9.73 ± 6.99
Radiographic measurements at baseline				
CEJ-BD	(means ± SD), mm	7.09 ±1.83	6.10 ±2.29	7.99 ±2.60
BC-BD	(means ± SD), mm	4.31 ± 1.29	4.01 ±1.59	4.35 ±2.44
Defect angle	(means ± SD), degrees	29.96 ± 7.40	33.59 ± 12.46	28.27 ± 11.39
Trans-operative measures				
Walls	1, n (%)	8 (40)	10 (50)	7 (35)
	2, n (%)	7 (35)	6 (30)	9 (45)
	3, n (%)	5 (25)	4 (20)	4 (20)
BC-ST	(means ± SD), mm	6.69 ± 1.82	7.29 ± 1.10	7.53 ± 1.97
BC-BD	(means ± SD), mm	5.64 ± 1.65	5.53 ± 1.74	5.72 ± 1.72
BC-RS	(means ± SD), mm	3.68 ± 1.04	3.56 ± 1.11	3.25 ± 1.22

*Indicate statistical difference between CP and AgP groups (one-way ANOVA test, p≤0.05). [τ]Indicate statistical difference to baseline (paired Student's t test, p≤0.05). [#]Indicate statistical difference to AgP+OFD/EMD group (Chi square test, p≤0.05).

The clinical parameters obtained at the end of 12 months are presented in Table 2. Additionally, no adverse effects have been reported. The clinical benefits achieved after 12 months for all groups are exemplified in Figure 4.

PPD

PPD has improved equivalently for all groups along the follow-ups with statistically significant difference over time, comparing to baseline ($p \leq 0.05$) but not among the groups ($p > 0.05$).

rCAL

In the AgP+OFD/EMD group, rCAL changed from 10.3 ± 1.8 mm at baseline to 7.8 ± 1.5 mm after 12 months corresponding to a gain of 2.4 ± 1.0 mm. AgP+OFD group changed from 10.5± 1.4 to 8.8 ± 1.5 mm in this period (rCAL gain of 1.6 ± 1.6 mm). Thus AgP patients that received EMD presented a lower rCAL at 12 months ($p \leq 0.05$) and a higher rCAL gain ($p \leq 0.05$) in relation to baseline when compared to AgP patients who did not receive. rCAL has improved for all groups along the follow-ups with statistically significant difference over time, comparing to baseline ($p \leq 0.05$). Additionally, the AgP+OFD/EMD group demonstrated rCAL gain over time, with an additional gain between 6 and 12 months (8.3± 1.9 to 7.8 ± 1.5 mm, $p \leq 0.05$).

GR

At AgP+OFD/EMD-treated sites, GR changed from 0.7 ± 0.9 mm at baseline to 0.7 ± 1.0 mm at 12 months postoperative, corresponding to gingival level maintenance (0.05 ± 0.9 mm in GR). Meanwhile, CP+OFD/EMD-treated sites GR increased from 1.3 ± 1.0 to 1.7 ± 0.9 mm, revealing a greater loss of the gingival margin and an increase in GR of 0.5 ± 0.9 mm. Thus, it was possible to observe statistically significant differences between AgP and CP patients that received EMD at 12 months ($p \leq 0.05$) and comparing baseline to 12 months ($p = 0.05$). The increase in GR over time was observed in the AgP+OFD (0.8 ± 1.0 at baseline to 1.6 ± 1.3 mm at 6 months, $p \leq 0.05$) and in the CP+OFD/EMD group (1.3 ± 1.0 at baseline to 1.7 ± 10.9 mm at 12 months, $p \leq 0.05$).

Table 2. Means (± SD) of PPD, rCAL and GR, in mm, at baseline, 6 and 12 months after, including the difference between baseline and 12 months.

		Baseline	6 months	12 months	0-12 difference
PPD	AgP+OFD/EMD	6.7 ± 1.1	4.7 ± 0.9*	4.3 ± 0.9*	2.3 ± 1.2
	AgP+OFD	6.7 ± 1.1	4.9 ± 1.0*	4.8 ± 1.0*	1.9 ± 1.5
	p-value (AgP+OFD/EMD x AgP+OFD)	**0.45**	**0.63**	**0.20**	**0.157**
	CP+OFD/EMD	6.6 ± 1.3	4.4 ± 1.4*	4.2 ± 1.4*	2.5 ± 0.9
	p-value (AgP+OFD/EMD x CP+OFD/EMD)	**0.77**	**0.46**	**0.81**	**0.66**
rCAL	AgP+OFD/EMD	10.3 ± 1.8	8.3 ± 1.9*	7.8 ± 1.5*$^{\Delta}$	2.4 ± 1.0
	AgP+OFD	10.5± 1.4	9.3 ± 1.9*	8.8 ± 1.5*	1.6 ± 1.6
	p-value (AgP+OFD/EMD x AgP+OFD)	**0.68**	**0.29**	**0.037**	**0.049**
	CP+OFD/EMD	11.3 ± 2.2	9.3 ± 2.3*	9.2 ± 2.2*	2.1 ± 0.9
	p-value (AgP+OFD/EMD x CP+OFD/EMD)	**0.09**	**0.41**	**0.17**	**0.44**
GR	AgP+OFD/EMD	0.7 ± 0.9	1.1 ± 1.3	0.7 ± 1.0	0.05 ± 0.9
	AgP+OFD	0.8 ± 1.0	1.6 ± 1.3*	1.1 ± 0.9	- 0.3 ± 1.1
	p-value (AgP+OFD/EMD x AgP+OFD)	**0.43**	**0.40**	**0.139**	**0.12**
	CP+OFD/EMD	1.3 ± 1.0	1.6 ± 0.9	1.7 ± 0.9*	- 0.5 ± 0.9
	p-value (AgP+OFD/EMD x CP+OFD/EMD)	**0.11**	**0.78**	**0.008**	**0.05**

Ancova test for comparison between groups in the follow-up times, considering the baseline as covariate, $p \leq 0.05$; To compare the baseline, a T test was performed, $p \leq 0.05$. *Intragroup differences comparing to baseline and $^{\Delta}$ indicate difference to 6 months (one-way repeated measures – *ANOVA/Tukey test*, $p \leq 0.05$)

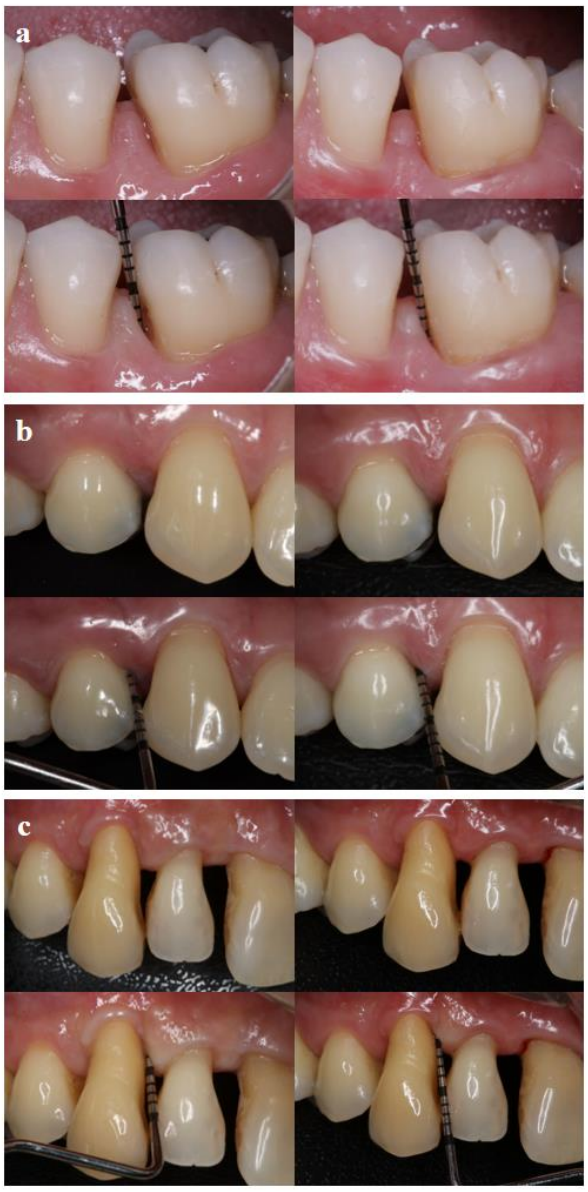


Figure 4. Baseline and final (left and right, respectively) clinical features at 12 months for (a) AgP+OFD/EMD, (b) AgP+OFD and (c) CP+OFD/EMD.

