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ESCOLA DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
DOUTORADO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM PRÓTESE DENTÁRIA

FÁBIO SÁ CARNEIRO SCZEPANIK

AVALIAÇÃO DA PERDA ÓSSEA MARGINAL COM O USO DE PILAR PERSONALIZADO DEFINITIVO EM TITÂNIO APÓS IMPLANTE E FUNÇÃO IMEDIATOS NA ZONA ESTÉTICA: UM ESTUDO DE COORTE PROSPECTIVO DE ATÉ 8 ANOS DE ACOMPANHAMENTO.

Porto Alegre
2019

PÓS-GRADUAÇÃO - *STRICTO SENSU*

Pontifícia Universidade Católica
do Rio Grande do Sul

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Tese apresentada como requisito para a obtenção do grau de Doutor pelo programa de Pós-Graduação da Faculdade de Odontologia da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientador: Prof. Dr. Márcio Lima Grossi

Coorientadores: Prof. Dr. Howard Tenenbaum

Prof. Dr. Michael Glogauer

Prof. Dr. Michael Goldberg

Linha de pesquisa: Técnicas e Aparelhos em Odontologia

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Aprovada em: ____ de _____ de _____.

BANCA EXAMINADORA:

_____
Prof. Dr. Márcio Lima Grossi – PUCRS

_____
Prof. Dr. José Pedro Peixoto de Oliveira – PUCRS

_____
Prof. Dr. Tomás Geremia – PUCRS

_____
Prof. Dr. Eduardo Reston – ULBRA

_____
Prof. Dr. Ricardo Smidt – ULBRA

Porto Alegre
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Dedico esta tese à minha avó materna, Lorena Luzia Gomes de Sá Carneiro.

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RESUMO

Objetivos: O objetivo deste estudo de coorte prospectivo com acompanhamentomédio de 3 anos foi avaliar a taxa de sobrevivência de implantes, níveis de perda óssea marginal (MBL) e complicações clínicas usando uma técnica minimamente invasiva para instalação e função imediatosepilar personalizado definitivo (OAOT)em titânio na zona estética. **Métodos:** Cinquenta e dois indivíduos (38 mulheres, 14 homens) com idade média de 58 anos receberam uma prótese unitária definitiva em média sete meses após o procedimento cirúrgico seguido de reconstrução com enxerto de matriz óssea bovina inorgânica. Cada paciente recebeu uma coroa temporária com um *abutment* definitivo personalizado colocado no mesmo dia da cirurgia não sendo removido durante todos os procedimentos restauradores subsequentes. Os pacientes foram examinados clinicamente para identificação de complicações mecânicas e biológicas durante os acompanhamentos e radiografias periapicais foram utilizadas para as medidas de MBL aos 6 meses, 12 meses e em uma avaliação final de acompanhamento que variou de 12 a 100 meses (média = 36,34 meses). Os dados foram analisados com teste t de Student e análise de variância (ANOVA) com nível de significância de $\alpha = 0,05$. **Resultados:** Dois implantes e uma coroa definitiva foram perdidos, resultando em uma taxa de sobrevivência dos implantes de 96,2% e taxa de sobrevivência das próteses definitivas de 98%. A MBL média nas faces mesial e distal foi de 0,19 ± 0,35 mm e 0,25 ± 0,47 mm, respectivamente, após um *follow-up*de 3 anos. Não houve diferenças estatisticamente significantes entre os níveis de MBL de 6 e 12 meses nos aspectos mesial e distal em relação ao acompanhamento final ($p = 0,832$ e $p = 0,958$, respectivamente). Apenas 8% dos implantes apresentaram MBL> 1mm e 65,6% dos implantes sem perda óssea marginal apresentaram contato osso/pilar protético verificado radiograficamente. A complicação mais frequente foi falha na cimentaçãoda restauração temporária com 30 ocorrências. **Conclusões:** Este estudo descreve um procedimento cirúrgico previsível de estágio único que oferece estética imediata e redução da morbidade combinada a resultados de remodelação óssea altamente estáveis na zona anterior.

Palavras-chave: implantes ósseos, substitutos ósseos, regeneração óssea, prótese dentária, técnicas cirúrgicas, biomateriais.

ABSTRACT

Objectives: The purpose of this 3-year cohort prospective study was to assess the implant survival rate, marginal bone loss (MBL) and clinical complications using a minimally invasive technique for immediate implant placement and restoration and the *one abutment-one time* (OAOT) protocol in the aesthetic zone. **Material and methods:** Fifty-two subjects (38 females, 14 males) with a mean age of 58 years received a single-tooth implant definitive prosthesis between both maxillary canines seven months after immediate implant placement and restoration, followed by a reconstructive bone graft with DBBM particles. Each patient received a temporary crown with a customized definitive abutment placed on the same day of surgery that was not removed throughout the subsequent restorative procedures. Patients were clinically screened for mechanical and biological complications during the follow-ups and periapical radiographs were used forMBL measurements at 6 months, 12 months and at a final follow-up assessment that ranged from 12 to 100 months (mean = 36.34 months). Data were analyzed with Student's t test and analysis of variance (ANOVA) at the significance level of $\alpha = 0.05$. **Results:** Two implants and one definitive crown were lost for a cumulative implant survival rate of 96.2% and definitive crown survival rate of 98%. The mean MBL at the mesial and distal aspects were 0.19 ± 0.35 mm and 0.25 ± 0.47 mm, respectively, after a 3-year follow up. There were no statistically significant differences between the 6-month and 12-month MBL at both mesial and distal aspects compared to the final follow-up ($p = 0.832$ and $p = 0.958$, respectively). Only 8% of the implants showed MBL > 1mm and 65.6% of implants with no marginal bone loss presented bone/prosthetic abutment contact radiographically.The most frequent complication was temporary crown loosening with 30 occurrences. **Conclusions:** This study describes a predictable one-stage surgical procedure that offers immediate aesthetics and reduced morbidity combined to highly stable bone remodeling results in the anterior zone.

Key-Words: dental implants, bone substitutes, bone regeneration, prosthodontics,surgical techniques, biomaterials.

SUMÁRIO

1. INTRODUÇÃO

A atual busca pela estética em reabilitações orais modificou a forma com que abordamos os pacientes com indicação de extração e instalação de implantes osseointegrados na região ânterosuperior. A instalação imediata de implantes é uma técnica consolidada na literatura e tem mostrado previsibilidade similar aos casos de instalação em osso cicatrizado,[1-3]não havendo diferença estatisticamente significativa em termos de taxa de sobrevivência quando comparados os implantes imediatos *versus* tardios.[3-5]

Tarnow e colaboradores relataram que após restauração com implantes utilizando a técnica convencional, houve migração do tecido ósseo de 1,4 – 2,0mm a partir da união implante-pilar dentro do primeiro ano de função, utilizando implantes de hexágono externo.[6,7] Atieh e colaboradores relatam que a média de reabsorção nos anos seguintes está na casa dos 0,2mm, porém a literatura ainda não estabeleceu um consenso. Em uma revisão sistemática da literatura realizada com estudos em humanos, presença de grupo controle e com um total de 1.239 implantes mostrou perda óssea marginal significativamente menor em implantes com mudança de plataforma, além de tecido duro substancialmente mais estável.[8] Comparativamente, Hurzeler e colaboradores relataram uma diferença de – 0,12mm ± 0,40mm para os implantes com mudança de plataforma contra – 0,29mm ± 0,34mm ($p \leq 0.0001$).[7] A razão para essa redução na perda de quantidade óssea marginal pode estar relacionada ao posicionamento mais apical da junção implante-*abutment*, afastando o infiltrado inflamatório da crista alveolar.[7,9,10] Além disso, a diferença entre os diâmetros do pilar protético e da plataforma do implante reduz a concentração de stress ósseo na região cervical, reduzindo a sua migração no sentido apical. Adicionalmente, Canullo e colaboradores relatam que implantes restaurados com o conceito de mudança de plataforma apresentaram uma redução significativa nos níveis de perda óssea marginal com correlação negativa entre perda óssea e diferença de diâmetro entre implante e abutment.[11] Uma redução de 0,45mm no diâmetro do pilar parece ser necessária para reduzir a perda óssea marginal.[7]

A interface implante-pilar é a região mais suscetível à contaminação bacteriana e a que mais sofre com o impacto mastigatório. Portanto, além da diferença de diâmetro, outro fator importante que influencia na manutenção de tecido ósseo periimplantar é o tipo de conexão protética utilizada. Em revisão sistemática da literatura avaliando a performance de conexões do tipo *cone morse*,foram detectados baixos níveis demicro movimentos do abutment sob forças verticais e oblíquas. Inclusive, este tipo de conexão mostrou maior resistência à perda de torque e à fratura, além de menor stress sobre o parafuso quando comparado com conexões

não cônicas. A geometria da conexão cone morse distribui mais homogeneamente o stress do impacto oclusal para o implante, melhorando o selamento, diminuindo a contaminação bacteriana e, por consequência, a reabsorção óssea circundante.[12]

Ao trabalharmos na região ântero superior, a altura e a espessura da parede óssea vestibular, presença de papila interdental e o biótipo gengival são considerados fatores chave para atingirmos níveis de estética satisfatórios,[2,13] especialmente devido a parede óssea vestibular dos dentes anteriores localizados na maxila geralmente apresentar-se fina ou ausente como consequência deimportante reabsorção após extração.[14]A manutenção da tábua óssea vestibular está diretamente ligada ao posicionamento vestíbulo-palatino desses implantes, devendo os mesmos estarem idealmente posicionados de 1-2mm palatinamente aos dentes adjacentes[13] e de4-5mm abaixo da margem gengival vestibular.[2]Evans e colaboradores relatam que o posicionamento mais vestibularizado ou ao nível dos dentes vizinhos mostrou chances três vezes maiores de perda da parede vestibular e, consequentemente, perda da arquitetura gengival quando comparado a um ideal posicionamento tridimensional.[2]Adicionalmente, uma distância mínima de 1,5mm entre os dentes adjacentes deve ser respeitada no momento da instalação do implante afim de minimizar a perda da crista alveolar e assegurar a presença de papila interdental.[5,13,15]

O biótipo gengival é definido pela visibilidade (fino) ou não (espesso) da sonda periodontal milimetrada através do tecido gengival quando a sondagem periodontal é realizada.[16] Os indivíduos com biótipo gengival mais fino têm menores chances de formação de papila interdental,[13] maior migração dos tecidos moles no sentido apical (45,8% *versus* 33,3%)[2] e maior recessão gengival quando comparados com indivíduos com biótipo gengival espesso (85,7% versus 66,7%). Por outro lado, os pacientes com biótipo gengival espesso apresentam alterações da mucosa vestibular significativamente menores pós instalação imediata de implantes osseointegrados.[16]No entanto, independentemente do biótipo gengival, o mínimo deperda da parede óssea vestibular pós extração dentária já representa consequências importantes, tendo em vista que a reabsorção do *bundle bone* se dá como um processo fisiológico e esperado. A literatura relata um mínimo de 2mm de espessura da tábua óssea vestibular afim de evitar a sua reabsorção, caso contrário, algum procedimento de enxertia deve ser utilizado.[14,17] Apesar deste consenso existir, clinicamente esse cenário é irreal, já que estudos comprovam uma média de 1mm de espessura da tábua óssea vestibular de dentes anteriores.[18]Dessa maneira, a utilização de enxertos ósseos no momento da instalação de implantesimediatos pode ser importante para tirar vantagem da cicatrização de tecido mole e diminuir o risco de reabsorção óssea vestibular.[4,5]

O processo de cicatrização pós extração vem acompanhado de uma série de eventos biológicos que podem influenciar negativamente o resultado final de um tratamento com implantes na zona anterior.[1]Recessão gengival, perda de papila interdental e de crista óssea marginal estão intimamente ligados[13,14] e são os principais fatores a serem controlados. Dentro desse conceito, extrações realizadas sem o descolamento muco periostal apresentam menor perda óssea marginal quando comparadas à técnica convencional,[19] além de reduzir o tempo de tratamento, o sangramento transoperatório e as chances de futuro desenvolvimento de periimplantite.[20]Adicionalmente, o descolamento mucoperiostal corta o aporte sanguíneo, reduz a quantidade de mucosa queratinizada[21]e altera a arquitetura gengival pós extração, aumentando os sinais inflamatórios clínicos e histológicos.[19,20,22]Portanto, técnicas minimamente invasivas ganharam notoriedade na reabilitação com implantes tendo em vista a conservação dos tecidos periimplantares.

Nessa linha, a instalação de um provisório imediato proporciona o acondicionamento dos tecidos moles através da manutenção de uma arquitetura gengival natural[5,10] através dapreservação do contorno e o volume periimplantar[23]e com taxas de sobrevivência estatisticamente semelhantes em comparação com implantes restaurados de acordo com o protocolo de carga convencional (3-6 meses após cirúrgicos).[8]Adicionalmente, estudos comparativos mostraram que as taxas de sucesso de implantes unitários instalados em zona estética com função tardia (97%) *versus* provisório imediato (98%) são similares,[5,16] fortalecendo a ideia de que a instalação de um provisório imediato é uma técnica segura e previsível.

Grandi e colaboradores demonstraram que a instalação de pilares provisórios mostrou sinais inflamatórios mais exacerbados, maior migração apical do epitélio juncional e maior perda de crista óssea marginal.[24] Os autores mostram que a não remoção dos pilares definitivos em titânio instalados no ato cirúrgico resultaram em uma redução estatisticamente significativa dos níveis de perda óssea e, consequentemente, redução na migração dos tecidos moles no sentido apical. Essa técnica está descrita na literatura como *one abutment-one time*[10,24,25]Igualmente, a forma dos pilares protéticos auxiliano estabelecimento de uma relação natural e harmônica dos tecidos moles circundantes, devendo os mesmos respeitarem os princípios biomecânicos dos preparos protéticos com o término cervical acompanhando a anatomia e arquitetura gengival para facilitar a remoção de excessos de cimento e propiciar um perfil de emergência adequado.[26]

Apesarda literatura atual apresentar diversas alternativas para intervenções minimamente invasivas na reabilitação de implantes unitários na zona estética,[5,10,23] não há

relatos de técnicas que unam todos os aspectos mencionados anteriormente, comocirurgia sem retalho, reconstrução com substitutos ósseos, pilares definitivos,e implante e função imediatos na região anterior. Da mesma forma, o conceito*one abutment-one time*, apesar do crescente interesse nos últimos anos, é representado na literatura quase em sua totalidade através de pilares pré-fabricados, tanto na região posterior como na anterior.[10,24-26]

Portanto, o presente estudo analisa os possíveis efeitos da utilização de pilares personalizados definitivos na região estética para a reabilitação de implantes unitários com implante e função imediatos.Este trabalho objetiva analisar as seguintes variáveis relacionadas à técnica que a ser descrita: a) taxa de sobrevivência dos implantes; b) níveis de perda óssea marginal, c) idade, d) gênero, e) região da cirurgia, f) características dos implantes instalados, g) tempo médio de acompanhamento, h) tempo médio de entrega das restaurações definitivas, i) taxa de sobrevivência das restaurações definitivas, j) complicações técnicas e k) complicações biológicas.

2. ARTIGO 1

CUSTOMIZED DEFINITIVE ABUTMENT FOLLOWING IMMEDIATE IMPLANT PLACEMENT AND RESTORATION IN THE ESTHETIC ZONE: A COHORT PROSPECTIVE STUDY UP TO 8 YEARS.

Sczepanik, FSC[1]; Tenenbaum, HC[2]; Grossi, ML[3], Dinato, JC[4]; Dinato, TR[5].

[1]DDS, MS, PhD Student, Post-Graduate Program in Prosthodontics, Faculty of Dentistry, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil.
[2]DDS, Dip. Perio, PhD, FRCD (C). Mount Sinai Hospital, Department of Dentistry; Centre for Advanced Dental Research and Care and Faculty of Dentistry, University of Toronto, Toronto, ON, Canada.
[3]DDS, MS, PhD, Professor, Post-Graduate Program in Dentistry, Faculty of Dentistry, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil.
[4]DDS, MS, PhD, Professor of the Specialization Program in Implantology, Brazilian Dental Association of Rio Grande do Sul (ABORS), Brazil.
[5]DDS, MS, PhD Student, Post-Graduate Program in Prosthodontics, Faculty of Dentistry, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil.

Corresponding author: Fábio Sá Carneiro Sczepanik, PhD Student, Post-Graduate Program in Prosthodontics, Faculty of Dentistry, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil, Avenida Ipiranga, 6681. Tel.: +5551 91974496 Fax.: +5551 33332717 Email: fabiosacarneiro@gmail.com

Running Title: One abutment - one time in the esthetic zone.

Key-Words: bone implant interactions, bone substitutes, bone regeneration, prosthodontics, soft tissue-implant interactions, surgical techniques, biomaterials.

Disclosure: The authors report no conflict of interests related to this study.

ABSTRACT

Objectives: The purpose of this 3-year cohort prospective study was to assess the implant survival rate, marginal bone loss (MBL) and clinical complications using a minimally invasive technique for immediate implant placement and restorationand the*one abutment-one time* (OAOT) protocol in the aesthetic zone.**Material and methods:**Fifty-twosubjects (38 females, 14 males)with a mean age of 58 years received a single-tooth implant definitive prosthesisbetween both maxillary canines seven months after immediate implant placement and restoration, followed by a reconstructive bone graft with DBBM particles. Each patient received a temporary crown with a customized definitive abutment placed on the same day of surgery that was not removed throughout the subsequent restorative procedures. Patients were clinically screened for mechanical and biological complications during the follow-ups and periapical radiographs were used for MBL measurements at 6 months, 12 months and at a final follow-up assessment that ranged from 12 to 100 months (mean = 36.34 months). Data were analyzed with Student's t test and analysis of variance (ANOVA) at the significance level of $\alpha = 0.05$.**Results:**Two implants and one definitive crown were lost for a cumulative implant survival rate of 96.2% and definitive crown survival rate of 98%.The mean MBLat the mesial and distal aspects were 0.19 ± 0.35mm and 0.25 ± 0.47 mm, respectively, aftera3-year follow up. There were no statistically significant differences between the 6-month and 12-month MBL at both mesial and distal aspects compared to the final follow-up ($p = 0.832$ and $p = 0.958$, respectively). Only 8% of the implants showed MBL > 1mm and 65.6% of implants with no marginal bone loss presented bone/prosthetic abutment contact radiographically.The most frequent complication was temporary crown loosening with 30 occurrences. **Conclusions:** This study describes a predictable one-stage surgical procedure that offers immediate aesthetics and reduced morbidity combined to highly stable bone remodeling results in the anterior zone.

Keywords: bone implant interactions, bone substitutes, bone regeneration, prosthodontics, soft tissue-implant interactions, surgical techniques, biomaterials.

INTRODUCTION

As the success criteria for dental implants were proposed by Albrektsson and colleagues[1]in 1986 as an addition to the first report on the subject,[2]the definition of a satisfactory implant-supported rehabilitation has additionally experienced significant changes[3] and will presumably face further supplements over the years as novel techniques emerge. Accordingly, immediate implant placement and restoration have already been widely described in the current literatureas a well-controlled and efficient approach for the replacement of failing teeth.[4-7]Reports on reduced number of interventions,[8] less morbidity,[9]immediate esthetics,[10]minimumamount of marginal bone loss and high success ratescomparable to early and conventional loading protocols[7,8,11]applythe placement of dental implants into fresh extraction socketsand their immediate loading as putative candidates as gold standard procedures in the aesthetic zone.

Marginal bone loss (MBL) has long been the subject of interest in implant dentistry research.[4-6,9,11] An initial 1.5mm bone loss within a year after implant placement in addition to 0.2mm annually thereafter represented the threshold for clinically acceptable results as dental implants showing bone remodeling within this range were considered successful.[1] The literature has consistently refuted this parameter.[7,8,12-15]For instance, Galindo-Moreno suggested a 0.44mm marginal bone loss at 6 months post loading should be includedfor assessment of implant success rates since odds of presentingMBL > 2mmat 18 months in this group increased.[16] Additionally, Arora and colleagues[8]reported bone levels within a 0.5mm range of either bone loss or bone gain. A representative number of studies have confirmed these findings.[7,12-15]

Thus, the focus of implant dentistry has shifted to long term preservation of both soft and hard tissues as a trend towards minimally invasive procedures to diminish the undesirable effects following tooth extractionhas been pointed out.[17]As such, in addition to the advantageous effects of an adequate 3D implant positioning[18,19] and platform-switching implants[20-22] on periimplant tissues,the use of a flapless surgeryhas been consistently associated with the maintenance of soft tissue contours and less pronounced vertical bone loss.[23-25]Furthermore, filling the post-extraction buccal void with bone grafts has been reported as limiting gingival contour change and preserving bone volume when placed above the implant’s platform and in contact with a definitive abutment, as described in previous reports.[26,27]

On the same line, abutment nonremoval has shown to result in improved soft tissue stability and hard tissue maintenance.[28]As a consequence of frequent provisional abutment dis/reconnections as part of the prosthetic phases, the implant-mucosal barrier is constantly being disrupted, shifting the junctional epithelium more apically and introducing higher loads of pathogens into the implant-abutment interface, which leads to multiple micro damage of the connective tissue, soft tissue trauma and eventually bone remodeling.[22,29]Therefore, the *one abutment-one time* (OAOT) protocol is characterized by the use of a definitive abutment immediately after implant insertion in replacement for the conventional provisional abutments or cover screws that wouldrequire multiple removals in subsequent phases.[30]Even though the literature is still controversial with the actual influence abutment removals exert[31,32] or with the minimum exact number of removalscapable of significantlychangingperiimplant-supporting tissues,[30,33]several authors have reported on the beneficial effects of this minimally invasive technique on longitudinal stability of soft tissues dimensions and bone levels.[17,29,34-36] However, current evidence is still lacking studies evaluating OAOT protocol using customized definitive abutment inasmuch as clinical[33,35] and review studies[17,30]have generally tested standard pre-fabricated abutments.

As such, the purpose of the present study is to describea minimally invasive technique for immediate implant placement and restoration by the use of a customized definitive abutment in the aesthetic zone. For such purpose, the following outcomes were investigated: a) implant survival rate; b) marginal bone loss,c) definitive restoration survivalrate, and d) clinical complications related to the presented technique.

METHODS

A population of 52 subjects (38 females, 14 males) presenting a hopeless tooth within both maxillary canines was screened for immediate implant placement and restoration in a private clinic (Porto Alegre, Brazil) from 2009 to 2018 according to specific selection criteria (Table 1). The mean age was 58 years (range = 24 to 79 years) and the mean follow-up period was36.3months (range = 12 to 100 months). Table 2displays the sample characteristics. All surgical procedures were performed by one experienced surgeon (JCD) who did not participate in the data collection. The study was performed according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects and the was reviewed and approved by the Research Ethics Committee (CEP# 1.878.772) of the São Lucas Hospital at the Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil.

Treatment

Prior to surgery, cone beam computerized tomographic (CBCT) images and periapical radiographs were evaluated. When necessary, patients underwent professional non-surgical periodontal therapy. The entire sample received prophylactic antibiotic medication (2g of amoxicillin 1h before the procedure) and 0.12% chlorhexidine gluconate mouthwashes.

After local anesthesia (Articaine 100, DFL, Rio de Janeiro, Brazil),a flapless tooth extraction was performed using a specific extractor (Benex Root Extraction System, Hager and Meisinger GMbH, Neuss, Germany) in an attempt to minimize surgical trauma to the surrounding tissues. Thenthe socket was vigorously debrided using curettes and a morse tapered implant (CM Drive, Neodent, Curitiba, Brazil) was placed 2 to 3 mm subcrestally and 2 mm palatal to the buccal bone wall with a minimum insertion torque of 35 N/cm. An implant impression was taken immediately after surgery using a piece of sterile rubber dam to avoid the impression material from entering the surgical site. A narrow diameter healing abutment was positioned and the gap between the implant's shoulder and the buccal bone wall was filled withdeproteinized bovine bone matrix (DBBM) particles(0.25 – 1 mm) (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland).

In the dental laboratory,a customized definitive titanium abutment and an acrylic provisional crown were then manufactured. However, beforeinsertion, the customized abutment was scanned using a chairside digital scanner (Neo Shape D700, 3Shape, Copenhagen, Denmark).Therefore, the future zirconia infrastructure will be digitally designed in advance, preventing additional abutment removals. Thereafter, within 24 hours after surgery, the abutment was screwed to 15 N/cm and the temporary crown wascemented (Dycal, Dentsply, York, United States) with a non-functional loading. A gap was deliberately left between the gingival contour and the cervical aspect of the temporarycrownto avoid tissue compression. Abutments had a reduced diameter in comparison to the implant's platform diameter, a conical connectionand presented 2.5 – 3.0mm in height.

After surgery, patients were instructed to follow a soft diet and to avoid using the area for the remaining duration of the implant healing phase. Drug therapy consisted of antibiotics (i.e., Amoxicillin 875mg, every 12h for 7 days), anti-inflammatory drugs (i.e., Nimesulide 100mg, every 12h for 3 days) and mouthwashes (i.e., 0.12% chlorhexidine gluconate, twice daily for 7-10 days).

After the healing process, the already digitallydesigned infrastructure was approved and amilled zirconium oxide coping (InLab MC XL, Sirona, Salzburg, Austria) was manufactured. The structure was then seated on the customized abutment and checked for possible marginal gaps, adequate occlusal space and proper thickness. Additionally, the abutment's finish line was evaluated to assure its subgingival position and a pick-up impression was taken (Regular Body Normal Set, Elite HD+, Zhermack, Rovigo, Italy). The all-ceramic definitive restoration was luted with a resin-modified glass ionomer cement (Relyx Luting 2, 3M ESPE, California, United States).All materials were handled according to the manufacturer's instructions.The treatment sequence is shown in Figure 1.

Follow-up controls

After the surgical procedure, follow-up consisted of radiographic and clinical recordingsonce a month. The following technical complications were recorded: a) temporary crown loosening; b)temporary crown fracture; c) abutment screw loosening; d) additional preparation of the abutment, and e)abutment replacement. Additionally, biological complications were also recorded: a) abscess, b)fistula, and c) peri-implantitis. After the definitive crown was delivered, patients were enrolled in a maintenance program with 6-month follow-up appointments during the first year and 1-year follow-up visits in the subsequent years.

Radiographic evaluation, measurement technique and data collection

For radiographic measurements, digital periapical radiographs (Vista Scan, Dürr Dental, Bietigheim-Bissingen, Germany) were taken with a standardized film holder (Cone Indicator Químico, Indusbello, Londrina, Brazil) and the parallel technique at three different times: at final crown delivery (6 months post-surgery), at 12 months and at the latest follow-up visit. Patients with less than 12 months of follow-up were not included in the study.

Marginal bone loss (MBL) was recorded as the measured distance from the implant-abutment interfaceto the first bone-to-implant contact in both mesial and distal aspects in each time point (6 months, 12 months, final follow-up). When bone was observed at the level or above the implant's platform, MBL was recorded as zero in order to not positively influence the results. The implant's length (i.e., 10mm, 11.5 mm, 13 mm, and 16 mm)and diameter (i.e.,

3.5mm, 4.3mm) served as control for adjusting possible image magnifications. Additionally, control of brightness and contrast were adjusted using a specific imaging software (DBSWIN Imaging Software, Dürr Dental, Bietigheim-Bissingen, Germany). Radiographic measurements were performed in two different days with at least 48 hours apart. Measurements of the mesial and distal marginal bone levels were made to the nearest 0.1 mm.

One independent examiner performed all examinations and data collection. The following variables were recorded: a) age, b) gender, c) surgical site, d) implant features, e) time of follow-up, f) time of definitive restoration delivery, g) implant survival, g) definitive restoration survival, h) marginal bone loss, i) technical complications, and j) biological complications.

Statistical analysis

SPSS® version 17 was used for the statistical analysis (IBM, Chicago, IL, USA). The Kolmogorov-Smirnov normality and the Levene's homogeneity of variance tests were used. Considering that all results had a parametric distribution, the Student's paired and independent t tests, and repeated-measures ANOVA were used at a significance level of 95%.

RESULTS

A total of 52 consecutive implants were immediately placed into fresh extraction sockets without flap elevation and restored with customized definitive abutments and a temporary crown. An implant survival rate of 96.2% was recorded for a sample size of 52 subjects (38 females, 14 males) with a mean age of 58.04 ± 12.91 years and a mean follow-up period of 36.34 ± 23.18 months (ranged from 12 to 100 months). Two implants were lost (3.8% failure rate). One due to lack of primary stability (one day after implant placement) and one due to occlusal trauma (2 months after implant placement). Both implants presented narrow platform diameters (3.5 x 13mm and 3.5 x 16mm) and were replaced on the same day without loading.Definitive crowns were delivered at 7.08 ± 2.63 months (ranged from 4 to 17 months) after implant placement with a 98% survival rate. Table 2 displays additional description concerning reasons for tooth removal and implant features.

The mean radiographic interproximal marginal bone loss levels at 6months, 12months and at final follow-up are presented in Table 3. Marginal bone loss at the mesial aspect

recorded at the final follow-up did not show statistically significant difference in comparison with the 6-month and 12-month recordings (p = 0.832). They were recorded as follows: MBL_m = 0.154 ± 0.340mm, MBL_m = 0.190 ± 0.357mm, and MBL_m = 0.192 ± 0.359mm. Similarly, marginal bone loss at the distal aspect recorded at the final follow-up did not show statistically significant difference in comparison with the 6-month and 12-month recordings (p = 0.958). They were recorded as follows: MBL_d = 0.230 ± 0.3489mm, MBL_d = 0.252 ± 0.474mm, and MBL_d = 0.256 ± 0.475mm.

The implants were divided as no marginal bone loss (MBL = 0), marginal bone loss less than 1mm (MBL < 1), and marginal bone loss higher than 1mm (MBL > 1mm) according to their marginal bone levels in reference to the outer implant shoulder. The descriptionof different marginal bone levels is shown in Table 4. Four radiographs for each of five patients were used to illustrate implants presenting MBL > 1mm (Figure 2), MBL < 1 (Figure 3), MBL = 0 (Figure 4), bone gain (Figure 5) and bone/abutment contact (Figure 6).