Radiographic evaluation

Table 3 contains the radiographic data analyzed. CEJ-BD and BC-BD improvements were observed after 12 months for all groups ($p \leq 0.05$), with no statistical difference among them ($p \leq 0.05$).

Table 3. Means (± SD) of CEJ-BD and BC-BD, in mm, at baseline and 12 months after, including the difference between baseline and 12 months, corresponding to bone filling and defect resolution, respectively.

		Baseline	12 months	0-12
CEJ-BD	AgP+OFD/EMD	7.09 ±1.83	5.95 ±1.71*	1.14 ±1.17
	AgP+OFD	6.10 ±2.29	5.56 ±2.63*	1.09 ±0.79
	p-value (AgP+OFD/EMD x AgP+OFD)	**0.595**	**0.94**	**0.447**
	CP+OFD/EMD	7.99 ±2.60	7.37 ±2.62*	0.78 ±1.43
	p-value (AgP+OFD/EMD x CP+OFD/EMD)	**0.313**	**0.116**	**0.453**
BC-BD	AgP+OFD/EMD	4.31 ±1.29	3.07 ±1.06*	1.24 ±1.14
	AgP+OFD	4.01 ±1.59	3.09 ±1.66*	0.92 ±1.01
	p-value (AgP+OFD/EMD x AgP+OFD)	**0.564**	**0.527**	**0.201**
	CP+OFD/EMD	4.35 ±2.44	3.72 ±2.15*	0.84 ±1.02
	p-value (AgP+OFD/EMD x CP+OFD/EMD)	**0.78**	**0.06**	**0.06**

Ancova test for comparison between groups in the follow-up times, considering the baseline as covariate, p≤0.05; To compare the baseline, a T test was performed, p≤0.05; T test one-tailed for comparison between groups in 0-12 months difference. *Intragroup differences comparing to baseline (T test p≤0.05)

Patient centered outcomes

In Table 4 it is possible to confer the assessment of the patient perception about the performed procedure and early post-therapy period. For this, the patients received a questionnaire on day 15 after the procedure, using a 100-mm horizontal visual analog scale (VAS). Performing paired comparisons among the AgP groups had higher root sensitivity than CP group (p≤0.05). The AgP+OFD group showed highest rates of pain/discomfort during procedure and Interference in daily activities when compared with the CP+OFD/EMD group (p≤0.05).

Table 4. Questionnaire to assess the patient's perception (0-100) regarding the procedure performed and the immediate postoperative period; mean (± SD).

	AgP+OFD/EMD	AgP+OFD	CP+OFD/EMD
Pain/discomfort during procedure	16.0 ± 22.8	**24.3 ± 28.7***	**5.0 ± 8.6**
Pain/discomfort after procedure	17.5 ± 24.5	11.0 ± 21.2	9.1 ± 15.7
Number of analgesic tablets	1.5 ± 2.4	0.6 ± 1.4	1.3 ± 2.8
Root hypersensitivity	**15.0 ± 30.7***	**16.7 ± 29.6***	**5.0 ± 17.9**
Edema	6.0 ± 17.0	3.3 ± 9.1	4.5 ± 21.3
Hematoma	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
High fever	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Interference in daily activities	10.0 ± 20.0	**19.0 ± 27.6***	**5.5 ± 17.9**

*Inter-group differences were observed from analysis of variance and Tukey test, p<0.05

For the late and global patient's perception evaluation, outcomes were estimated using the OHIP-14 (Table 5). All groups showed a positive impact for OHIP-14 in most parameters evaluated and the total score was better for all groups ($p \leq 0.05$) after 6 months. Only for psychologic discomfort was observed a difference between the groups AgP+OFD/EMD and CP+OFD/EMD at baseline ($p \leq 0.05$), however after 6 months this difference was not detectable ($p > 0.05$).

Table 5. OHIP total score (0-70) and segmented (0-10), means (± SD), at baseline and 6 months later

		Baseline	**6 months**
OHIP-14 total score	AgP+OFD/EMD	35.4 ± 14.3	21.9 ± 10.9$^{\Delta}$
	AgP+OFD	31.6 ± 11.0	22.7 ± 9.0$^{\Delta}$
	CP+OFD/EMD	28.2 ± 8.2	18.3 ± 6.2$^{\Delta}$
Functional limitations	AgP+OFD/EMD	3.3 ± 1.9	2.6 ± 1.5
	AgP+OFD	3.2 ± 1.8	2.4 ± 0.8
	CP+OFD/EMD	3.5 ± 1.8	2.4 ± 0.8$^{\Delta}$
Physical pain	AgP+OFD/EMD	6.0 ± 2.6	3.0 ± 1.2$^{\Delta}$
	AgP+OFD	5.8 ± 2.2	3.87 ± 2.1$^{\Delta}$
	CP+OFD/EMD	5.8 ± 1.7	2.9 ± 1.45$^{\Delta}$
Psychologic discomfort	AgP+OFD/EMD	7.6 ± 2.3*	4.4 ± 2.9
	AgP+OFD	7.4 ± 1.7	5.1 ± 2.6$^{\Delta}$
	CP+OFD/EMD	6.0 ± 1.4	3.2 ± 1.5$^{\Delta}$
Physical disability	AgP+OFD/EMD	4.9 ± 2.3	2.8 ± 1.6$^{\Delta}$
	AgP+OFD	4.2 ± 2.0	2.8 ± 1.5$^{\Delta}$
	CP+OFD/EMD	3.7 ± 1.8	2.4 ± 1.0$^{\Delta}$
Psychologic disability	AgP+OFD/EMD	5.6 ± 3.0	3.7 ± 2.3$^{\Delta}$
	AgP+OFD	4.7 ± 2.5	3.8 ± 2.0
	CP+OFD/EMD	4.2 ± 2.3	3.0 ± 1.7
Social disability	AgP+OFD/EMD	4.2 ± 2.9	2.6 ± 1.5$^{\Delta}$
	AgP+OFD	3.2 ± 1.9	2.5 ± 1.3$^{\Delta}$
	CP+OFD/EMD	2.4 ± 0.6	2.2 ± 0.5$^{\Delta}$
Handicap	AgP+OFD/EMD	3.7 ± 2.4	2.8 ± 2.0
	AgP+OFD	3.1 ± 1.8	2.3 ± 0.7
	CP+OFD/EMD	2.7 ± 1.5	2.2 ± 0.6 $^{\Delta}$

*Inter-group differences were observed from one-way Kruskall-Wallis test, $p \leq 0.05$. $^{\Delta}$Intra-group differences were observed from Wilcoxon test, $p \leq 0.05$.

DISCUSSION

The purpose of this study was to measure the impact of EMD in the treatment of intrabony defects in patients with AgP, comparing them to individuals with CP. It reveals that the

use of EMD during the OFD approach promotes additional benefits in the regeneration of intrabony defects in the AgP. Now, comparing the application of EMD in patients with different diagnoses, AgP or CP, it was possible to observe a difference between the behaviors of the gingival margin in which AgP sites presented a discreet decrease while in CP sites GR increased significantly over time.