Mechanical complications almost entirely consisted of temporary crown loosening, which occurred thirty times during treatment. Abutment loosening occurred three times (6%) and twotemporary crowns were replaced after fracture (4%). Patients 39 and 43 had their abutments removed for additional preparation and to subgingivally reposition the finish line (4%). The abutments were removed once. Soft tissue and bone levels in patients 6, 27, 35, and 44 were clinically stable at 6 months after implant placement but their abutments were replaced for thinner ones for proper definitive crown thickness (8%).Four patients (8%) showed peri-implantinflammatory reactions that were treated with 0.12% chlorhexidine gluconate irrigation and hygiene instructions. Mechanical and biological complications are displayed in the Table 5.

The comparison between MBL with age showed non-significant differences between subjects under sixty-years old and over sixty-years old in the three follow-ups and for both aspects. Similarly, the comparison between MBL with gender showed non-significant differences between males and females in the three follow-ups and for both aspects. The comparisons between MBL with age and gender are presented in Tables 6 and 7.

DISCUSSION

The presented study evaluates the clinical and radiographic results of a minimally invasive surgical procedure that combines immediate implant placement and immediate implant loading with customized definitive abutmentsin the aesthetic zone. A cumulative implant

survival rate of 96.2% was achieved for 52 implantsin a three-year follow-up. Definitive crowns were deliveredafter7 months of implant placement.

The most frequent mechanical complication was temporary crown loosening (30 occurrences). Immediately after surgery, the amount of temporary cement was purposely reduced to avoid excess of cement into the wound. That might be the reason why 46% of the sample presented this clinical complication, which is in accordance with the study by Hartlev, that reported the same temporary crown loosening frequency.[37] Abutment loosening occurred three times and two temporary crowns were replaced after fracture. Only two patients presented important gingival recession, so their abutments were re-customized, as previously described (see Results). In addition, other four abutments were replaced for reasons other than soft tissue recession or significant bone loss (also see Results).

Several studies have evaluated implant survival rates following different protocols of implant placement and loading.[4,6,9,11,12]Hartog and colleagues, in a systematic review and meta-analysis found a 95.5% survival rate for single-tooth implants inserted in the aesthetic zone irrespective of the time of placement.[4] On the same line, 3082 implants were evaluated in another systematic review gathering only prospective studies with a 98.4% survival rate for immediate implant placement in both immediate and early restoration protocols.[9]In contrast, Atieh and colleagues reported a survival rate for immediate implant placement ranging from 82.4% to 100%. In this systematic review, however, four out of five selected articles reported 100% survival rates and only one 82.4%.[5] Two additional studies with similar methodological designs strongly disagree with these findings. One found no statistically significant differences between immediate and conventional loading after 2, 3, and 5 years of follow-up[6] andthe other reported a small greater risk of implant failure for immediate againstdelayed loading (98.2% *versus*99.6%, respectively), after selecting 37 randomized clinical trials with a follow-up time ranging from 6 to 84 months.[11] These findings are in agreement with retrospective studies and longitudinal clinical trials reporting high survival rates for immediate restoration of implant placed into fresh extraction socketsafter different follow-ups.[7,8,13-15,37-39] They also agree with the 96.2% survival rate yielded by the technique described in our study.

Our results also report a mean marginal bone loss of 0.19 ± 0.36mm at the mesial aspect and 0.26 ± 0.47mm at the distal aspect at the final follow-up with no statistically significant differences between the 6-month, 12-month and final follow-upat both aspects ($p = 0.832$ and $p = 0.958$, respectively). This marginal bone change is according to the range reported as high success criteria by Arora and colleagues.[8]Additionally, it is important to

mention that the described implants presenting bone at the level or even coronally to the implant's platform were classified as presenting no bone loss (MBL = 0) in order to not positively influence the data. Even thoughour results share a small disagreement with similar papers,[37-39] they are in accordance with the available data from a greater number of the studies evaluating similar approaches.[7-9,11,13-15] They will be further addressed.

Two of the aforementioned studies reported MBL of 2.0mm[39] and 1.0mm[37] 3 and 7 years after immediate implant loading. The increase in bone remodeling might be due to the use of a platform-matched implant-abutment connection and the absence of bone graft procedures in both studies.[27]Conversely, Calvo-Guirado and colleagues also did not use bone grafts in their study but the simple fact that they used an internal connection with non-matched abutments might have been the reason they reported lower levels of MBL (0.86 ± 0.29mm) after a 3-year follow-up.[38]The internal displacement of the implant-abutment interface away from the implant's shoulder decreases the inflammatory effects ofthe surroundingstructures,[40]re-establishes the biologic width,[30] creates a more stable environment[41] and reduces crestal bone loss,[42] meaning reduced biomechanical and biological complications following implant placement.[43]The use of a platform-switched connection explains the minimal bone loss found in our study. It might also explaina non-significant ($p = 0.832$ and $p = 0.958$) change in bone levels between the 6-month ($MBL_m = 0.15 \pm 0.34$mm and $MBL_d = 0.23 \pm 0.49$mm), 12-month ($MBL_m = 0.19 \pm 0.36$mm and $MBL_d = 0.25 \pm 0.47$mm), and the final follow-up ($MBL_m = 0.19 \pm 0.36$ mm and $MBL_d = 0.26 \pm 0.47$mm).

Our findings agree with the results from an animal experimental study showing the use of DBBM particles modified the hard tissue remodeling after immediate implant placement and improved bone-to-implant contact.[26]Similarly,reconstructive bone graft to fill the void between the buccal bone wall and the implant resulted in reduced marginal bone loss in two other similar studies.[13,14] After immediate placement and restoration of 24 consecutive implants, Cristalli found MBL levels of 0.38 ± 0.75mm mesially and 0.27 ± 0.59mm distally with an open flap procedure and a DBBMgraft.[13]On the same line, although using a different xenograft (Endobon® xenograft granules), Nimwegen reported reduced mean levels of MBL (0.31 ± 0.20mm) in 51 implants.[14]Their radiographic findings are in agreement with the present study; however, both studies included first premolars in their esthetic zone assessment. Our conception of esthetic zone differs as our described sample selection included exclusively teeth within both maxillary canines.

In addition to optimal MBL levels described in the current study,32/50 implants showed no interproximal marginal bone loss (64%), 14/50 implants showed marginal bone

loss less than 1mm (28%) and only 4/50 implants showed marginal bone loss higher than 1mm (8%) after a mean follow-up period of 3 years post-loading. Accordingly, Cristalli reported 8.7% of his sample with MBL > 1mm.[13]Also evaluating immediate implant placement and restoration in the esthetic zone, Cosyn and colleagues described 7/17 patients (41%) with either no marginal bone loss or bone gain using DBBM.[7] Conversely, a retrospective analysis found 16 out of 30 (53%) implants presenting bone at the level or coronally to the implant's platform, which is slightly lower than our 64% results, anda mean bone gain of 0.26mm using DBBM.[8] Both studies presented a higher mean follow-up than the present study (5 years); however, part of their sample was lost during the course of theirevaluation.

Finally, Noelken and colleagues described bone gain of 0.04mm, ranging from 1.37mm of bone loss to 1.19mm of bone gain, in a sample of 33 implants placed into fresh extraction sockets. They used CBCT scans and reported a 1mm marginal bone loss in 27% of their sample after 5 years of follow-up.[15]Our results account for only 8% of the sample presenting MBL > 1 mm. Furthermore, thirty two out of fifty implants placed showed no interproximal marginal bone loss. Assessing these 32 implants separately, 5/32 (15.6%) showed bone at the platform level and 27/32 (84.4%) exhibited bone level coronally to the implant shoulder and even in contact with the prosthetic abutment(21/32 patients (65.6%). The presence of preexistent or newly formed bone growing beyond the implant's platform and in an intimate contact with the abutment surface is in agreement with the literature;[26,27,40,44] however, to the best of the author's knowledge, this is the first clinical report showing such bone level stability with a representative sample size fora mean follow-up of 3 years.

The subcrestal positioningof implants with conical abutments significantlydecreases MBL levels when compared to external hexagon implants placed equicrestally, as confirmed by experimental studies in dogs[24,44] and clinical reports.[23,45]In a histomorphometric analysis of retrieved implants removed for psychologic distress reasons, Degidi and colleagues found areas of new bone formation and 0.5 – 3mm of bone gain in implants placed subcrestally.[23]Accordingly, the present study used customized abutments with 2.5 – 3.0mm in height, reflecting an adequate subcrestal implant positioning that is in accordance with the findings from Galindo-Moreno showing lower MBL rates when prosthetic abutments higher than 2mm are used.[16]Thatmight also justify the bone preservation observe in the current work over time.

The present study used definitive abutments immediately after implant placement. The concept, defined as *one abutment-one time* (OAOT) protocol, has been increasingly reported

over the years and consists of the non-removal of the prosthetic abutment throughout the entire rehabilitation phases.[17,29,35,36]The number of abutment removals capable of causing negative effects on bone is yet to be defined; however, an OAOT systematic review and meta-analysis, apart from finding positive effects of the assessed technique, also found no differences in the subgroup analysis comparing less than two abutment disconnections and more than three.[30] Their findings are in accordance with another recent systematic review and meta-analysis that also highlighted the benefit of such protocol when platform-switched implants are placed below the bone crest.[17]Moreover, Grandi reported statistically significant differences in MBL when definitive abutments (DA) were compared to provisional abutments (PA) (0.094mm *versus* 0.435mm) in 28 implants.[33]Another RCT from the same research group also found a 0.5mm bone loss difference in favor of the abutments inserted on the day of surgery.[34] They performed a conventional impression using the double-chord packing technique to copy the customized definitive abutments placed in premolar sites. In contrast, our technique consisted only of a pick-up impression of the Zr framework that had already been manufactured, which is a less time-consuming option and prevents additional disruption of the epithelium seal. Their results are in accordance with another prospective RCT showing a statistically significant bone resorption between DA and PA (MBL = 0.61 ± 0.40 mm*versus* MBL = 1.24 ± 0.79 mm) during healing period.[35]

Finally, Canullo and colleagues, in two recently published studies on OAOT prosthetic approach, found stable bone resorption results over a five-year period,as well as improved soft tissue dimensions using digital scanning analysis.[29,36]However, in both studies patients had to undergo a three-step surgical procedure in three different times (i.e. tooth removal and ridge preservation;flapped delayed implant placement; and a small incision for abutment insertion) and were provisionalized using their adjacent teeth as retainers for a temporary adhesive prosthesis. A 0.31 ± 0.29mm of marginal bone loss after a higher five-year follow-up was reported in their study, which is slightly greater than the bone remodeling reported in the current investigation after a mean follow-up of three years (mean MBL = 0.22 ± 0.44mm); however, our surgical/prosthetic procedures were significantly less invasive as a flapless immediate implant placement and restoration in addition to an OAOT approach were delivered for the entire sample.

CONCLUSIONS

This is the first clinical trial on the use of customized definitive abutments in conjunction with a flapless immediate implant placement and restoration in the aesthetic zone. Within the limitations of the present study, it can be concluded that the described technique is a predictable, effective alternative for the replacement ofafailing tooth in the esthetic zone with high implant survival rates and minimal tissue remodeling results over a three-year period. Further long-term clinical trials are neededto confirm the encouraging results found in our study.

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ANEXOS – ARTIGO 1

ANEXO 1 – Tabela 1 mostrando os critérios de exclusão e inclusão.

Table 1. Inclusion and exclusion criteria (adapted from Canullo et. al):

Subject inclusion criteria: • Age > 18 years • No relevant medical conditions • Non-smoking or smoking ≤ 10 cigarette/day • Full mouth plaque score and full mouth plaque bleeding score < 25%
Exclusion criteria: • Patients with history of IV Bisphosphonate therapy • Patients with uncontrolled diabetes (HbA1c > 6%, glycemic level > 110 mg/dl

ANEXO 2 – Tabela mostrando as características da amostra.

Table 2. Sample characteristics.

Independent variables	
Age (years) Mean (±SD)	**(n=52)** 58.04 (12.91)
Follow-up (months) Mean (±SD)	**(n=50)** 36.34 (23.18)
Definitive crown delivery Mean (±SD)	**(n=50)** 7.08 (2.63)
Gender (%) Women Men	**(n=52)** 73.1 26.9
Implant features (%) 3.5 x 13.0 3.5 x 16.0 4.3 x 10.0 4.3 x 13.0 4.3 x 16.0	**(n=52)** 17.3 13.5 1.9 40.4 25.0

Implant failure (%)	**(n=52)**
No implant loss	96.2
Implant loss	3.8
Reasons for tooth extraction (%)	**(n=52)**
Fracture	75.0
Resorption	15.4
Caries	3.8
Endodontic failure	3.8
Prosthetic failure	1.9
Definitive crown failure (%)	(n=50)
No failure	98.0
Failure	2.0
Implant site (%)	(n=50)
#11	31.0
#12	19.0
#13	6.0
#21	23.0
#22	15.0
#23	6.0

ANEXO 3 – Tabela mostrando os níveis de perda óssea marginal nas faces mesial e distal nos diferentes tempos de acompanhamento (6 meses, 12 meses e *follow-up* final).

Table 3. Comparison of marginal bone loss at 6 months, 12 months and at final follow-up both at mesial and distal aspects.

Independent variables		**N**	**Mean (mm)**	**Std. Deviation**	**P value**
MBL mm (mesial)	6 months	50	0.1540	0.34059	0.832 ^{NS}
	12 months	50	0.1900	0.35700	
	final	50	0.1920	0.35961	
MBL mm (distal)	6 months	50	0.2300	0.48959	0.958 ^{NS}
	12 months	50	0.2520	0.47434	
	final	50	0.2560	0.47559	

Analysis of Variance (ANOVA): ***** $p < 0.05$, NS (non-significant).
Post-hoc Tukey-b test: a = mesial, b = distal, and c = mesial distal (letters separated by comma are significantly different between them at $p<0.05$).

ANEXO 4 – Tabela mostrando a descrição dos níveis ósseos em relação à plataforma do implante.

Table 4. Description of marginal bone levels in comparison with the implant's platform.

Groups / Variables	Implants N = 50
Marginal bone level equals 1 mm (%):	
0 = negative	36.0
1 = positive	64.0
Marginal bone level less than 1 mm (%):	
0 = negative	72.0
1 = positive	28.0
Marginal bone level more than 1 mm (%):	
0 = negative	92.0
1 = positive	8.0
MBL = 0 mm	**Implants N = 32**
At implant's platform (%):	
0 = negative	84.4
1 = positive	15.6
Coronal do implant platform (%):	
0 = negative	15.6
1 = positive	84.4
Bone/abutment contact (%):	
0 = negative	34.4
1 = positive	65.6

ANEXO 5 – Tabela descrevendo as complicações mecânicas e biológicas.

Table 5. Description of mechanical and biological complications.

Groups / Variables	Implants N = 50
Temporary crown loosening (%):	
0 = negative	40.0
1 = positive	60.0
Temporary crown fracture (%):	
0 = negative	96.0
1 = positive	4.0
Abutment loosening (%):	
0 = negative	94.0
1 = positive	6.0
Abutment repreparation (%):	
0 = negative	96.0
1 = positive	4.0
Abutment replacement (%):	
0 = negative	92.0
1 = positive	8.0

Biological peri-implant complications (%):	
0 = negative	92.0
1 = positive	8.0

ANEXO 6 – Tabela mostrando a comparação da MBL com a variável idade.

Table 6. Comparison of marginal bone loss at 6 months, 12 months and at final follow-up measured mesially (M) and distally (D) with age.

Independent variables	Age	N	Mean (mm)	Std. Deviation	P value
MBL 6M (M)	less than 60 years	23	0.1696	0.33769	0.769 *NS*
	more than 60 years	27	0.1407	0.34891	
MBL 6M (D)	less than 60 years	23	0.2609	0.43037	0.685 *NS*
	more than 60 years	27	0.2037	0.54171	
MBL 12M (M)	less than 60 years	23	0.2217	0.35414	0.567 *NS*
	more than 60 years	27	0.1630	0.36390	
MBL 12M (D)	less than 60 years	23	0.3043	0.44054	0.477 *NS*
	more than 60 years	27	0.2074	0.50530	
MBL FINAL (M)	less than 60 years	23	0.2565	0.36906	0.373 *NS*
	more than 60 years	27	0.1630	0.36390	
MBL FINAL (D)	less than 60 years	23	0.3130	0.44242	0.439 *NS*
	more than 60 years	27	0.2074	0.50530	

Student's t test: * $p < 0.05$, NS (non-significant).

ANEXO 7 – Tabela mostrando a comparação da MBL com a variável gênero.

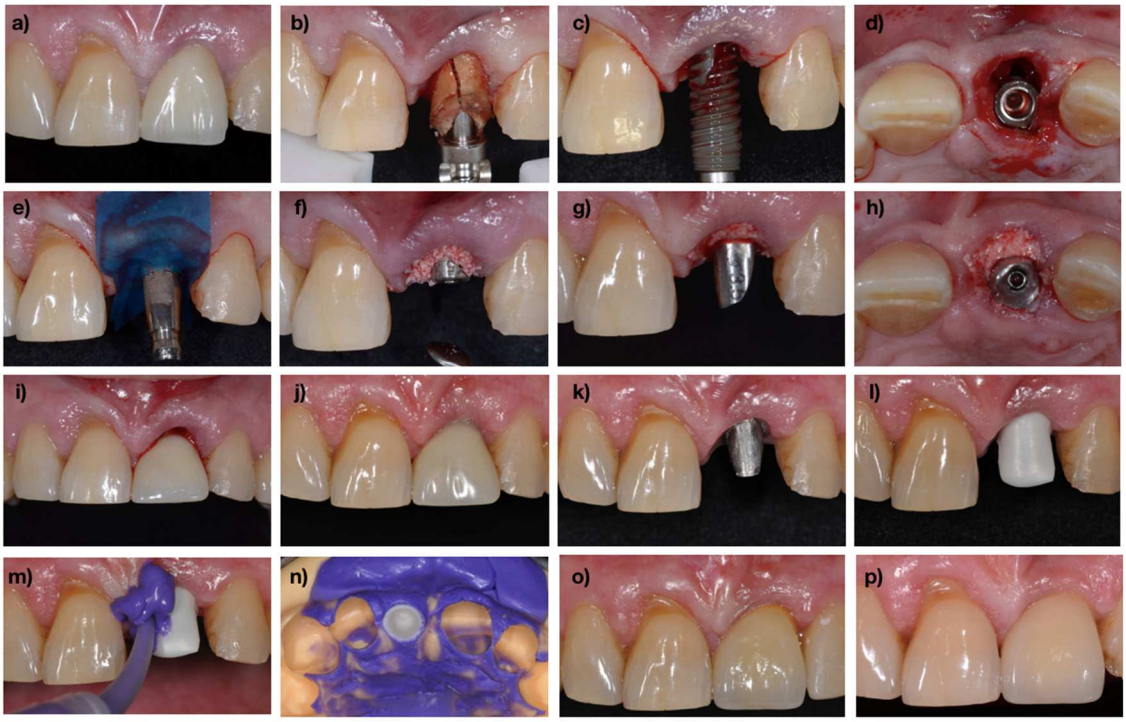
Table 7. Comparison of marginal bone loss at 6 months, 12 months and at final follow-up measured mesially (M) and distally (D) with gender.

Independent variables	Gender	N	Mean (mm)	Std. Deviation	P value
MBL 6M (M)	Male	13	0.2077	0.40919	0.514 *NS*
	Female	37	0.1351	0.31729	
MBL 6M (D)	Male	13	0.3308	0.60192	0.394 *NS*
	Female	37	0.1946	0.44780	
MBL 12M (M)	Male	13	0.2462	0.41153	0.515 *NS*
	Female	37	0.1703	0.33982	
MBL 12M (D)	Male	13	0.4154	0.58715	0.151 *NS*
	Female	37	0.1946	0.42226	
MBL FINAL (M)	Male	13	0.3000	0.42230	0.286 *NS*
	Female	37	0.1730	0.34371	
MBL FINAL (D)	Male	13	0.4154	0.58715	0.162 *NS*
	Female	37	0.2000	0.42492	

Student's t test: ***** *$p < 0.05$, NS (non-significant).*

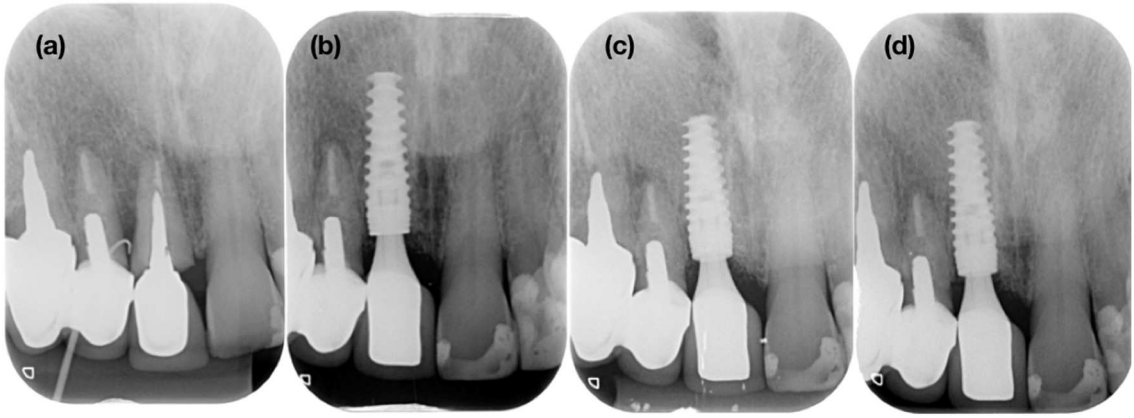
ANEXO 8 – Figura ilustrando a sequência clínica.

Figure 1. Illustration of the treatment sequence:a) Initial clinical aspect of the maxillary left central incisor with a longitudinal root fracture prior to removal. b) Flapless tooth extraction with a specific extractor (Benex Root Extraction System) for minimal disturbance of the surrounding tissues. c) Immediate implant placement (CM Drive, Neodent). d) Occlusal view of the gap between the buccal bone wall and implant demonstrating a 2 mm palatal position. e) Sterile rubber dam protecting the socket during the impression. f) Narrow diameter healing abutment for DBBM particles filling the void after implant placement. g-h) Buccal and occlusal aspects of the customized definitive abutment in place. i) Temporary crown delivered immediately after surgery. Note the distance from the cervical area and the gingival contour avoiding soft tissue compression. j) Clinical aspect of the temporary crown 7 days after immediate implant placement and restoration. k) Clinical aspect of the customized abutment 7 months after the surgical procedure. l) Trying the Zr framework for adaptation and gingival contour. m) Pick-up impression. o) Clinical aspect of the definitive crown at 3-year follow-up. p) Clinical aspect of the definitive crown at 5-year follow-up.



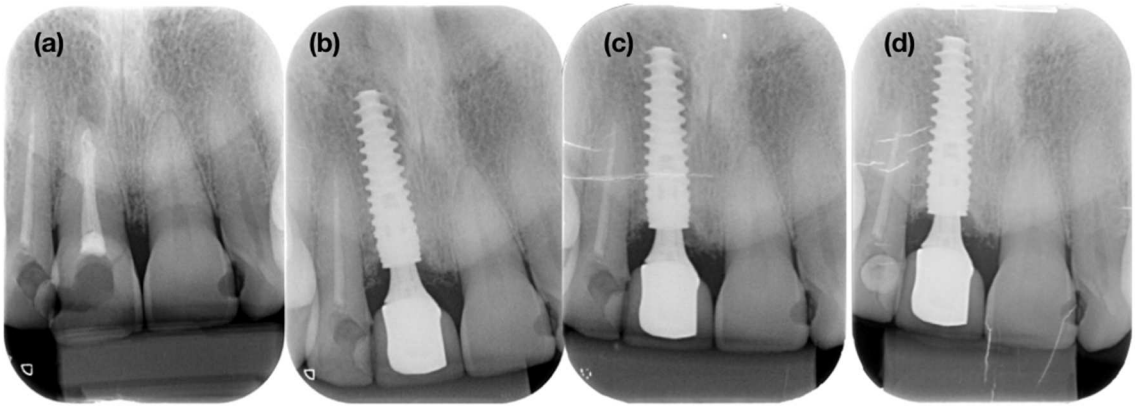
ANEXO 9 – Figura ilustrando exames radiográficos de pacientes com perda óssea marginal maior do que 1 mm.

Figure 2. Preoperative (a), 6-month (b), 12-month (c) and final follow-up (d) digital radiographs showing MBL > 1 mm at distal aspect.



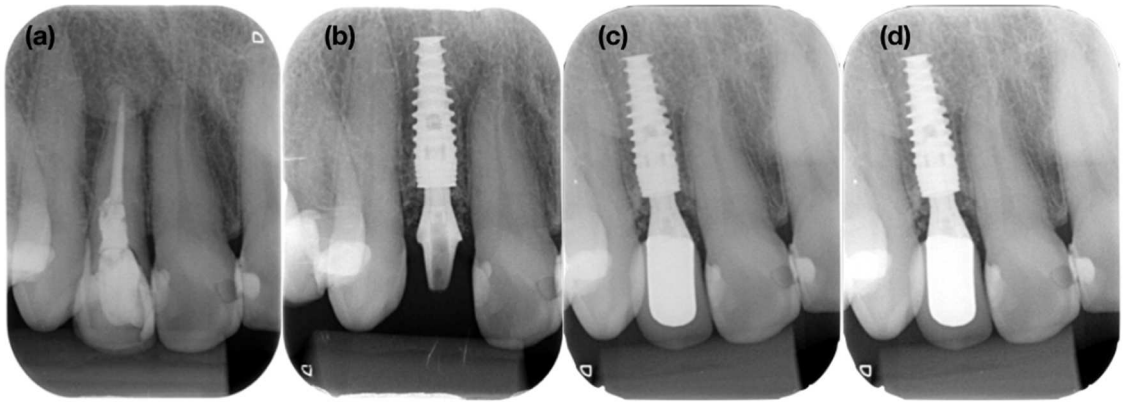
ANEXO 10 – Figura ilustrando exames radiográficos de pacientes com perda óssea marginal menor do que 1 mm.

Figure 3. Preoperative (a), 6-month (b), 12-month (c) and final follow-up (d) digital radiographs showing MBL < 1 mm at both aspects.



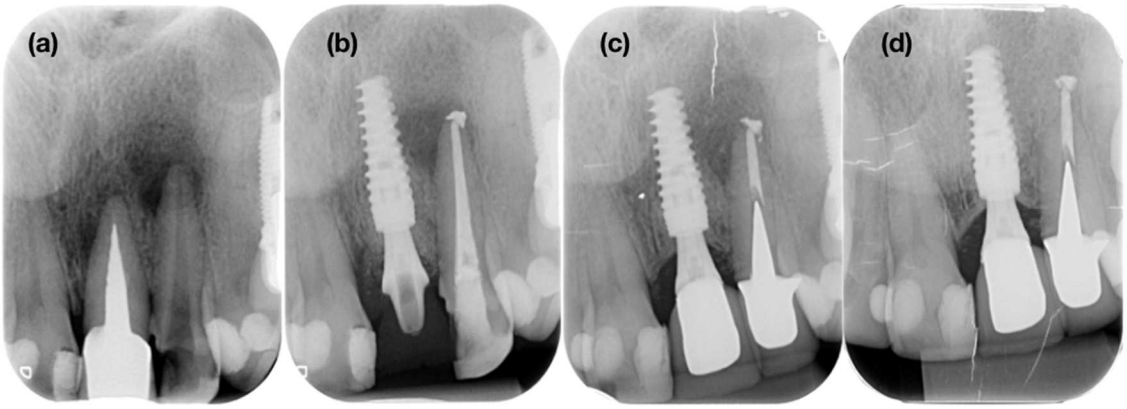
ANEXO 11 – Figura ilustrando exames radiográficos de pacientes com perda óssea marginal igual à zero.

Figure 4. Preoperative (a), 6-month (b), 12-month (c) and final follow-up (d) digital radiographs showing MBL = 0.



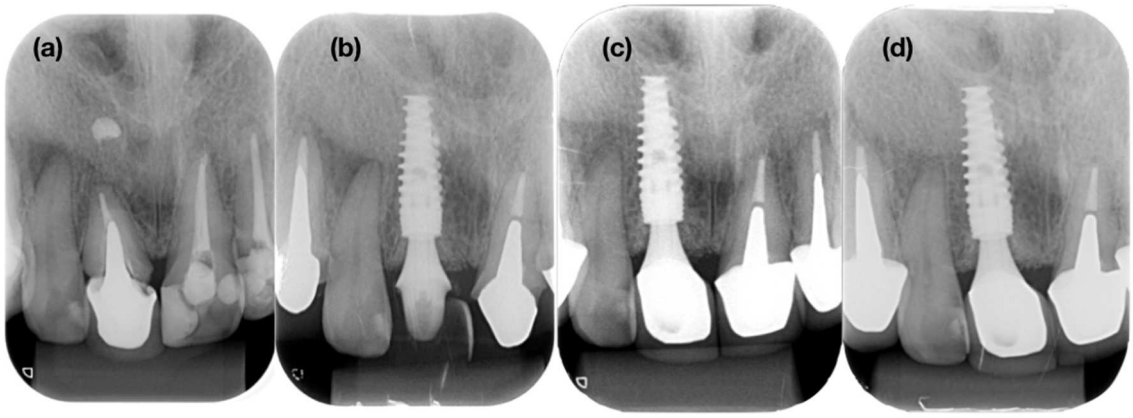
ANEXO 12 – Figura ilustrando exames radiográficos de pacientes com ganho ósseo.

Figure 5. Preoperative (a), 6-month (b), 12-month (c) and final follow-up (d) digital radiographs showing bone gain.



ANEXO 13 – Figura ilustrando exames radiográficos de pacientes demonstrando contato osso-pilar.

Figure 6. Preoperative (a), 6-month (b), 12-month (c) and final follow-up (d) digital radiographs showing bone/abutment contact.



DISCUSSÃO ARTIGO 1

No presente estudo, implantes cônicos com mudança de plataforma foram instaladosatravés de um protocolo de função imediata,sem descolamento de retalho mucoperiostal e restaurados com pilares definitivos personalizados e coroa provisória no dia da cirurgia. Uma taxa de sobrevivência dos implantes de 96,2% foi registrada para uma amostra de 52 indivíduos (38 mulheres, 14 homens) com idade média de 58,04 ± 12,91 anos em um período médio de acompanhamento de 36,3 ± 23,2 meses (variação de 12 a 100 meses). Dois implantes foram perdidos (taxa de falha de 3,8%). Um implantenão apresentou travamento de 35 N/cme foi removido um dia após a suainstalação e outro devido a trauma oclusal (dois meses após a instalação do implante). Ambos eram implantes de plataforma estreita (3,5 x 13 mm e 3,5 x 16 mm) e foram substituídos no mesmo dia de sua remoção.