The results presented in this study corroborate with a recent systematic review of the literature, in which the results for patients with AgP were in agreement with those demonstrated in patients with CP, with regard to periodontal regeneration and could be successfully implemented in patients with AgP (3). A significant limitation of the related literature is the low amount of published RCTs. Just eight papers (21–29) from five RCTs, that tested distinct biomaterials combined with different surgical techniques in AgP patients and in none of the these was used EMD. Comparing guided tissue regeneration (GTR) with a modified perforated collagen membrane (MPM) or a standard collagen membrane (CM) in AgP intrabony defects, Górski et al. (22) found improvements for PPD, CAL, radiographic defect depth (DD), and change in alveolar crest position (ACP) values in both groups however, statistical differences between them were not observed. Additionally, Rakmanee et al. (26) reported PPD reduction and CAL gain of 2.4mm and 1.6mm, respectively, at the GTR sites with CM without fillers and PPD reduction and CAL gain of 2.5mm and 2.1mm, respectively, at the OFD sites after 12 months of surgical therapy with no statistical differences either. In this case, the low gain of CAL for the test group, in relation to the present study (AgP+OFD/EMD rCAL gain of 2.4mm and AgP+OFD rCAL gain of 1.6mm) can be attributed to the fact that 72% of this group had membrane exposure, common intercurrence in GTR procedures that significantly affected the post-operative results obtained. Thus, it is impossible to infer that, based on significant results (CAL gain = 2.4 ± 1.2 mm) found in the present study, compared to GTR, the application of EMD in patients with AgP can be considered an easy-to-use and accessible technique with excellent results and safe in the immediate and late postoperative.

Meanwhile, although EMD therapy could be considered an easy-to-do technique, its benefits appears to be related to bone defect (30). Factors such as the anatomy of the intrabony defect and its retention property were considered determinant for additional benefits using regeneration in patients with AgP. (3). Although only rare studies included, it is possible that AgP subjects also present similar impact of bone defect on clinical-radiographic results. In the present

study the presence of 1-wall, or non-contained defects, was massive (41.6%), mainly due to the large extent and severity of the cases included in the sample. In spite of that, this fact did not seem to be impesive for the clinical success of the application of EMD compared to other regenerative therapies in AgP patients documented in the literature. Bajaj et al. (24) found clinical results after 9 months similar to those of the present study, both for the test group (CAL gain = 2.66 ± 1.07) in which platelet rich fibrin (PRF) was used, and in the control group (CAL gain = 1.59 ± 1.01) that was submitted to OFD. However, it should be pointed that, even with similar results, only 3-wall defects were selected, a classically more predictable defect. This comparison reinforce that EMD therapy in intrabony defects, especially in the more advanced stages of the disease, could be an important therapeutic alternative for AgP patients.

On the other hand, the morphology of the defects in the baseline, including not only the number of walls, but also the angulation of the defects, another defect pattern seemed to influence the therapy results. Statistically significant differences were found for all groups after 12 months of therapy, in relation to baseline for bone filling (BF) and defect resolution (DR), similarly between the groups, as well as Rakmanee et al. (25,26) and Losada et al. (2). Interestingly, the Losada's study tested EMD plus bone graft (BG) or EMD alone in patients with CP, exclusively in non-contained (one-wall) defects. Meanwhile, bone filling or defect resolution could depends not only of therapy, but also radiographic technique, period of evaluation and type of defects (22,23,25). One study (23) show that a change of 4.9% in bone density for test group, between 6 and 12 months follow-up, by radiographic subtraction, was necessary for a statistically significant relevance to be found in relation to the control group (-0.8%). Thus, the clinical benefits observed in EMD therapy might be followed-up to determine its radiographic changes.

The combination of the large amount of bone loss present, early tooth mobility and the age of the patients may have a strong impact on their quality of life, a reason that immediately points to the need for a more advanced treatment plan, comprehensive and predictable, using regenerative procedures for AgP cases.

The comparison between the responses obtained after the surgical treatment, by means of periodontal regeneration for patients with AgP and CP was performed in two non-RCTs studies (31,32). It is important to emphasize during the interpretation of the results, that these studies have significant limitations because it is not a randomized, blind study. Statistically significant

differences were not detected between both clinical conditions and PPD reduction, CAL gain and defect resolution. The results found for the different groups were comparable to those of the present study, regarding the EMD-groups. However, considering gingival recession, Zucchelli et al. (32) did not find statistically significant differences between AgP- and CP-patients using GTR, opposing to the found in the present study which AgP sites presented a discreet GR decrease of 0.05 ± 0.9 mm after 12 months while CP sites an increase of 0.5 ± 0.9 mm ($p \leq 0.05$). This result suggest a greater effect of EMD on the stability of the gingival margin for patients with AgP, and proving that the different diagnoses of periodontitis can behave differently in the face of regenerative procedures. However, this fact should be evaluated in future studies, included in vitro analysis, to explain the cells behavior differences.

All the significant results found in this study can be attributed to the great clinical applicability of the EMD in the literature for the regeneration of intrabony defects in CP. Kao et al. (33), in a systematic review, have shown that the studies evaluating the efficacy of OFD+EMD versus OFD in the management of intrabony defects, confirm that EMD application resulted in substantial improvements in clinical measurements and bone filling with EMD. Moreover, the authors highlighted that neither postoperative antibiotics, nor EDTA root conditioning, improved the clinical outcome of EMD therapy.

Two recent systematic reviews (4,5) reporting this kind of approach argue that the combined use of EMD with bone substitutes can get better clinical improvments. Moreover, recent RCTs (2,34), with follow-ups of up to 3 years, comparing the use of EMD in combination with BG and EMD applied singly, failed to prove an additional benefit of graft combination, suggesting that the use of EMD was decisive for the improvements achieved in the long term (PPD reduction ranging from 3.14 and 4.07 mm, and CAL gain, 2.62 and 4.10 mm). Additionally, Ribeiro et al. (10) analyzed the impact of EMD on a minimally invasive surgical technique (MIST) for the treatment of intrabony defects, including a group without the use of EMD or bone graft as control. They also presented significant PPD reductions, CAL gains, and no changes in GR, without differences between therapies at any time. These results differ from those found in the present study, in relation to CAL gains. At 12 months for EMD/OFD- and OFD-treated sites in AgP patients, the mean rCAL gain was 2.4 ± 1.0 mm and 1.6 ± 1.6 mm, respectively, with statistically significant differences between them.

In an expanded context, considering the last papers cited, it is possible to suggest that CAL gain is smaller comparing AgP patients to CP, although in the present RCT the gains were similar after 12 months (2.4 ± 1.0 mm and 2.1 ± 0.9 mm, respectively), and that the EMD can be considered an important approach to AgP patients as for CP. Interestingly, in the present study, only the AgP group that received EMD continued having rCAL gain over time, with statistical difference between 6 and 12 months. Studies that used EMD in CP patients (9,35) observed an additional benefit only after longer follow-up, indicating the impact of evaluation in clinical/radiographic evaluation. This aspect reinforce the need for longer follow-up determining among the regenerative potential in patients with AgP in relation to CP.

The clinical gains achieved may be associated with the positive impact that the proposed therapies for this study had on patients' quality of life. About patient's perception regarding the procedure performed and the immediate postoperative period, reduce indices of pain/discomfort during and after the procedure, edema, low interference in daily activities were reported 2 weeks after procedures in this study, with no statistical difference when compared patients who received EMD with those who have not received, or both groups that received EMD, AgP versus CP, in this study. These findings corroborate with others that also compared the use of EMD with conservatives surgical techniques, only in CP patients (18,19). Tonetti et al. (19) additionally found that the root hypersensitivity was the most frequent post-operative adverse event, affecting 45% of test and 35% of controls. On the other hand, in the present study root hypersensitivity showed levels as low as the other parameters, but comparing the groups, patients with AgP showed this symptom more pronounced than CP, suggesting that AgP patients has greater root hypersensitivity, regardless of the application of EMD. For the oral health-related quality of life, outcomes were estimated using the OHIP-14. In this study, it was possible to observe an improvement in the quality of life for all patients after 6 months, regardless of the therapy, corroborating with a previous study (36), before and after covering the application root of EMD, in patients with CP.

Analyzing the results and discussions presented it is possible to infer that EMD has proven to be a viable therapy for the treatment of individuals with AgP, with a rate of regeneration similar to that of patients with CP.

ACKNOWLEDGMENTS

This study was supported by the São Paulo Research Foundation (FAPESP), São Paulo, SP, Brazil (process 2015/19731-0). The authors report no conflicts of interest related to this study.

REFERENCES

1. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol. 1999;4(1):1–6.

2. Losada M, Gonzalez R, Garcia AP, Santos A, Nart J. Treatment of Non-Contained Infrabony Defects With Enamel Matrix Derivative Alone or in Combination With Biphasic Calcium Phosphate Bone Graft: A 12-Month Randomized Controlled Clinical Trial. J Periodontol. United States; 2017 May;88(5):426–35.

3. Corbella S, Weinstein R, Francetti L, Taschieri S, Del Fabbro M. Periodontal regeneration in aggressive periodontitis patients: A systematic review of the literature. J Investig Clin Dent 2017;8(4):e12245.

4. Esposito M, Grusovin MG, Papanikolaou N, Coulthard P, Worthington H V. Enamel matrix derivative (Emdogain) for periodontal tissue regeneration in intrabony defects. A Cochrane systematic review. Eur J Oral Implantol. England; 2009;2(4):247–66.

5. Matarasso M, Iorio-Siciliano V, Blasi a., Ramaglia L, Salvi GE, Sculean a. Enamel matrix derivative and bone grafts for periodontal regeneration of intrabony defects. A systematic review and meta-analysis. Clin Oral Investig. 2015 Sep;19(7):1581-93.

6. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. J Clin Periodontol. 2018;89 Suppl 1:S1-8.

7. Hammarstrom L. Enamel matrix, cementum development and regeneration. J Clin Periodontol. 1997 Sep;24(9 Pt 2):658–68.

8. Cardaropoli G, Leonhardt AS. Enamel matrix proteins in the treatment of deep intrabony defects. J Periodontol. 2002;73:501–4.

9. Casarin RCV, Del Peloso Ribeiro É, Nociti FH, Sallum AW, Ambrosano GMB, Sallum EA, et al. Enamel matrix derivative proteins for the treatment of proximal class II furcation involvements: A prospective 24-month randomized clinical trial. J Clin Periodontol. 2010;37:1100–9.

10. Ribeiro FV, Casarin RCV, Júnior FHN, Sallum EA, Casati MZ. The role of enamel matrix derivative protein in minimally invasive surgery in treating intrabony defects in single-rooted teeth: a randomized clinical trial. J Periodontol. 2011;82(4):522–32.

11. Koop R, Merheb J, Quirynen M. Periodontal Regeneration With Enamel Matrix Derivative in Reconstructive Periodontal Therapy: A Systematic Review. J Periodontol. 2012;83:707–20.

12. Inglehart MR. Enhancing periodontal health through regenerative approaches: a commentary on the need for patient-reported outcomes. J Periodontol. 2015 Feb;86(2 Suppl):S4-7.

13. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. J Am Coll Dent. United States; 2014;81(3):14–8.

14. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement : Updated guidelines for reporting parallel group randomised trials. Int J Surg. 2011;9(8):672-7.

15. Cortellini P, Tonetti MS. Improved wound stability with a modified minimally invasive surgical technique in the regenerative treatment of isolated interdental intrabony defects. J Clin Periodontol. United States; 2009 Feb;36(2):157–63.

16. Sangiorgio JPM, Neves FL da S, Rocha Dos Santos M, Franca-Grohmann IL, Casarin RCV, Casati MZ, et al. Xenogenous Collagen Matrix and/or Enamel Matrix Derivative for Treatment of Localized Gingival Recessions: A Randomized Clinical Trial. Part I: Clinical Outcomes. J Periodontol. United States; 2017 Dec;88(12):1309–18.

17. Linares A, Cortellini P, Lang NP, Suvan J, Tonetti MS. Guided tissue regeneration/deproteinized bovine bone mineral or papilla preservation flaps alone for treatment of intrabony defects. II: radiographic predictors and outcomes. J Clin Periodontol. United States; 2006 May;33(5):351–8.

18. Ribeiro FV, Nociti Júnior FH, Sallum EA, Sallum AW, Casati MZ. Use of enamel matrix protein derivative with minimally invasive surgical approach in intra-bony periodontal defects: Clinical and patient-centered outcomes. Braz Dent J. 2010;21:60–7.

19. Tonetti MS, Fourmousis I, Suvan J, Cortellini P, Brägger U, Lang NP. Healing, post-operative morbidity and patient perception of outcomes following regenerative therapy of deep intrabony defects. J Clin Periodontol. 2004;31(March):1092–8.

20. Slade GD. Derivation and validation of a short-form oral health impact profile. Community Dent Oral Epidemiol. 1997;25:284–90.

21. Rakmanee T, Calciolari E, Olsen I, Darbar U, Griffiths GS, Petrie A, et al. Expression of growth mediators in the gingival crevicular fluid of patients with aggressive periodontitis undergoing periodontal surgery. Clinical Oral Investigations; 2018.

22. Górski B, Jalowski S, Górska R, Zaremba M. Treatment of intrabony defects with modified perforated membranes in aggressive periodontitis: a 12-month randomized controlled trial. Clin Oral Investig. 2018 Nov;22(8):2819-2828.

23. Gorski B, Jalowski S, Gorska R, Zaremba M. Treatment of intrabony defects with modified perforated membranes in aggressive periodontitis: subtraction radiography outcomes, prognostic variables, and patient morbidity. Clin Oral Investig. Germany; 2018 Oct.

24. Bajaj P, Agarwal E, Rao NS, Naik SB, Pradeep AR, Kalra N, et al. Autologous Platelet Rich Fibrin in the Treatment of 3-Wall Intrabony Defects in Aggressive Periodontitis - A Randomized Controlled Clinical Trial. J Periodontol. 2017;88(11):1–9.

25. Rakmanee T, Griffiths GS, Auplish G, Darbar U, Petrie A, Olsen I, et al. Radiographic outcomes following treatment of intrabony defect with guided tissue regeneration in aggressive periodontitis. Clin Oral Investig. 2016;20(6):1227–35

26. Rakmanee T, Griffiths GS, Auplish G, Darbar U. Treatment of intrabony defects with guided tissue regeneration in aggressive periodontitis : clinical outcomes at 6 and 12 months. Clin Oral Investig. Clinical Oral Investigations; 2016;1217–25.

27. Thorat M, Baghele ON, SRP. Adjunctive Effect of Autologous Platelet-Rich Fibrin in the Treatment of Intrabony Defects in Localized Aggressive Periodontitis Patients: A Randomized Controlled Split-Mouth Clinical Trial. Int J Periodontics Restorative Dent. United States; 2017;37(6):e302–9.

28. Gupta G. Clinical and radiographic evaluation of intra - bony defects in localized aggressive periodontitis patients with platelet rich plasma / hydroxyapatite graft : A comparative controlled clinical trial. 2014;5(4):445–51.

29. Queiroz AC, da Nóbrega PB, Oliveira FS, Novaes AB, Taba M, Palioto DB, et al. Treatment of intrabony defects with anorganic bone matrix/P-15 or guided tissue regeneration in patients with aggressive periodontitis. Braz Dent J. 2013;24:204–12.