As restaurações definitivas foram entregues em média 7,08 ± 2,63 meses (variando de 4 a 17 meses) após a colocação dos implantes com uma taxa de sobrevivência de 98%. A descrição amostral, assim como os motivos para as extrações dentárias e as características dos implantes estão descritos naTabela 2 (Table 2 – artigo 1).A complicação mecânica mais frequente foi falha na cimentação das coroas temporárias (30 vezes). Imediatamente após a cirurgia, a quantidade de cimento provisório foi propositalmente reduzida para evitar o extravasamento do mesmo dentro da ferida cirúrgica. Essa pode ser a razão pela qual 46% da amostra apresentou essa complicação clínica, o que está de acordo com o estudo de Hartlev que relatou a mesma frequência desta complicação clínica.[1] O afrouxamento do pilar personalizado ocorreu três vezes e duas coroas temporárias foram substituídas após a fratura. Apenas dois pacientes apresentaram recessão gengival importante, de modo que os seus *abutments* foram removidos ume única vez para repreparo subgengival e instalados novamente. Além disso, outros quatro pilares foram substituídos por espessura insuficiente para recobrimento do material cerâmico.

Diversos estudos avaliaram as taxas de sobrevivência de implantes seguindo diferentes protocolos de instalação e função.[4,6,9,11,12] Hartog e colaboradores, em uma revisão sistemática e metanálise da literatura, descrevem uma taxa de sobrevivência de 95,5% para implantes unitários na zona estética para função imediata, precoce e tardia.[4]Na mesma linha, 3082 implantes foram selecionados em outra revisão sistemáticareunindo apenas estudos prospectivos.A taxa de sobrevivência de 98,4% foi reportada para implantes imediatos efunção imediata e precoce.[9]Por outro lado, Atieh e colaboradores relataram uma taxa de

sobrevivência para função imediata variando de 82,4% a 100%. Nesta revisão sistemática, no entanto, quatro dos cinco artigos selecionados relataram taxas de sobrevivência de 100% e apenas um 82,4%.[5]Outros dois estudos com desenhos metodológicos semelhantes discordam fortemente desses achados. Em um deles, não foram encontradas diferenças estatisticamente significativas entre instalação imediata e convencional após 2, 3 e 5 anos de acompanhamento.[6]O outro relatou um risco ligeiramente maior para implantescomfunção imediata *versus*tardia (98,2% e 99,6%, respectivamente), após a seleção de 37 ensaios clínicos randomizadoscom um tempo de acompanhamento de 6 a 84 meses.[11] Esses achados estão de acordo com estudos retrospectivos e ensaios clínicos longitudinais relatando altas taxas de sobrevivência para função imediata em diferentes *follow-ups*.[7,8,13-15,37-39] Eles também estão de acordo com a taxa de sobrevivência de 96,2% através da técnica descrita em nosso estudo.

Nossos resultados também relatam uma perda óssea marginal média de 0,19 ± 0,36mm naface mesial e 0,26 ± 0,47mm na face distal no*follow-up* final, não havendo diferenças estatisticamente significativas entre 6 meses, 12 meses e *follow-up*final em ambas as faces (p = 0,832 e p = 0,958, respectivamente). Esta alteração óssea marginal está de acordo com os critérios de de sucesso relatados por Arora e colaboradores.[8] No entanto, é importante mencionar que implantes apresentando osso no nível ou mesmo coronalmente à plataforma do implante foram classificados como apresentando perda óssea marginal zero (MBL = 0), a fim de não influenciar positivamente os dados do presente trabalho. Dessa forma, nossos resultados estão de acordo com os dados disponíveis em estudos avaliando abordagens semelhantes[7-9,11,13-15]ecompartilham um pequeno desacordo com outros artigos que serão tratados mais a fundo.[37-39]

Dois dos estudos acima mencionados relataram MBL de 2,0 mm[39] e 1,0 mm[1] 3 e 7 anos após função imediata. O aumento do remodelamento ósseo pode ser devido ao uso de uma conexões de hexágono externo e a ausência de procedimentos de enxertia em ambos os estudos.[27]Por outro lado, Calvo-Guirado e colaboradores também não usaram enxertos ósseos, porém o simples fato de usarem uma conexão cônicae mudança de plataforma pode ter sido a razão pela qual relataram níveis mais baixos de MBL (0,86 ± 0,29mm) após um acompanhamento de 3 anos.[38] O deslocamento do infiltrado inflamatório da interface implante-pilar diminui os seus efeitos deletérios nas estruturas circundantes,[40] restabelece o espaço biológicoperi-implantar,[30] cria um ambiente mais estável[41] e reduz a perda óssea marginal,[42] o que significa redução das complicações biomecânicas e biológicas após a colocação de implantes dentro deste conceito.[43] O uso de conexões com mudança de plataforma ajuda a explicar a perda óssea mínima encontrada em nosso estudo. Também pode

explicar uma mudança não significativa (p = 0,832 ep = 0,958) nos níveis ósseos entre os 6 meses (MBL_m = 0,15 ± 0,34mm e MBL_d = 0,23 ± 0,49mm), 12 meses (MBL_m = 0,19 ± 0,36mm e MBL_d = 0,25 ± 0,47mm) e o controle final (MBL_m = 0,19 ± 0,36 mm e MBL_d = 0,26 ± 0,47mm).

Nossos achados concordam com os resultados de um estudo experimental em animais mostrando que o uso de partículas de matriz óssea bovina inorgânica modificou o remodelamento do tecido duro após função imediata e melhorou o contato osso-implante.[26] Da mesma forma, enxertos ósseos reconstrutivos para preenchimento do *gap* entre a tábua óssea vestibular e o implante resultaram em redução da perda óssea marginal em dois outros estudos semelhantes.[13,14] Após função imediata de 24 implantes, Cristalli encontrou níveis de MBL de 0,38 ± 0,75mm mesialmente e 0,27 ± 0,59mm distalmente com um procedimento de retalho aberto eenxerto xenógeno.[13] Na mesma linha, embora usando um enxerto xenógeno diferente (Endobon®), Nimwegen relatou níveis médios reduzidos de MBL (0,31 ± 0,20mm) em 51 implantes.[14] Seus achados radiográficos estão de acordo com o presente estudo; no entanto, ambos os estudos incluíram primeiros pré-molares na sua avaliação da zona estética. Nossa concepção de zona estética difere, já que a presente seleção amostral incluiu exclusivamente dentes de canino a canino.

Além dos níveis reduzidos de MBL descritos no presente estudo, 32/50 implantes não mostraram perda óssea marginal (64%), 14/50 implantes apresentaram perda óssea marginal menor que 1 mm (28%) e apenas 4/50 implantes mostraram perda óssea maior que 1mm (8%) após um período médio de acompanhamento de 3 anos pós-carga. Comparativamente, Cristalli relatou 8,7% da amostra com MBL> 1mm.[13] Também avaliando implante e função imediatos na zona estética, Cosyn descreveu 7/17 de seus pacientes (41%) não apresentando perda óssea marginal ou apresentando ganho ósseo usando matriz óssea bovina inorgânica.[7]Em uma análise retrospectiva, 16 de 30 (53%) implantes apresentaram osso no nível ou coronalmente à plataforma do implante, além de um ganho ósseo médio de 0,26 mm usando enxertos xenógens.[8] Ambos os estudos apresentaram um acompanhamento médio maior do que o presente estudo (5 anos).No entanto, parte de suas amostras foram perdidas durante o curso do estudo.

Finalmente, Noelken e colaboradores descreveram ganho ósseo de 0,04mm, variando de 1,37mm de perda óssea a 1,19mm de ganho ósseo, em uma amostra de 33 implantes colocados em alvéolos frescos. Analisando tomografia computadorizada, eles relataram uma perda óssea marginal de 1mm em 27% de sua amostra após 5 anos de acompanhamento.[15]Em nosso trabalho, trinta e dois dos cinquenta implantes (64%) colocados não apresentaram perda

óssea marginal interproximal. Avaliando esses 32 implantes separadamente, 5/32 (15,6%) apresentaram osso no nível da plataforma e 27/32 (84,4%) apresentaram nível ósseo coronalmenteà plataforma do implante e até mesmo em contato com o pilar protético (21/32 pacientes (65,6%). A presença de osso preexistente ou formado crescendo além da plataforma do implante e em contato íntimo com a superfície do *abutment* está de acordo com a literatura;[26,27,40,44]Para o conhecimento dos autores, este é o primeiro relato clínico mostrando tal estabilidade óssea com um tamanho de amostral representativo e um tempo médio de acompanhamento de 3 anos.

O posicionamento subcrestal de implantes com pilares cônicos diminui significativamente os níveis de MBL quando comparado a implantes hexagonais colocados à nível ósseo, como confirmado por estudos experimentais em cães,[24,44]um estudo com análise histomorfométrica em implantes removidos[40] e em outros relatos clínicos.[23,45]Avaliando implantes removidos por motivos de sofrimento psicológico, Degidi e colaboradores encontraram áreas de formação de osso novo e 0,5 à 3 mm de ganho ósseo em implantes instalados subcrestalmente.[23]Comparativamente, todos os pilares personalizados em nosso estudo apresentaram 2,5 - 3,0 mm de altura, demonstrando um posicionamento infra ósseo adequado e de acordo com os achados de Galindo-Moreno que mostram menores taxas de MBL em pilares protéticos superiores a 2mm.[16] O que também ajuda a justificar a preservação óssea vista no presente estudo.

Adicionalmente, este trabalho utilizou *abutments* definitivos imediatamente instalados após a colocação dos implantes. O conceito, definido como *one abutment-one time* (OAOT), tem sido cada vez mais relatado ao longo dos anos e consiste na não remoção do pilar protético ao longo de todas as etapas de reabilitação.[17,29,35,36]O número de remoções de abutments capazes de causar efeitos negativos no osso ainda está para ser definido.[3]Uma revisão sistemática e metanálise, além de encontrar efeitos positivos da técnica avaliada, não encontrou diferenças estatisticamente significativas na análise de subgrupo comparando menos de duas remoções e mais de três.[3] Suas descobertas estão de acordo com outra recente revisão sistemática e metanálise que também destacou o benefício de tal protocolo quando implantes com mudança de plataforma são instalados apicalmenteà crista óssea.[17] Além disso, Grandi relatou diferenças estatisticamente significativas na MBL quando os abutments definitivos (AD) foram comparados com os abutments provisórios (AP) (0,094 mm versus 0,435 mm) em 28 implantes.[33]Outro ensaio clínico randomizado do mesmo grupo também encontrou uma diferença de perda óssea de 0,5mm em favor dos pilares inseridos no dia da cirurgia.[34] Eles realizaram uma moldagem convencional usando a técnica de duplo fio para

copiar os pilares definitivos personalizados colocados em região de pré-molares. Em contraste, nossa técnica consiste apenas em uma moldagem de localizaçãousando o coping de Zr que já havia sido fabricado, evitando ruptura adicional do selamento epitelial. Seus resultados estão de acordo com outro ensaio clínico randomizado prospectivo mostrando uma reabsorção óssea estatisticamente significativa entre AD e AP (MBL = 0.61 ± 0.40 mm versus MBL = 1.24 ± 0.79 mm) durante o período de cicatrização.[35]

Finalmente, Canullo e colaboradores em dois estudos recentemente publicados sobre OAOT, encontraram resultados estáveis de reabsorção óssea ao longo de um período de cinco anos, bem como melhores dimensões de tecido mole usando análise de escaneamento digital.[29,36] No entanto, em ambos os estudos, os pacientes tiveram que ser submetidosa três etapas cirúrgicas em três momentos distintos (isto é, extração dentária e preservação do rebordo, instalação tardia do implante e uma pequena incisão para inserção do pilar) e foram reabilitados provisoriamente usando seus dentes adjacentes como pilares para uma prótese adesiva. Uma perda óssea marginal de 0,31 ± 0,29 mm após cinco anos foi relatada, que é ligeiramente maior que a remodelação óssea relatada na presente investigação após um *follow-up* médio de três anos (MBL médio = 0,22 ± 0,44 mm).No entanto, nossos procedimentos cirúrgicos e protéticos foram significativamente menos invasivos, uma vez que a colocação imediata de implantes sem retalho e a restauração através de uma abordagem OAOTforam realizados para todos os participantes do estudo em até 24 horas após o procedimento cirúrgico.

Este é o primeiro ensaio clínico sobre o uso de pilares definitivos personalizados em conjunto com um protocolo de implante e função imediatos sem retalho na zona estética. Dentro das limitações do presente estudo, pode-se concluir que a técnica descrita é uma alternativa previsível e eficaz para a substituição de um dente perdido na zona estética com altas taxas de sobrevivência e mínima alteração tecidual durante um período médio de três anos. Novos estudos clínicos de longa duração são necessários para confirmar os resultados encorajadores encontrados em nosso trabalho.

3. ESCLARECIMENTOS- ARTIGO 2

O segundo artigo incluído na presente tese de doutorado trata-se de uma revisão da literatura intitulada “Periodontitis is an Inflammatory Disease with Oxidative Stress: we should treat it that way”. O artigo foisubmetido à revista Periodontology 2000 (fator de impacto 6,22/classificação A1 segundo a CAPES) a convite do professor Frank Scannapieco, escritor responsável pela elaboração do capítulo “Prevention of Periodontal Disease”.

A cada ano o periódico publica três capítulos sobre diversos temas relacionados à doença periodontal. O corpo editorial identifica tópicos significativos e cientistas renomados para a elaboração de cada capítulo. O desenvolvimento do trabalho foi feito em conjunto com alunos e professores da Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Universidade de Campinas (UNICAMP) e Universidade de Toronto.

Como a seleção dos autores participantes em cada capítulo é feita através de convite, o método de submissão dos trabalhos é feito diretamente com o responsável pela elaboração do mesmo. Portanto, a sequência de e-mails confirmando o envio ao autor responsável encontra-se em anexo nesse trabalho. A previsão de publicação do periódico é junho de 2019.

4. ARTIGO 2

Periodontitis is an Inflammatory Disease with Oxidative Stress: we should treat it that way.

Short title: periodontitis, oxidative stress, ROS, resveratrol, antioxidant therapy.

Authors:

Fábio Sá Carneiro Sczepanik DDS, MSc. Department of Prosthodontics, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil. (Communicating author)

Márcio Lima Grossi DDS, MSc, PhD. Professor, School of Health Sciences, Dentistry; Post-Graduate Program in Dentistry, Prosthodontics, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Márcio Casati DDS, PhD. Dental Research Division, School of Dentistry, Paulista University (UNIP); Department of Prosthodontics and Periodontics, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil.

Michael Goldberg DDS, MSc, Dip. Perio. Associate Professor, Discipline of Periodontology, Faculty of Dentistry, University of Toronto; Department of Dentistry, Clinical Director, Centre for Advanced Dental Research and Care; Head, Division of Periodontology, Mount Sinai Hospital, Toronto, ON, Canada.

Michael Glogauer DDS, PhD, Dip. Perio.Professor, Faculty of Dentistry and Matrix Dynamics Group, University of Toronto; Interim Chief of Dentistry, Princess Margaret Cancer Centre; Department of Dentistry, Centre for Advanced Dental Research and Care, Mount Sinai Hospital, Toronto, ON, Canada.

Noah Fine MSc, PhD.Matrix Dynamics Group, Faculty of Dentistry, University of Toronto; Centre for Advanced Dental Research and Care, Mount Sinai Hospital, Toronto, ON, Canada.

Howard C Tenenbaum DDS, Dip. Perio, PhD, FRCD (C). Mount Sinai Hospital, Department of Dentistry; Centre for Advanced Dental Research and Care and Faculty of Dentistry, University of Toronto, Toronto, ON, Canada.

Communicating author: Fábio Sá Carneiro Sczepanik, DDS, MSc; Department of Prosthodontics, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil, Avenida Ipiranga, 6681. Tel.: +5551 991974496 Fax.: +5551 33332717 Email: fabiosacarneiro@gmail.com.

ABSTRACT

Periodontitis is a highly prevalent disease. As it progresses, it causes serious morbidity in the form of oral periodontal abscesses, loss of teeth, and when more severe, pain. It is also now known that periodontitis is associated strongly with several non-oral diseases. In this regard, patients with periodontitis are at greater risk for the development and/or exacerbation of diabetes, chronic obstructive pulmonary disease, as well as cardiovascular diseases, among other conditions. Although there is no question that specific groups of oral bacteria that populate dental plaque play a causative role in the development of periodontitis**,** it is now thought that once the disease has been triggered, other factors play an equal and possibly more important role in the progression of periodontitis, particularly severe cases or cases of periodontitis that prove difficult to treat. In this regard we refer to the so-called host response. This refers to the notion that the host, once infected with oral periodontal pathogenic bacteria, will mount a defense response mediated largely through the innate immune system. The major cells of the innate immune system, polymorphonuclear neutrophils (PMNs) can, when protecting the host from microbial invasion, mount a response that includes upregulation in the production of cytokines, matrix metalloproteinases, as well as reactive oxygen species (ROS), all of which then contribute to the tissue damage (and for example loss of teeth) commonly associated with periodontitis. Of the mechanisms referred to here we suggest that upregulation of the production of ROS might play one of the most important roles in the establishment and progression of periodontitis as well as other diseases of inflammation by way of the development of oxidative stress. In this overview, we present various factors both innate and epigenetic that lead to the development of oxidative stress (e.g. diabetes, smoking). The latter then provides the environment that leads to the destructive processes observed in periodontitis. Therefore, we shall describe some of the fundamental characteristics of oxidative stress and its effects on the periodontium, what diseases or other factors that cause oxidative stress and finally potentially novel therapeutic approaches for management of periodontitis, and possibly even reversing the condition that rely on the use of therapies that upregulate antioxidant activity including the use of resveratrol and other antioxidants.

1. Oxidative stress and its relation to chronic Periodontitis.

In recent years, there has been an increasing body of evidence that has reported on the role of reactive oxygen species (ROS) in the establishment of an oxidative environment related to the pathogenesis of a wide range of chronic inflammatory conditions, such as type-2 diabetes,[1] atherosclerosis, rheumatoid arthritis,[2] cancer,[3] inflammatory lung disease, and also periodontitis .[4] In particular, periodontitis is an inflammatory condition that affects 10 – 15% of the adult population[5] and when not properly treated, leads to chronic pain, loss of tooth-supporting tissues, and, consequently, tooth loss.[4,6,7] The persistent presence of plaque attached to the dental surface and its migration to the surrounding periodontal pockets leads to the recruitment of leukocytes from the bloodstream into the site of infection.

The polymorphonuclear infiltrate acts as the first line of defense against these bacterial pathogens. In particular, neutrophils, which represent 50% to 70% of this infiltrate,[8] play an essential role in periodontal health and in the innate immune system by acting as first-responder cells functioning through different defense mechanisms, such as degranulation, chemotaxis, phagocytosis, NETosis, and the release of ROS. However, the hyper-activated neutrophil phenotype associated with periodontal disease is characterized by overproduction of ROS and proteases making this subset of patients more susceptible to transitioning to periodontitis.[9-12] This complex interplay between the presence of sub-gingival biofilm and the degree of the host immune response facing this threat is the key to the establishment of a dysbiotic environment and the pathogenesis of periodontitis.[13-15]

Primarily acting as an antimicrobial defense, the generation of ROS is reported as a double-edged sword because of their capability of acting as a protective mechanism in physiological conditions and also presenting cytotoxic effects when overproduced. Despite their role in cell signaling, gene regulation, and antimicrobial defense,[16] an increased oxidant load together with either unaltered or reduced antioxidant capacity results in an oxidative stressed environment which is now responsible for tissue destruction.[3,17] Intracellularly, ROS damage biomolecules, cell membrane and, finally, cause cell death. Moreover, the release of ROS into the extracellular environment promotes degradation of the connective tissue and, consequently, destruction of the tooth-supporting structures.

As an end-product of the mitochondrial respiratory burst in neutrophils, the production of free radicals, specially O_2^-, H_2O_2 and $OH^\bullet$,[18] during phagocytosis,acts mainly through lipid peroxidation [19] and both protein[20] and DNA damage [21] leading to an oxidative imbalance that

triggers pro-inflammatory mechanisms and osteoclastogenesis.[22-25] Additionally, ROS affects the master antioxidant regulator nuclear factor erythroid 2-related factor 2 (NrF2),[12,26] which, when downregulated, is correlated with some of the previously mentioned inflammatory diseases and their progression, specially periodontitis and rheumatoid arthritis.[26,26] Finally, ROS production is responsible for attachment loss through direct damage to the extracellular connective tissue that leads to periodontal destruction.[18,28,29]

The destructive pattern that clinically characterizes chronic periodontitis as a disease affecting the tooth-supporting tissues is a result from an active connective tissue destruction and progressive bone resorption as the presence of different stimulating factors in plasma is responsible for increasing the respiratory burst, neutrophil priming and neutrophil life span in patients with chronic periodontitis.[10,30] Accordingly, the production of ROS has been associated with enhanced expression of pro-inflammatory cytokines that are directly and indirectly responsible for connective tissue destruction and bone resorption. While mineralized and non-mineralized destruction processes occur concomitantly, specific pro-inflammatory products are associated with either one or both levels of tissue damage. As an example, the bone uncoupling resulting from the ROS-mediated increased in RANKL/OPG ratio breaks the physiological bone dynamic in favour of bone remodeling as one major characteristic of disease progression.[31-33] Through the differentiation of macrophage precursor cells into osteoclasts and its following maturation caused by the increased levels of RANKL mRNA that disrupts the balance between RANKL and OPG, the bone physiologic conditions are altered. Therefore, the RANKL/OPG axis is in the center of bone resorption in periodontal diseases as well as in several chronic inflammatory diseases that are related to bone remodeling. Moreover, the reduced collagen production by ROS-affected fibroblasts and the wide range of different metalloproteinases (MMPs) excessively released into the site of infection during the immune response leads to both connective tissue and bone matrix degradation.[34-36] Following the increased release of MMPs, an imbalance between them and their endogenous inhibitors (TIMPs) also plays an important role in tissue destruction. Together, these cascade of events leads to a constant degradation of the mineral and organic matrix by which chronic inflammatory diseases are characterized.[37]

On the same line, Graves et al.[33] performed a literature review evaluating the groups of cytokines who influence bone uncoupling and showed that different groups of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, have been reported to either stimulate osteoclast activity, osteoblast death or influence bone remodeling through RANKL induction in periodontal destruction.[33,37]

The reports from different studies addressing the increased levels of oxidative markers in saliva, gingival crevicular fluid and plasma from periodontitis patients strengthens the relation between the establishment of an oxidative stressed environment and periodontal diseases progression. Konopka and colleagues[21] analysed the concentration of the oxidant-induced DNA damage biomarker 8-hydroxy-2'-deoxyguanosine (8OHdG) in patients with chronic periodontitis and found a significantly increased level of the oxidative biomarker in diseased patients when compared with healthy controls.[21] These findings agree with previous reports.[17] Similarly, 58 periodontal patients were compared with 234 healthy controls in terms of a different oxidative stress marker (protein carbonyl levels) in unstimulated saliva.[20] The authors found significantly higher levels of total protein carbonyls through enzyme-linked immunosorbent assay (ELISA) and, consequently, greater loss of periodontal attachment.Accordingly, the levels of malondialdehyde (MDA), a highly specific marker for polyunsaturated fatty acid peroxidation, were found increased in serum, saliva, and gingival crevicular fluid (GCF) samples from patients presenting chronic periodontitis in comparison with their healthy counterparts.[17,39] In the same experiment, the non-surgical therapy significantly modified the levels of MDA into levels compared the healthy samples, highlighting the interplay between the expression of oxidative biomarkers and periodontitis. Moreover, Novaković et al.[39] reported on the beneficial effects of non-surgical therapy in the levels of salivary antioxidant glutathione peroxidase (GPx) assessing periodontitis patients before and after treatment.[39] The correlation between non-surgical periodontal treatment and the reduction in the oxidative stress represented by the overall decreased ROS production reflects the direct relation between the release of free radicals and periodontal tissue destruction.

To act against the excess of ROS, antioxidant enzymes, such as superoxide dismutases, catalases and glutathione peroxidases are released into the oral cavity as an attempt to balance and re-establish the oxidative status and prevent tissue destruction.[40,41] Along with those enzymatic antioxidants, endogenous albumin and uric acid also play fundamental roles in the maintenance of the redox state in favour of a positive antioxidant balance.[42] Accordingly, a significant number of case-control and longitudinal studies have compared the levels of different antioxidant markers in health and periodontitis along the effectiveness of non-surgical therapy.[19,39,43] Analysing salivary markers in patients suffering from periodontitis, Banasova and colleagues[19] have found a tendency towards reduced DNA integrity and significant reduced antioxidant status (54% reduction) in diseased subjects when compared with their healthy controls.[19] Moreover, the antioxidant levels for glutathione

peroxidase (GPx), albumin (ALB), uric acid (UA), and total antioxidant capacity (TAOC) were measured before and after non-surgical therapy.[39,41] The research group concluded that conventional periodontal treatment positively influenced the levels of all the antioxidant markers in comparison with the levels before intervention. On the other hand, Chapple et al.,[9] addressing the longitudinal changes in GCF and plasma TAOC, showed no statistical differences between periodontally compromised and healthy subjects' plasma samples.[9] Interestingly, the GCF samples from periodontitis patients after non-surgical treatment reached control levels, leading the authors to suggest that decreased TAOC is more likely to be a consequence of the inflammatory condition rather than a cause for periodontal diseases. However, there is no consensus established thus far when comparing other similar studies.[44-46]

Although there are varied opinions when assessing the role of ROS production and in terms of creating the precondition allowing for the development of periodontal disease, the excess of free radicals in conjunction with host antioxidant incapability plays a central function in the pathogenesis and progression of chronic periodontitis.[9,47,48] This imbalance extends to a broader range of other inflammatory conditions and behaviors that stimulate oxidative stress and present correlation with oral manifestations, such as diabetes, smoking, obesity, and rheumatoid arthritis. Thus, the next section's aim is to demonstrate that the aforementioned are not only associated with more severe periodontitis but that they likely have their effects on account of their ability to upregulate oxidative stress.

2. Conditions and Behaviors that Stimulate Oxidative Stress

The establishment of systemic environment characterized by oxidative stress can cause an exaggerated pro-inflammatory condition which is the center of a wide range of metabolic disorders that present destructive patterns as a consequence.[49] These complex multifactorial disorders (which include diabetes, obesity and rheumatoid arthritis),[50-54] share ROS and its subsequent antioxidant imbalance as a common feature for their development and progression.[55-56] Thus, establishing a connection between this group of diseases, their correlation with severe periodontal disease, and their pro-inflammatory similarities is of great value.

2.1. Diabetes

Diabetes Mellitus is a chronic disorder that affects over 340 million people worldwide.[57] In North American, 9% of the population suffer with this chronic metabolic disorder. Type-1 Diabetes Mellitus (T1DM) is characterized by the autoimmune destruction of pancreatic ß-cells and the complete lack of insulin production, representing 5 – 10% of the total cases and with a relatively early onset. Hyperglycemia and hypoglycemia are two possible conditions patients presenting T1DM might face.[49] The most common subtype (85 – 90%) is Type-2 Diabetes Mellitus (T2DM). With a more prolonged onset, T2DM presents different degrees of ß-cell dysfunction and insulin resistance, and is usually accompanied by either obesity, overweight, a sedentary lifestyle, genetic predisposition, or during pregnancy (gestational Diabetes Mellitus).[57] In T2DM, glucose and lipid metabolisms are chronically dysregulated. Pancreatic ß-cells, responsible for secretion of insulin, fail to compensate insulin resistance by peripheral cells, leading to hyperglycaemia, disturbing the blood vessels physiological activity and increasing reactive oxygen species production, which dysregulate the redox state, causing loss of the homeostatic balance.[1,49,58,59] As a consequence of insulin resistance, the pancreas ineffectively starts secreting higher loads of insulin, in order to compensate the non-absorption by peripheral cells in the muscles, adipose tissues, and in the liver. The latter then starts releasing glucose into the blood, increasing blood sugar levels.[60-61] Secondary complications to non-controlled T2DM are chronic hyperglycemia and its subsequent results, such as microangiopathies (nephropathy, neuropathy, retinopathy, cardiomyopathy, and periodontal disease), macrovascular diseases (cardiovascular diseases, hypertension, ischemic heart disease and stroke, infertility, and necrosis), and several impaired immune responses.[57,62-64]

Within the broad scope of secondary conditions related to T2DM, chronic periodontal disease is considered the sixth diabetic complication, playing a two-way part with the chronic metabolic disorder.[65] The relationship between impaired blood glucose levels and periodontitis is widely established in the literature.[49,66,67] Both are chronic and inflammatory diseases that share common risk factors and mutually interact with one another, presenting increased oxidative stress and exacerbated pro-inflammatory mediator release.[67,68] There is a consistent body of literature reporting on how increased diabetic parameters, such as glycated hemoglobin (HbA1C), correlates positively with oral inflammatory biomarkers and polymorphonuclear activity, disease progression, and probability to develop periodontitis.[1,16,57,68-71] On one side, the immune defense mechanisms of patients systemically affected by T2DM fail to act against microbial challenge, particularly due to a more pathogenic subgingival microbial profile in those subjects, collapsing to tooth surrounding

tissues destruction.[67] On the other, periodontal disease parameters, such as probing depth (PD), clinical attachment loss (CAL), bleeding on probing (BoP), and gingival index (GI), are negatively affected by chronic hyperglycemia and ß-cell dysfunction.[1,66] Rovai et al.,[72] in a systemic review of the literature, have shown that non-surgical periodontal treatment of T1DM and T2DM patients significantly improved CAL and reduced PD levels.[72] Furthermore, the mutual relationship between periodontitis and T2DM is highlighted in different reports concluding that periodontal treatment results in a positive response on previously increased glycemic levels in T2DM patients, systemic oxidative stress levels improve after non-surgical periodontal treatment in diabetic patients and insulin resistance and altered ß-cells function may predict the progression and severity of chronic periodontal disease.[66,70,71,73]