30. Casarin RCV, Ribeiro EDP, Ribeiro FV, Nociti FHJ, Sallum AW, Sallum EA, et al. Influence of anatomic features on the effectiveness of enamel matrix derivative proteins in the treatment of proximal Class II furcation involvements. Quintessence Int. Germany; 2009 Oct;40(9):753–61.

31. Vandana KL, Shah K, Prakash S. Clinical and radiographic evaluation of Emdogain as a regenerative material in the treatment of interproximal vertical defects in chronic and aggressive periodontitis patients. Int J Periodontics Restorative Dent. United States; 2004 Apr;24(2):185–91.

32. Zucchelli G, Brini C, De Sanctis M. GTR treatment of intrabony defects in patients with early-onset and chronic adult periodontitis. Int J Periodontics Restorative Dent. United States; 2002 Aug;22(4):323–33.

33. Kao RT, Nares S, Reynolds MA. Periodontal Regeneration – Intrabony Defects: A Systematic Review From the AAP Regeneration Workshop. J Periodontol. 2015;86(1):S77–104.34. Hoffmann T, Al-machot E, Meyle J. Three-year results following regenerative periodontal surgery of advanced intrabony defects with enamel matrix derivative alone or combined with a synthetic bone graft. 2016;357–64.

35. Casarin RCV, Del Peloso Ribeiro É, Nociti FH, Sallum AW, Sallum EA, Ambrosano GMB, et al. A double-blind randomized clinical evaluation of enamel matrix derivative proteins for the treatment of proximal class-II furcation involvements. J Clin Periodontol. 2008;35(1991):429–37.

36. Rocha Dos Santos M, Sangiorgio JPM, Neves FL da S, Franca-Grohmann IL, Nociti FHJ, Silverio Ruiz KG, et al. Xenogenous Collagen Matrix and/or Enamel Matrix Derivative for Treatment of Localized Gingival Recessions: A Randomized Clinical Trial. Part II: Patient-Reported Outcomes. J Periodontol. United States; 2017 Dec;88(12):1319–28.

3 CONCLUSÃO

A aplicação de EMD provou ser uma terapia periodontal regenerativa viável para o tratamento de indivíduos com PAg, com uma taxa de regeneração similar dos pacientes com PC.

REFERÊNCIAS*

1. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol. 1999;4(1):1–6.

2. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS, et al. A new classification scheme for periodontal and peri - implant diseases and conditions – Introduction and key changes from the 1999 classification. 2018;45(March):1–8.

3. Albandar JM. Aggressive periodontitis: Case definition and diagnostic criteria. Periodontol 2000. 2014;65:13–26.

4. Adams D a., Barrington EP, Caton J, Genco RJ, Goodman SF, Hildebrand CN, et al. Parameters of Care Supplement. J Periodontol. 2000;71(5).

5. Kulkarni C, Kinane DF. Host response in aggressive periodontitis. Periodontol 2000. 2014;65:79–91

6. Susin C, Albandar JM. Aggressive Periodontitis in an Urban Population in Southern Brazil. J Periodontol. 2005;76(3):468–75.

7. Susin C, Haas AN, Albandar JM. Epidemiology and demographics of aggressive periodontitis. Periodontol 2000. 2014;65:27–45.

8. Teughels W, Dhondt R, Dekeyser C, Quirynen M. Treatment of aggressive periodontitis. Periodontol 2000. 2014;65:107–33.

9. Priyadarshini D, Nadig P, Deshpande N, Deshpande A. Role of psychotherapy in managing a case of generalised aggressive periodontitis. BMJ Case Rep. England; 2014;2014.

*De acordo com as normas da UNICAMP/FOP, baseadas na padronização do International Committee of Medical Journal Editors - Vancouver Group. Abreviatura dos periódicos em conformidade com o PubMed.

10. Aimetti M, Romano F, Guzzi N, Carnevale G. Full-mouth disinfection and systemic antimicrobial therapy in generalized aggressive periodontitis: A randomized, placebo-controlled trial. J Clin Periodontol. 2012;39:284–94.

11. Casarin RCV, Peloso Ribeiro É Del, Sallum EA, Nociti FH, Gonçalves RB, Casati MZ. The Combination of Amoxicillin and Metronidazole Improves Clinical and Microbiologic Results of One-Stage, Full-Mouth, Ultrasonic Debridement in Aggressive Periodontitis Treatment. J Periodontol. 2012;83(8):988–98.

12. Guerrero A, Griffiths GS, Nibali L, Suvan J, Moles DR, Laurell L, et al. Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: A randomized placebo-controlled clinical trial. J Clin Periodontol. 2005;32(June):1096–107.

13. Haas AN, De Castro GD, Moreno T, Susin C, Albandar JM, Oppermann R V., et al. Azithromycin as an adjunctive treatment of aggressive periodontitis: 12-Months randomized clinical trial. J Clin Periodontol. 2008;35:696–704.

14. Heller D, Varela VM, E Silva-Senem MX, Torres MCB, Feres-Filho EJ, Colombo APV. Impact of systemic antimicrobials combined with anti-infective mechanical debridement on the microbiota of generalized aggressive periodontitis: A 6-month RCT. J Clin Periodontol. 2011;38:355–64.

15. Mestnik MJ, Feres M, Figueiredo LC, Duarte PM, Lira EAG, Faveri M. Short-term benefits of the adjunctive use of metronidazole plus amoxicillin in the microbial profile and in the clinical parameters of subjects with generalized aggressive periodontitis. J Clin Periodontol. 2010;37:353–65.

16. Mestnik MJ, Feres M, Figueiredo LC, Soares G, Teles RP, Fermiano D, et al. The effects of adjunctive metronidazole plus amoxicillin in the treatment of generalized aggressive periodontitis: A 1-year double-blinded, placebo-controlled, randomized clinical trial. J Clin Periodontol. 2012;39:955–61.

17. Purucker P, Mertes H, Goodson JM, Bernimoulin J. Local Versus Systemic Adjunctive Generalized Aggressive Periodontitis. J Periodontol. 2001 Sep;72(9):1241-5.

18. Yek EC, Cintan S, Topcuoglu N, Kulekci G, Issever H, Kantarci A. Efficacy of amoxicillin and metronidazole combination for the management of generalized aggressive periodontitis. J Periodontol. 2010;81(7):964–74.

19. Slots J. Systemic Antibiotics in Periodontics. J Periodontol. 2004;75(November):1553–65.

20. Califano JV. Position paper: periodontal diseases of children and adolescents. J Periodontol. 2003;74(11):1696–704.

21. Sgolastra F, Petrucci A, Gatto R, Monaco A. Effectiveness of Systemic Amoxicillin/Metronidazole as an Adjunctive Therapy to Full-Mouth Scaling and Root Planing in the Treatment of Aggressive Periodontitis: A Systematic Review and Meta-Analysis. J Periodontol. 2012;83(June):731–43.

22. Keestra JAJ, Grosjean I, Coucke W, Quirynen M, Teughels W. Non-surgical periodontal therapy with systemic antibiotics in patients with untreated aggressive periodontitis: a systematic review and meta-analysis. J Periodontal Res. 2014 Dec.

23. Merchant SN, Vovk A, Kalash D, Hovencamp N, Aukhil I, Harrison P, et al. Localized Aggressive Periodontitis Treatment Response in Primary and Permanent Dentitions. J Periodontol. 2014;85(12):1722–9.

24. Rabelo CC, Feres M, Goncalves C, Figueiredo LC, Faveri M, Tu Y-K, et al. Systemic antibiotics in the treatment of aggressive periodontitis. A systematic review and a Bayesian Network meta-analysis. J Clin Periodontol. 2015 Jul;42(7):647-57.

25. Kao RT, Nares S, Reynolds M a. Periodontal Regeneration – Intrabony Defects: A Systematic Review From the AAP Regeneration Workshop. J Periodontol. 2015;86(1):S77–104.

26. Cortellini P, Labriola A, Tonetti MS. Regenerative periodontal therapy in intrabony defects: state of the art. Minerva Stomatol. Italy; 2007 Oct;56(10):519–39.

27. Corbella S, Weinstein R, Francetti L, Taschieri S, Del Fabbro M. Periodontal regeneration in aggressive periodontitis patients: A systematic review of the literature. J Investig Clin Dent. 2017;8(4):e12245.

28. De Marco TJ, Scaletta LJ. The use of autogenous hip marrow in the treatment of juvenile periodontosis. A case report. J Periodontol. 1970;41:683–4.

29. Evian CI, Amsterdam M, Rosenberg ES. Juvenile periodontitis - healing following therapy to control inflammatory and traumatic etiologic components of the disease. J Clin Periodontol. 1982 Jan;9(1):1–21.

30. Sugarman MM, Sugarman EF. Precocious periodontitis: a clinical entity and a treatment responsibility. J Periodontol. 1977;48:397–409.

31. Queiroz AC, da Nóbrega PB, Oliveira FS, Novaes AB, Taba M, Palioto DB, et al. Treatment of intrabony defects with anorganic bone matrix/P-15 or guided tissue regeneration in patients with aggressive periodontitis. Braz Dent J. 2013;24:204–12.

32. Evans GH, Yukna RA, Sepe WW, Mabry TW, Mayer ET. Effect of various graft materials with tetracycline in localized juvenile periodontitis. J Periodontol. 1989;60:491–7.

33. Mabry TW, Yukna RA, Sepe WW. Freeze-dried bone allografts combined with tetracycline in the treatment of juvenile periodontitis. J Periodontol. UNITED STATES; 1985 Feb;56(2):74–81.

34. Yukna RA, Sepe WW. Clinical evaluation of localized periodontosis defects treated with freeze-dried bone allografts combined with local and systemic tetracyclines. Int J Periodontics Restorative Dent. UNITED STATES; 1982;2(5):8–21.

35. Kumar PG, Kumar JA, Anumala N, Reddy KP, Avula H, Hussain SN. Volumetric analysis of intrabony defects in aggressive periodontitis patients following use of a novel composite alloplast: a pilot study. Quintessence Int. 2011;42(5):375–84.

36. Mengel R, Schreiber D, Flores-de-Jacoby L. Bioabsorbable membrane and bioactive glass in the treatment of intrabony defects in patients with generalized aggressive periodontitis: results of a 5-year clinical and radiological study. J Periodontol. 2006;77(October):1781–7.

37. DiBattista P, Bissada NF, Ricchetti P a. Comparative effectiveness of various regenerative modalities for the treatment of localized juvenile periodontitis. J Periodontol. 1995;66:673–8.

38. Mengel R, Soffner M, Flores-de-Jacoby L. Bioabsorbable membrane and bioactive glass in the treatment of intrabony defects in patients with generalized aggressive periodontitis: results of a 12-month clinical and radiological study. J Periodontol. United States; 2003 Jun;74(6):899–908.

39. Sirirat M, Kasetsuwan J, Jeffcoat MK. Comparison between 2 surgical techniques for the treatment of early-onset periodontitis. J Periodontol. 1996;67:603–7.

40. Zucchelli G, Brini C, De Sanctis M. GTR treatment of intrabony defects in patients with early-onset and chronic adult periodontitis. Int J Periodontics Restorative Dent. United States; 2002 Aug;22(4):323–33.

41. Rakmanee T, Griffiths GS, Auplish G, Darbar U, Petrie A, Olsen I, et al. Radiographic outcomes following treatment of intrabony defect with guided tissue regeneration in aggressive periodontitis. Clin Oral Investig. 2016;20(6):1227–35.

42. Górski B, Jalowski S, Górska R, Zaremba M. Treatment of intrabony defects with modified perforated membranes in aggressive periodontitis: a 12-month randomized controlled trial. Clin Oral Investig. 2018;1–10.

43. Okuda K, Kawase T, Mormose M, Murata M, Saito Y, Suzuki H, et al. Platelet-Rich Plasma Contains High Levels of Platelet-Derived Growth Factor and Transforming Growth Factor-

β and Modulates the Proliferation of Periodontally Related Cells In Vitro. J Periodontol. 2003;74(June):849–57.

44. Ripamonti U, Petit J-C. Bone morphogenetic proteins, cementogenesis, myoblastic stem cells and the induction of periodontal tissue regeneration. Cytokine Growth Factor Rev. 2009;20:489–99.

45. Teare JA., Petit JC, Ripamonti U. Synergistic induction of periodontal tissue regeneration by binary application of human osteogenic protein-1 and human transforming growth factor-β 3 in Class II furcation defects of Papio ursinus. J Periodontal Res. 2012;47:336–44.

46. Gupta G. Clinical and radiographic evaluation of intra - bony defects in localized aggressive periodontitis patients with platelet rich plasma / hydroxyapatite graft : A comparative controlled clinical trial. 2014;5(4):445–51.

47. Mauro S, Orlando L, Panzoni R, Orlando PF. Platelet gel biotechnology applied to regenerative surgery of intrabony defects in patients with refractory generalized aggressive peridontitis. Case report. Minerva Stomatol. Italy; 2003;52(7-8):401–12.

48. Bajaj P, Agarwal E, Rao NS, Naik SB, Pradeep a. R, Kalra N, et al. Autologous Platelet Rich Fibrin in the Treatment of 3-Wall Intrabony Defects in Aggressive Periodontitis - A Randomized Controlled Clinical Trial. J Periodontol. 2017;88(11):1–9.

49. Deas DE, Mealey BL. Response of chronic and aggressive periodontitis to treatment. Periodontol 2000. 2010;53:154–66.

50. Roshna T, Nandakumar K. Generalized Aggressive Periodontitis and Its Treatment Options: Case Reports and Review of the Literature. Case Rep Med. 2012;2012:1–17.

51. Losada M, Gonzalez R, Garcia AP, Santos A, Nart J. Treatment of Non-Contained Infrabony Defects With Enamel Matrix Derivative Alone or in Combination With Biphasic Calcium Phosphate Bone Graft: A 12-Month Randomized Controlled Clinical Trial. J Periodontol. United States; 2017 May;88(5):426–35.

52. Hammarstrom L. Enamel matrix, cementum development and regeneration. J Clin Periodontol. 1997 Sep;24(9 Pt 2):658–68.

53. Corrêa MG, Gomes Campos ML, Marques MR, Casati MZ, Nociti FH, Sallum E a. Histometric Analysis of the Effect of Enamel Matrix Derivative on the Healing of Periodontal Defects in Diabetic Rats. J Periodontol. 2012;1(September):1–18.

54. Sallum EA, Pimentel SP, Saldanha JB, Nogueira-Filho GR, Casati MZ, Nociti FH, et al. Enamel Matrix Derivative and Guided Tissue Regeneration in the Treatment of Dehiscence-Type Defects: A Histomorphometric Study in Dogs. J Periodontol. 2004;75(October):1357–63.

55. Ribeiro FV, Casarin RCV, Júnior FHN, Sallum EA, Casati MZ. The role of enamel matrix derivative protein in minimally invasive surgery in treating intrabony defects in single-rooted teeth: a randomized clinical trial. J Periodontol. 2011;82(4):522–32.

56. Casarin RCV, Del Peloso Ribeiro É, Nociti FH, Sallum AW, Ambrosano GMB, Sallum EA, et al. Enamel matrix derivative proteins for the treatment of proximal class II furcation involvements: A prospective 24-month randomized clinical trial. J Clin Periodontol. 2010;37:1100–9.

57. Koop R, Merheb J, Quirynen M. Periodontal Regeneration With Enamel Matrix Derivative in Reconstructive Periodontal Therapy: A Systematic Review. J Periodontol. 2012;83(June):707–20.