As one of the main disease mechanisms, Advanced Glycation Endproducts (AGEs) are one of the major links between diabetes mellitus and its complications. This end-product originates from the irreversible non-enzymatic glycation and oxidation of proteins, lipids, and nucleic acids by the addition of sugar to their polypeptide chain, which alters their structure and functionality.[74] AGEs are produced in general in conjunction with aging, with the endproduct carboxymethyl lysine being the most common produced.[75] Elevated levels of AGEs are a result of chronic hyperglycemia and promote a pro-inflammatory state by increasing the production of specific cytokines, such as TNF-α, IL-6, IL-1ß, and prostaglandin E_2, which alter oxygen diffusion by changing membrane structure and permeability, and are associated with a state of enhanced oxidant stress.[49,57,58,74,76,77] An increase in binding of AGE products to their receptors (RAGEs), is thought to explain the increased inflammatory environment observed in poorly-controlled diabetics with chronic periodontal disease. Consequently, the release of cross-linking collagen thickens the membrane of blood vessels with AGE-modified collagen accumulation, changing the transport between the endothelial membrane, increasing production of vascular endothelial growth factor (VEGF) and causing micro and macro vasculature complications.[58] Additionally, Mealey and colleagues[58] performed a review with over 200 articles evaluating the relationship between diabetes mellitus and periodontal disease and concluded that AGE-RAGE interaction on monocytes increases cellular oxidant stress and activates the transcription factor nuclear factor- kappa B (NF-κB).[58] On a similar line, the 2013 EFP/AAP Consensus Report[43] on periodontitis and systemic diseases evaluated clinical studies and animal experiments and concluded that AGE–RAGE interaction leads to the exaggerated inflammatory response and periodontal tissue destruction in T2DM patients.[43]

As previously mentioned, a representative group of oxidative stress stimulating mechanisms and antioxidant markers have been analysed and proposed to play important roles in T2DM pathogenesis and in its interplay with periodontal disease. Evaluating either protein, DNA, or lipid oxidation end-products, antioxidant markers, or enzymatic antioxidant mechanisms, and utilizing different methods of analysis, the current literature has established a consistent link between both disorders in terms of reactive oxygen species over production and their oxidative pathways.

In an environment characterized by the overproduction of free radicals, the release of different enzymatic antioxidants in an attempt to prevent oxidative damage is represented by various acting molecules. One of the most prominent enzymes is the family of the superoxide dismutase (SOD), representing important indicators of the oxidative processes. T2DM individuals with periodontal disease show a decrease in the antioxidant capacity represented by an inefficient SOD activity.[78] A case-control study with a sample size of 150 plasma analysis revealed that SOD activity is decreased in periodontally compromised patients but showing that its level increases when T2DM patients are also affected by periodontitis.[79] Interestingly, Akalin and colleagues[80] have also demonstrated that SOD activity in T2DM periodontitis patients is higher in comparison with systemically healthy patients presenting periodontal disease, suggesting that diabetes increases gingival SOD activity as an adaptive mechanism, while periodontitis patients keep their antioxidant defenses diminished.[80] Finally, Duarte et al.,[81] using gingival tissue sampling for mRNA levels and qPCR analysis, showed similar results, reporting on SOD2 genes being only slightly influenced by periodontal disease, whereas in poorly controlled T2DM individuals this enzymatic mechanism being significantly induced.[81]

Another key antioxidant enzyme that acts together with SOD in the dismutase of superoxide to hydrogen peroxide is the glutathione peroxidase (GPx).[82] Those enzymatic antioxidants are associated with the glycation of hemoglobin in such a way that when HbA1c levels increase, GPx activity decreases.[83] Similar results were reported for salivary GPx levels before and after periodontal treatment.[39] Duarte and colleagues[81] used 49 gingival biopsies harvested from poorly and well-controlled T2DM patients for mRNA analysis to demonstrate that GPx levels are up-regulated by periodontal disease and independent from the diabetic status of the individual.[81] On the other hand, different observational studies have concluded that poor glycemic control and untreated periodontal disease are directly correlated to the worsening of oxidative stress markers for both diseases and that a similar decreased pattern is found for C-reactive protein and protein carbonyl levels.[1,49,59,73,78,84]

On the same line, the levels of malondialdehyde (MDA), a marker for lipid peroxidation, has been reported to be significantly increased in periodontal tissues from patients presenting T2DM, representing excessive free radical activity.[59,78] Accordingly, the same pattern of increased MDA is found in systemically healthy patients with chronic periodontitis.[17,38] Most interestingly, MDA parameters were found in significantly lower levels in serum samples after T2DM subjects went through periodontal treatment and lycopene administration demonstrating how both diseases interchangeably relate.[85] Additionally, the DNA damage biomarker 8-hydroxy-2'-deoxyguanosine (8OHdG), which is found increased in patients presenting periodontal disease in comparison with their healthy counterparts, is also affected positively in T2DM patients after scaling and root planning.[21,86] Analyzing gingival crevicular fluid (GCF) from 48 individuals, the authors concluded that the group presenting the most prominent reduction in oxidative stress parameters (represented by 8-OHdG), as well as clinical parameters, was the one presenting both diabetes and periodontitis.[86]

Total antioxidant capacity (TAOC), represented by the capacity to inhibit the production of TBARS (thiobarbituric acid reacting substances), is the balance between the presence of antioxidant markers protecting host cells against oxidative agents and is widely reported in the literature as being decreased both in peripheral blood samples and GCF samples in T2DM patients with periodontitis.[9,44-46,78,79,87] Thus, the generation of oxidative stress may be an underlying systemic condition directly related to alveolar bone loss in periodontitis in T2DM patients.[88] Aryl hydrocarbon receptor (AhR) ligands are environmental contaminants found in a wide range of pollutants used in agriculture, such as pesticides and herbicides, burning of garbage, by-products of combustion processes, and also in cigarette smoke.[89] Tetrachlorodibenzo-para-dioxin (TCDD) is a prototype of dioxin and, as well as other dioxin-like compounds, binds to AhR and mediates a variety of toxic effects, such as increased risk of cancer and stroke, suppression of the immune system, hormonal imbalances, and T2DM.[90] Different studies have reported on the effects of AhR ligands as a putative mechanism of T2DM development through TCDD-induced impaired glucose levels,[89] impaired β-cell glucose metabolism[91] and insulin sensitivity.[92] The majority of studies relating AhR ligands to T2DM were performed using animal models, such as the experimental study performed by Takuma and colleagues[92] in which TCDD was repeatedly administrated to mice to assess the influence on insulin sensitivity. The authors found the AhR activation by TCDD caused insulin resistance and elevated plasma insulin concentration.[92] Their findings agree with the results from a case-control study that measured

serum AhR activity in T2DM subjects. There was a significant higher AhR activity in the T2DM in comparison age -, sex -, and BMI - matched subjects presenting impaired glucose tolerance and healthy controls. The authors suggested insulin resistance might be a possible link between AhR and T2DM development.[89]Additionally, there is evidence that ARNT/HIF-1β, a regulator of β-cell function required to keep pancreatic β-cells in a glucose-responsive state, are profoundly reduced in islets obtained from T2DM patients.[93] Most interestingly, Wang[91] have demonstrated that even in patients with no history of diabetes, serum AhR concentrations are negatively associated with β-cell function.[91] Those findings taken together suggest that AhR may be involved in the pathogenesis of an abnormal glucose tolerance and, consequently, in T2DM development.

Using a chick periosteal osteogenesis model, Singh and colleagues[90] reported significant reductions in mineralization mediated by TCDD, decreases in calcium accumulation and an approximate 80% reduction in alkaline phosphatase activity mediated by dioxin exposure.[90] The authors supported the hypothesis that TCDD may further predispose smokers to osteoporosis and periodontal bone loss.[90] Additionally, as Gram-negative bacteria colonization plays a fundamental role in periodontitis pathogenesis, Andreou and colleagues evaluated the putative synergistic effect of lipopolysaccharide (LPS) from *Porphyromonas gingivalis* with aryl hydrocarbons (BaP). They found significantly reduced bone nodule formation adding smoke-derived aryl hydrocarbons and bacterial LPS. Most interestingly, the effects of the combination between LPS and BaP were considered additive in terms of inhibiting bone nodule formation (9-fold) when compared to their separate administration.[94] Similar results were found in human periodontal ligament cells. In this recent study, the addition of BaP decreased mRNA expression of osteogenic genes and alkaline phosphatase activity.[95] Similarly, there is evidence in the current literature on how the inhibitory effects of AhR and RANKL signaling pathways interact in bone metabolism, more specifically, osteoclastogenesis.[96,97] Conclusively, the destructive effects on the structure of the tooth-supporting tissues are enhanced with the long-term exposure to aryl hydrocarbons in a dose-dependent manner and a representative group of experimental in vitro and in vivo studies have tested different AhR antagonists to counteract their deleterious effects,[90,94 -97] which will be deeply discussed in the following section.

Due to the similarities in terms of release of oxidative markers, disease progression and resolution, the establishment and maintenance of a continued oxidative stress environment is in the central axis connecting Type-2 diabetes mellitus and chronic periodontal disease.

2.2. Smoking

Tobacco is one of the greatest emerging health disasters in human history.[98] It is estimated that there are more than $200 billion in annual cost for smoking-related consequences in the United States. Five million people die annually from cigarette smoking and its deadly consequences globally, with almost one in every two smokers likely to die from secondary effects of tobacco consumption.[98,99] In the face of those alarming numbers, cigarette smoking consumption has been decreasing in developed countries, but still encompasses a significant portion of the world population, especially those in low-income countries.[98] The negative health effects of smoking include ischaemic heart disease, cerebro-vascular disease, chronic obstructive pulmonary disease, multiple types of cancer, and periodontal diseases.[69,98-100]

Although different mechanisms through which smoking affects the progression of periodontitis have been ascribed, a definitive theory explaining this process remains unclear. One of the mechanisms suggests there is a shift in the microbiota composition to a highly pathogenic one within periodontal tissues. Smoking habits have also been related to a change in neutrophil migration and chemotaxis dysfunction, leading to a deficient immune response facing the microbial threat. Finally, tobacco smoking may lead to a shift in neutrophil activity to a more hyperactive state, which increases the release of proinflammatory cytokines and over production of reactive oxygen species through respiratory burst, followed by gingival tissue destruction.[99,101,102]

The negative effect of smoking on periodontal health have been extensively reported in the literature with a vast number of clinical trials, systematic reviews, meta-analysis and epidemiological studies. The highly significant positive association between heavy smoking and disease incidence, progression and severity as well as low success rates for periodontal treatment makes cigarette smoking the most preventable risk factor for periodontal disease.[102] A national cross-sectional survey in the U.S. has estimated that current smokers are at four times higher risk of developing periodontitis. Similarly, evaluating the risk of tooth loss in a sample of health professionals, Dietrich and colleagues[103] have found an increased number of missing teeth with a progressive pattern of tooth loss as smoking intensity increased. They have also shown that smokers had twice the risk of tooth loss when compared with never smokers.[103] Later on, the same group updated the odds ratio to 3.6.[104] Their findings are consistent with the current literature.[105-108] In a systematic review and meta-regression, the

pooled adjusted ratios have estimated that smoking habits increased the risk for periodontitis in 85% (OR: 1.85, 95% CI = 1.5 – 2.2) for clinical studies with follow-up periods ranging from 2 to 37 years. Studies with similar designs have presented comparable results with one reporting a 30% radiographic bone level improvement among quitters.[109,110] Smokers are also at a higher risk for clinical attachment loss and recession, deeper probing depths, lower tooth retention, and severity of periodontal destruction.[102,111,112] Interestingly, the literature seems consistent with lower GI and BOP parameters in smokers due to the suppressive effect of smoking on periodontal blood vessels.[113,114]

The literature is still controversial regarding the GCF biomarkers profile of smokers that present periodontal disease. While the majority of the studies report a depressive effect of smoking on the expression of pro-inflammatory cytokines, others report no significant differences and even an increased cytokine profile in smokers.[115] Evaluating GCF, Tymkiw and colleagues[115] found decreased pro-inflammatory cytokine (IL-1β, IL-6) and chemokine profiles.[115] Similarly, smokers showed no statistically significant difference in expression of IL-1β, IL-6 and TNF-α in peri-implant sulcus fluid when compared to non-smokers.[116]This may be the reflection of the immunosuppressant effects of smoking which in turn may increase susceptibility to peri-implant periodontal and destruction.Even though TNF-α and IL-1β are secreted through similar mechanisms, IL-1β did not seem to be influenced by cigarette smoking in periodontitis patients.[113] Their findings are contradicted by the results from Liu et al.[117] In their experimental study, non-smokers were given nicotine supplements after they quit smoking. The GCF IL-1β levels were found higher at the final follow-up in comparison with one month after quitting (baseline).[117] Interleukin 1β (IL-1β) stimulates bone resorption, inhibits bone formation and is considered even more potent than TNF-α in terms of effects on bone metabolism. Similarly, a multiple linear regression analysis showed significant correlations between GCF cytokine levels and smoking. Additionally, Giannopoulou and colleagues analysed GCF samples and observed associations between smoking and total amounts of IL-6 and IL-8 but not with IL-1β levels.[118] These findings are in accordance with subsequent studies.[119]

Periodontal disease progression is mainly due to bone resorption and tissue destruction. One of the main discoveries in bone biology is the RANK/RANKL/OPG system. A fundamental mechanism for bone health which, if disrupted, causes several bone diseases, such as chronic periodontal disease. Bone remodeling is a dynamic mechanism mostly regulated by osteoblasts, who mediate osteoclastogenesis through different signaling

pathways, such as balancing the ratio between RANKL and OPG. In general, the attachment of RANKL on the surface of osteoclast precursors (OCP) is needed in order to differentiate them into osteoclasts (RANKL-induced osteoclast activation). Osteoprotegerin (OPG), a bone protector expressed by osteoblasts, is a natural inhibitor of osteoclast differentiation that binds to the RANKL surface and prevents OCP from differentiating into osteoclasts; thus, preventing bone resorption. The imbalance in the RANKL/OPG ratio has been positively associated with bone loss in smoker-related periodontitis patients[24,119-121] Gingival crevicular fluid samples from 149 periodontitis patients divided into 3 groups (never smokers, former smokers and current smokers) were evaluate through ELISA. Osteoprotegerin was significantly reduced and consequently RANKL/OPG ratio significantly increased in the current smoker's periodontitis group.[122] The authors concluded the suppression of OPG production may have led to bone loss. These results agree with similar studies using both gingival biopsies and serum.[119,120,123] Additionally, OPG concentration in whole saliva samples was compared with full-mouth clinical periodontal measurements and correlated positively with PD, CAL and BOP.[121] Finally, the RANKL/OPG ratio may act as an indicator of the extent of periodontal breakdown and thus treatment modalities capable of "switching off" this mechanism should be considered as an adjunctive tool for periodontal disease management.[24,124] Interestingly, a recent study has compared GCF total oxidant status (TOS) with RANKL, OPG and the ratio between both.[25] They have shown a significant association between increased TOS, RANKL and RANKL/OPG values in both local and systemic samples, suggesting that oxidative stress might be a common link between bone resorption markers and periodontitis severity.[25]