58. Cardaropoli G, Leonhardt AS. Enamel matrix proteins in the treatment of deep intrabony defects. J Periodontol. 2002;73:501–4.

59. Matarasso M, Iorio-Siciliano V, Blasi A, Ramaglia L, Salvi GE, Sculean a. Enamel matrix derivative and bone grafts for periodontal regeneration of intrabony defects. A systematic review and meta-analysis. Clin Oral Investig. 2015 Sep;19(7):1581-93.

60. Rasperini G, Silvestri M, Schenk RK, Nevins ML. Clinical and histologic evaluation of human gingival recession treated with a subepithelial connective tissue graft and enamel

matrix derivative (Emdogain): a case report. Int J Periodontics Restorative Dent. United States; 2000 Jun;20(3):269–75.

61. Pimentel SP, Sallum AW, Saldanha JB, Casati MZ, Nociti FH, Sallum E a. Enamel matrix derivative versus guided tissue regeneration in the presence of nicotine: A histomorphometric study in dogs. J Clin Periodontol. 2006;33:900–7.

62. Corrêa MG, Gomes Campos ML, Marques MR, Bovi Ambrosano GM, Casati MZ, Nociti FH, et al. Outcome of Enamel Matrix Derivative Treatment in the Presence of Chronic Stress: Histometric Study in Rats. J Periodontol. 2014;85(July):e259–67.

63. Corrêa MG, Campos MLG, Benatti BB, Marques MR, Casati MZ, Nociti FH, et al. The impact of cigarette smoke inhalation on the outcome of enamel matrix derivative treatment in rats: histometric analysis. J Periodontol. 2010;81(12):1820–8.

64. Bonta H, Llambes F, Moretti AJ, Mathur H, Bouwsma OJ. The use of enamel matrix protein in the treatment of localized aggressive periodontitis: a case report. Quintessence Int. England; 2003 Apr;34(4):247–52.

65. Miliauskaite A, Selimovic D, Hannig M. Successful Management of Aggressive Periodontitis By Regenerative Therapy: A 3-Year Follow-Up Case Report. J Periodontol. 2007;78(October):2043–50.

66. Kaner D, Bernimoulin J-P, Kleber B-M, Friedmann A. Minimally invasive flap surgery and enamel matrix derivative in the treatment of localized aggressive periodontitis: case report. Int J Periodontics Restorative Dent. United States; 2009 Feb;29(1):89–97.

67. Siqueira SJ, Ribeiro FV, Villalpando KT, Cirano FR, Pimentel SP. Maintenance periodontal therapy after systemic antibiotic and regenerative therapy of generalized aggressive periodontitis. A case report with 10-year follow-up. Dent Update. England; 2015 May;42(4):385–6,389–90,392–3.

68. Vandana KL, Shah K, Prakash S. Clinical and radiographic evaluation of Emdogain as a regenerative material in the treatment of interproximal vertical defects in chronic and

aggressive periodontitis patients. Int J Periodontics Restorative Dent. United States; 2004 Apr;24(2):185–91.

69. Artzi Z, Tal H, Platner O, Wasersprung N, Weinberg E, Slutzkey S, et al. Deproteinized bovine bone in association with guided tissue regeneration or enamel matrix derivatives procedures in aggressive periodontitis patients: a 1-year retrospective study. J Clin Periodontol. 2015;42:547–56.

ANEXO 1: PARECER CONSUBSTANCIADO CEP-FOP

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Proteínas derivadas da matriz do esmalte para o tratamento de defeitos infra-ósseos em pacientes com periodontite agressiva e crônica: estudo clínico, controlado e randomizado**", CAAE **48351115.9.0000.5418**, dos pesquisadores **Ana Lívia Fileto Santana, Antonio Wilson Sallum** e **Mauro Pedrine Santamaria**, satisfaz as exigências das resoluções específicas sobre ética em pesquisa com seres humanos do Conselho Nacional de Saúde – Ministério da Saúde e foi aprovado por este comitê em 19/10/2015 (protocolo original) e em 02/04/2017 (emenda ao protocolo).

The Research Ethics Committee of the Piracicaba Dental School of the University of Campinas (FOP-UNICAMP) certifies that research project "**Enamel matrix proteins in the treatment of intrabony defects in patients with aggressive and chronic periodontitis: Randomized clinical trial**", CAAE **48351115.9.0000.5418**, of the researcher's **Ana Lívia Fileto Santana, Antonio Wilson Sallum** and **Mauro Pedrine Santamaria**, meets the requirements of the specific resolutions on ethics in research with human beings of the National Health Council - Ministry of Health, and was approved by this committee on 19th of October of 2015 (original protocol) an on 2nd of April of 2017 (amended protocol).

Profa. Fernanda Miori Pascon
Vice Coordenador
CEP/FOP/UNICAMP

Prof. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo e a lista de autores aparecem como fornecidos pelos pesquisadores, sem qualquer edição.
Notice: The title and the list of researchers of the project appears as provided by the authors, without editing.

ANEXO 2: VERIFICAÇÃO DE ORIGINALIDADE E PREVENÇÃO DE PLÁGIO

PROTEÍNAS DERIVADAS DA MATRIZ DO ESMALTE PARA O TRATAMENTO DE DEFEITOS INFRA-ÓSSEOS EM PACIENTES COM PERIODONTITE AGRESSIVA E CRÔNICA

ORIGINALITY REPORT

13%	5%	12%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Bartłomiej Górski, Stanisław Jalowski, Renata Górska, Maciej Zaremba. "Treatment of intrabony defects with modified perforated membranes in aggressive periodontitis: a 12-	2%

ANEXO 3: COMPROVANTE DE SUBMISSÃO DO ARTIGO

CLOI: CLOI-D-19-00227 - Submission Confirmation for CLINICAL, RADIOGRAPHIC AND PATIENT-CENTERED OUTCOMES AFTER USE OF ENAMEL MATRIX DERIVATIVE PROTEINS FOR THE TREATMENT OF INTRABONY DEFECTS IN PATIENTS WITH AGGRESSIVE PERIODONTITIS: A 12-MONTHS MULTIC...

Clinical Oral Investigations <em@editorialmanager.com>
Dom, 17/02/2019 19:09
Para: Ana Lívia Fileto Mazzonetto <anafileto@hotmail.com>

Dear Mrs. Mazzonetto,

Your submission entitled "CLINICAL, RADIOGRAPHIC AND PATIENT-CENTERED OUTCOMES AFTER USE OF ENAMEL MATRIX DERIVATIVE PROTEINS FOR THE TREATMENT OF INTRABONY DEFECTS IN PATIENTS WITH AGGRESSIVE PERIODONTITIS: A 12-MONTHS MULTICENTER CLINICAL TRIAL" has been received by Clinical Oral Investigations

The submission id is: CLOI-D-19-00227
Please refer to this number in any future correspondence.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is https://www.editorialmanager.com/cloi/. Alternatively, please call us at 001-630-468-7784 (outside the US)/(630)-468-7784 (within the US) anytime from Monday to Friday.

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to our journal.

Kind regards,

Editorial Office
Clinical Oral Investigations

Now that your article will undergo the editorial and peer review process, it is the right time to think about publishing your article as open access. With open access your article will become freely available to anyone worldwide and you will easily comply with open access mandates. Springer's open access offering for this journal is called Open Choice (find more information on www.springer.com/openchoice). Once your article is accepted, you will be offered the option to publish through open access. So you might want to talk to your institution and funder now to see how payment could be organized; for an overview of available open access funding please go to www.springer.com/oafunding.
Although for now you don't have to do anything, we would like to let you know about your upcoming options.

Recipients of this email are registered users within the Editorial Manager database for this journal. We will keep your information on file to use in the process of submitting, evaluating and publishing a manuscript. For more information on how we use your personal details please see our privacy policy at https://www.springernature.com/production-privacy-policy. If you no longer wish to receive messages from this journal or you have questions regarding database management, please email our publication office, stating the journal name(s) and your email address(es): PublicationOfficeSPI@springernature.com

ANEXO 4: QUESTIONÁRIOS

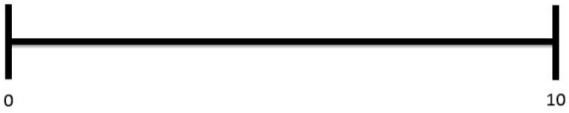
"Proteínas derivadas da matriz do esmalte para o tratamento de defeitos infra-ósseos em pacientes com periodontite agressiva e crônica"

Nº do paciente:

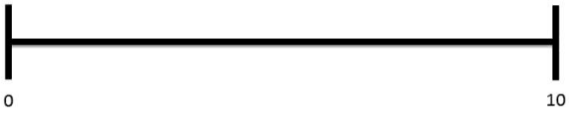
Avaliação do paciente quanto ao procedimento realizado e o período pós-operatório

15 dias pós-operatório >>> Aplicação:____/____/____

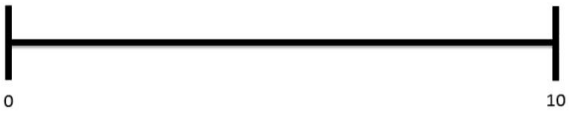
Em relação a **dor/desconforto**, se "0" significa *Nenhum* e "10" significa *Severo*, como você classifica este sintoma durante o procedimento?



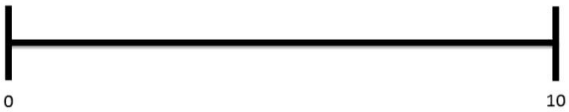
Em relação a **dor/desconforto**, se "0" significa *Nenhum*a e "10" significa *Severa*, como você classifica este sintoma no pós-operatório?



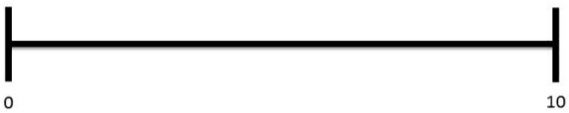
Em relação a **quantidade de analgésicos administrados**, se "0" significa *Nenhum* e "10" significa *Máximo prescrito*, como você quantifica esta administração no pós-operatório?



Em relação a **hipersensibilidade radicular**, se "0" significa *Nenhuma* e "10" significa *Severa*, como você classifica este sintoma no pós-operatório?



Em relação a **edema**, se "0" significa *Nenhum* e "10" significa *Severo*, como você classifica este sintoma no pós-operatório?



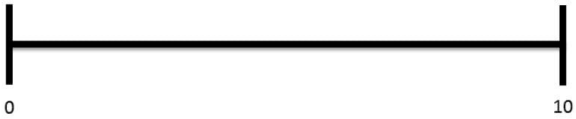
"Proteínas derivadas da matriz do esmalte para o tratamento de defeitos infra-ósseos em pacientes com periodontite agressiva e crônica"

Nº do paciente:

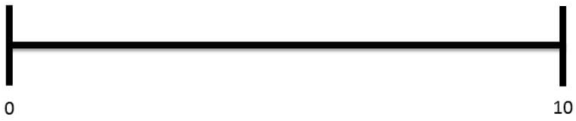
Avaliação do paciente quanto ao procedimento realizado e o período pós-operatório

15 dias pós-operatório >>> Continuação

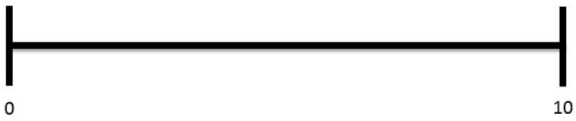
Em relação a **hematoma**, se "0" significa *Nenhum* e "10" significa *Severo*, como você classifica este sintoma no pós-operatório?



Em relação a **febre**, se "0" significa *Nenhuma* e "10" significa *Severa*, como você classifica este sintoma no pós-operatório?



Em relação a **interferência na execução de atividades diárias**, se "0" significa *Nenhuma* e "10" significa *Severa*, como você classifica este sintoma no pós-operatório?



"Proteínas derivadas da matriz do esmalte para o tratamento de defeitos infra-ósseos em pacientes com periodontite agressiva e crônica"

Nº do paciente:

Questionário de Qualidade de Vida OHIP 14 – Modificado

Pré-operatório >>> Aplicação:____/____/____

Formulário OHIP-14 modificado	Nunca	Raramente	Às vezes	Frequentemente	Sempre
1. Você teve problemas para falar alguma palavra por causa de problemas com seus dentes ou sua boca?					
2. Você sentiu que o sabor dos alimentos ficou pior por causa de problemas com seus dentes ou sua boca?					
3. Você sentiu dores em sua boca ou nos seus dentes?					
4. Você se sentiu incomodado ao comer algum tipo de alimento por causa de problemas com seus dentes ou sua boca?					
5. Você ficou preocupado por causa de problemas com seus dentes ou sua boca?					
6. Você sentiu-se estressado por causa de problemas com seus dentes ou sua boca?					
7. Sua alimentação ficou prejudicada por causa de problemas com seus dentes ou sua boca?					
8. Você teve que parar suas refeições por causa de problemas com seus dentes ou sua boca?					
9. Você encontrou dificuldades para relaxar por causa de problemas com seus dentes ou sua boca?					
10. Você sentiu-se envergonhado por causa de problemas com seus dentes ou sua boca?					
11. Você ficou irritado com outras pessoas por causa de problemas com seus dentes ou sua boca?					
12. Você teve dificuldades em realizar suas atividades diárias por causa de problemas com seus dentes ou sua boca?					
13. Você sentiu que a vida, em geral, ficou pior por causa de problemas com seus dentes ou sua boca?					
14. Você ficou totalmente incapaz de fazer suas atividades diárias por causa de problemas com seus dentes ou sua boca?					

"Proteínas derivadas da matriz do esmalte para o tratamento de defeitos infra-ósseos em pacientes com periodontite agressiva e crônica"

Nº do paciente:

Questionário de Qualidade de Vida OHIP 14 – Modificado

6 meses pós-operatório >>> Aplicação:____/____/____

Formulário OHIP-14 modificado	Nunca	Raramente	Às vezes	Frequentemente	Sempre
1. Você teve problemas para falar alguma palavra por causa de problemas com seus dentes ou sua boca?					
2. Você sentiu que o sabor dos alimentos ficou pior por causa de problemas com seus dentes ou sua boca?					
3. Você sentiu dores em sua boca ou nos seus dentes?					
4. Você se sentiu incomodado ao comer algum tipo de alimento por causa de problemas com seus dentes ou sua boca?					
5. Você ficou preocupado por causa de problemas com seus dentes ou sua boca?					
6. Você sentiu-se estressado por causa de problemas com seus dentes ou sua boca?					
7. Sua alimentação ficou prejudicada por causa de problemas com seus dentes ou sua boca?					
8. Você teve que parar suas refeições por causa de problemas com seus dentes ou sua boca?					
9. Você encontrou dificuldades para relaxar por causa de problemas com seus dentes ou sua boca?					
10. Você sentiu-se envergonhado por causa de problemas com seus dentes ou sua boca?					
11. Você ficou irritado com outras pessoas por causa de problemas com seus dentes ou sua boca?					
12. Você teve dificuldades em realizar suas atividades diárias por causa de problemas com seus dentes ou sua boca?					
13. Você sentiu que a vida, em geral, ficou pior por causa de problemas com seus dentes ou sua boca?					
14. Você ficou totalmente incapaz de fazer suas atividades diárias por causa de problemas com seus dentes ou sua boca?					