The mechanisms through which cigarette smoking influences periodontal destruction are complex. As mentioned above, the clinical effects of smoking are singular and unique in their own manner as smoke-induced disease progression is likely to present minor BOP measurements, but significant CAL and PD changes. Additionally, nicotine is not the only chemical compound negatively influencing periodontal tissues as the role played by AhR ligands in bone destruction has been confirmed in different studies.[90,94,95]

As there is evidence of RANKL-induced tooth-supporting tissue destruction, there is also evidence of smoke-induced oxidative stress caused mainly by an increased generation of reactive oxygen species within gingival tissues.[125] Screening tests for markers of DNA (8-Hydroxy-2′-deoxyguanosine (8-OHdG), lipid (malondialdehyde (MDA) and protein (C-reactive protein (CRP) oxidation have been compared to the smoking status of periodontally diseased patients, as well as the formation of antioxidant compounds, such as superoxide

dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Malondialdehyde is widely accepted as one of the most representative biomarkers for lipid peroxidation reflecting the presence of oxidative stress within different tissues. Tonguç and colleagues[126] screened the oxidative profile in gingival and serum samples from 65 patients with different smoking status and periodontal conditions. They have found significant correlations between periodontal parameters, smoking-related parameters, and increased MDA levels both in blood and local tissues.[126] Their findings agree with the results from another comparative study showing that the combination of periodontal disease with smoking have demonstrated significantly higher MDA concentrations in comparison with the non-smoking controls.[127] This pattern of higher lipid peroxidation markers in blood, saliva and gingival samples is in accordance with the current available evidence.[125,128]Has already confirmed in previous studies,[125,129] both smoking and periodontal disease affect C-reactive protein levels in a separate manner. Nonetheless, a small number of studies evaluated the effects of the two conditions combined on the level of the protein damage marker. A recent retrospective cohort data collection, demonstrated that the effect of periodontal status on CRP is significantly influenced by the pack year values (PYV), showing that a PYV > 30 is significantly associated with higher CRP levels in periodontitis patients.[130] Pack year value is calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. Their findings are in accordance with another publication from the same group, which had observed that smokers with chronic periodontitis exhibit elevated oxidative stress compared to non-smokers with chronic periodontitis.[129,130]Even though the 8-OHdG levels in patients with chronic periodontitis is significantly increased in comparison with healthy controls, smoking status does not seem to play a role in terms of increasing DNA damage in whole saliva samples.[21,131,132] Robust evidence on the combined effects of smoking and periodontal disease on 8-OHdG levels are still needed as a recent Korean study has reported a higher odds ratio between periodontitis and the DNA damage marker in whole saliva.[132]

While some studies imply that smoking increases gingival antioxidant activities as a result of a protective and adaptive mechanism developed in the tissue, even though they are not capable of reversing smoked-related periodontal destruction, another suggested mechanism relates smoking habits to a decrease in locally and systemically antioxidant defenses which in turn result in progressive tissue destruction. As an example, the SOD levels of smokers and non-smokers presenting periodontal disease was compared by Tonguç et al.[126] The authors reported insignificant changes in blood SOD levels but significantly higher

gingival SOD levels. Similar findings were found by other authors.[125,133] However, in a recent observational study evaluating the effects of periodontal treatment on oxidative biomarkers, a significant interaction between smoking status and salivary SOD levels at baseline and after treatment was reported. Smokers had significant lower reductions in SOD levels after treatment in comparison with non-smokers and former smokers. The authors implied cigarette smoking does influence redox homeostasis and alters antioxidant levels in favor of ROS.[134] Their findings agree with other studies.[128,135]In both reports, SOD levels were found significantly lower in smokers when compared with non-smokers and, most interestingly, antioxidant levels of heavy smokers differed from light smokers, leading the authors to imply that tobacco consumption influenced SOD levels in a dose-dependent manner.[128,135] The same pattern is reported by other groups using blood and saliva samples.[136] Similar discrepancies in the literature are shared for other antioxidant markers[125,127,128,134] but the current available evidence seems to confirm a significant reduction of TAOC in the combination of periodontal disease and smoking.[21] Additionally, cigarette smoking has been proven to affect neutrophil function, which stimulates ROS release and oxidative stress mediated tissue damage.[137] Consequently, with the capacity of protection diminished in smokers, it is plausible that the use of antioxidant compounds that are capable of acting against the overproduction of ROS within this setting should be addressed.

2.3. Obesity

Obesity is considered one of the main public health concerns with approximately 600 million people suffering with the disease worldwide and 31% of North American adults also affected. It is usually caused by excessive food intake, lack of physical activity, genetic susceptibility or a combination of those and other factors, such as endocrine and mental disorders.[138] Obesity is characterized by the deposition of excessive or abnormal fat in adipose tissues and diagnosed according to the World Health Organization criteria by using mainly body mass index (BMI), the ratio between body weight and body height. BMI is divided into three categories, class I (30.00-34.9 kg/m^2), class II (35.00-39.99 kg/m^2) and class III/morbid obesity (>40.00 kg/m^2). Additional measures, such as waist circumference (WC), waist/hip ratio (WHR) and measurement of subcutaneous skin fold can be used for complementary screening.[87,139,140] It is a chronic metabolic disease associated with subclinical inflammatory response in adipocytes and the release of adipose-tissue-derived hormones and cytokines (adipokines), which leads to altered hormonal activity, a pro-inflammatory state,

and, consequently, secondary consequences, such as hypertension, increased cholesterol and triglycerides levels, insulin resistance, and continued oxidative stress. It is also strongly associated with other chronic diseases, such as Type-2 diabetes, cardiovascular diseases, osteoarthritis, respiratory disorders, and periodontitis.[139,141]

There is a strong association between body fat measurements and periodontitis.[142] One group analysed longitudinal and experimental studies only and concluded that, especially in longitudinal studies (with a follow-up > 20 years), overweight, obesity, weight gain, and increased weight gain may be risk factors for development of periodontitis.[143] Additionally, a systematic review and meta-analysis delineated the profile of high BMI subjects as more likely to present greater mean attachment loss.[144] A similar pattern of association is also found between high levels of serum triglycerides and low high-density lipoprotein-cholesterol (HDL) with deepened periodontal pockets in obese patients.[145] In terms of clinical periodontal parameters, there are several clinical trials and comparative studies relating different levels of periodontal disease with high BMI. Obese patients are also described as presenting higher GI and gingival bleeding index (GBI) levels when compared to non-obese patients with periodontal disease.[141] Buduneli et al.[146] found a significantly higher PD and CAL values in the obese subjects ($p < 0.05$) and a tendency for a positive correlation between BMI and CAL.[146] Moreover, a cohort study with over one thousand participants in Brazil identified a higher risk for unfavorable periodontal outcomes, represented by BOP and CAL, in obese patients (RR: 1.45).[147] Those results are in accordance with previous studies.[148,149]

There is also evidence on the effects of SRP on obese and normal-weight individuals presenting chronic periodontal disease in terms of before and after treatment comparison showing that clinical parameters (PI, BOP, PD, and CAL) significantly reduce in both groups, as well as the expression of pro-inflammatory cytokines (IL-1ß, IL,6 and TNF-□), even though the improvement does not seem to be modified by obesity.[150-151] In both studies the sample comprised of mainly class I and II obese individuals. Their findings agree with a meta-review of the literature which have shown inconsistent evidence on the response to non-surgical periodontal therapy in obese patients.[152] However, studies on the effects of obesity on periodontal disease show clinical parameters that improve less in obese individuals rather than in normal-weight periodontitis individuals and indicate high BMI as a significant predictor of periodontal treatment success.[148,153-155] Gerber et al.,[156] in a systematic review of the literature on the effects of obesity on non-surgical periodontal therapy outcomes, noted the subject is still controversial and that there may be a negative association between obesity and periodontal treatment outcomes, as five out of eight studies included reported results along

this line. The authors, however, indicate a potentially inferior healing response for high BMI individuals based on their pathophysiological inflammatory models.[156] The results from that study agree with the findings from Gonçalves and colleagues[157] who have found patients with obesity presenting lower reductions in periodontal disease after SRP in comparison with non-obese chronic periodontal disease group.[157] Similarly, another systematic review showed no statistical differences in clinical periodontal measures after SRP but significant differences in inflammatory and metabolic parameters in obese individuals before and after treatment compared to periodontally health patients.[158] Results following a similar line were reported in another recent comparative study.[159]

Obesity is considered to be a modifying factor for periodontal disease through the promotion of a more pro-inflammatory state, which may affect their susceptibility to pathogenic bacteria and favor a shift towards promotion of periodontitis.[84,141] Tumour necrosis factor-a (TNF-α) is considered the main candidate connecting both conditions.[160] One proposed model linking obesity to periodontitis describes the increased secretion of pro-inflammatory cytokines, especially TNF-α, that inhibits insulin signaling, causing insulin resistance and the development of T2DM, which leads to a hyperinflammatory state, priming of periodontal tissues, exaggerated response to microbial colonization, and finally periodontal disease destruction.[161] Additionally, Lundin and colleagues[162] have found a positive association between the levels of GCF TNF-α and high BMI in periodontally healthy subjects, suggesting that this specific cytokine might originate from another tissue rather than the periodontium and might affect different structures than just the adipose tissue.[162] Most interestingly, one study has shown that the increased expression of TNF-α was detected in GCF samples of obese children before the development of periodontitis was diagnosed.[163] Conversely, Saxlin et al.[164] suggested that not TNF-α serum levels but IL-6 might mediate the connection between body weight and deepened periodontal pocket mainly due to C-reactive protein expression.[164] Their findings are contradicted by different authors who suggest IL-6 might act as a contributor factor instead of playing a major.[160,165] Nevertheless, even though the underlying mechanisms relating obesity with periodontitis remain unclear and their relation is considered bidirectional, high BMI is a significant risk factor for periodontal disease, suggesting obese subjects have a 35% increased chance of developing periodontitis, and chronic oxidative stress might be the common link between both conditions.[51,149,166]

Oxidative stress is characterized as a persistent imbalance between the release of highly reactive molecular species (ROS and reactive nitrogen species (RNS) and anti-oxidant

responses.[49] As such, the role of obesity in overproduction of ROS is consistent in the literature.[167] Obese individuals present an exacerbated inflammatory response facing microbial threat that leads to an exaggerated production of ROS, confirmed by a significant, positive correlation between oxidative markers and GI, PD, and CAL.[87,124,149,154] Most interestingly, increased circulating ROS may induce gingival oxidative stress and potentiate the onset and/or progression of obesity-induced gingival inflammation.[154] Studies tracking the most relevant oxidative markers in obese subjects presenting different levels of periodontal disease show that MDA, MPO, protein carbonyl, and 8-OhdG levels are significantly increased both systemically (serum) and locally (gingival crevicular fluid).[87,149] Local markers for lipid peroxidation (MDA) and for protein carbonylation are found to be higher in obese versus normal weight individuals regardless of their periodontal status, whereas their total antioxidant capacity (TAOC) is found diminished, indicating that increased BMI might act as a periodontitis modifying factor.[87] Interestingly, in a prospective clinical study evaluating tooth alignment in obese and normal-weight orthodontic patients and their markers for oral inflammation and hormone activity, myeloperoxidase (MPO), resistin and leptin levels were found higher in obese GCF samples.[168] Their findings agree with other conclusions.[169] The presence of these markers in periodontal samples, even in the absence of periodontal infection, could be interpreted as indicative of a bidirectional intimate connection between obesity and periodontitis. On one side, those adipokines excessively secreted into the blood stream in obese patients help in the establishment of an inflammatory state causing overproduction of oxide end-products within periodontal tissues. Concomitantly, periodontal infection releases a wide range of pro-inflammatory cytokines, contributing for the manifestation of other chronic diseases, such as obesity.[139]

Therefore, obese individuals are statistically more susceptible to develop periodontal disease through a continuous inflammatory state and a hyper-oxidative environment that negatively influence the immune response facing periodontal pathogens. Once the destruction of the tooth supporting tissues begins, a wide range of pro-inflammatory cytokines is released into the blood stream contributing to the expansion of both inflammatory conditions.

2.4. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that leads to joint swelling, joint tenderness, synovial inflammation and subsequent destruction of cartilage and bone, leading to severe disability and premature mortality.[170] The combination of genetic

and environmental factors, such as smoking and alcohol intake, can increase the likelihood of its development.[50,171,172] Even though some authors state that its etiology and pathogenesis still remain unknown,[173] there is some evidence suggesting that genetic risk factor associated with environmental triggers can induce molecular changes to host proteins leading to loss of immune tolerance through protein citrullination.[172,174]

RA and periodontitis display various pathogenic similarities. These include parallels in relation to the dysregulation of host immune response leading to soft tissue inflammation with subsequent hard tissue destruction. There are even shared risk factors, including smoking and excess body weight or obesity.[2] Additionally, early studies indicate that patients with RA may have a higher incidence of periodontal disease and *vice versa*, and the possibility exists that both conditions result from common underlying pathologic features, resulting in a strong association between both.[2,36] Moreover, both diseases have common aspects in terms of the pattern of soft and hard tissue destruction. While rheumatoid arthritis is responsible for inflammation of the synovial fluid and destruction of the joints, periodontitis causes inflammation of the periodontal tissues and bone loss.[175]

Interestingly, although RA is not considered an infectious disease, it has been shown that oral bacteria strongly associated with periodontitis, such as *P. gingivalis* and *A. actinomycetemcomitans* can be found in the serum of patients with RA. It has been postulated that these microbes could contribute to chronic and more generalized inflammation including the generation of autoantibodies that might then trigger RA. Also, the production of deamination enzymes by *P. gingivalis* causes the citrullination of proteins, further inducing autoantibody formation, which is reported as a link between periodontal infection and the development or progression of RA.[36]

Patients presenting RA have been compared with non-RA patients for the assessment of their periodontal status. Clinical attachment loss and probing depth have been the most evaluated periodontal measures used for this comparison with reports of RA patients presenting significantly deeper probing depths and 4.28 more chances of having periodontitis.[175] Kaur and colleagues,[36] in a systematic review of the literature, found 10 studies that made this comparison. Seventy percent of them showed a statistical difference between CAL in non-RA and RA patients with periodontal disease.[36] The analyses also have indicated an increased tooth loss associated with RA patients when compared against their non-RA counterparts. Some of the studies included in this review showed not just a statistically significant difference but RA patients presented with two times more CAL and almost double chance of showing CAL > 5mm in comparison with non-RA patients.[176-179] To

further highlight their relationship, experimental studies assessing the effects of periodontal treatment on biochemical markers for RA have shown a statistically significant improvement for erythrocyte sedimentation rate (ESR), C-reactive protein, and DAS28 (RA activity marker) in RA patients.[180,181] Their findings are in accordance with another study and a systematic review.[170,175,182] Even though some of those studies had relatively small samples, they may represent a significant indicatory trend in terms of a possible RA-periodontitis relationship and, most importantly, RA additional treatment tools.

The profile of inflammatory cytokines seen in periodontitis is quite similar to that found in RA. Specifically, there are persistently high levels of pro-inflammatory cytokines, including interleukin (IL)-1β, matrix metalloproteinase-8 (MMP-8), and tumour necrosis factor (TNF)-α, and low levels of cytokines which suppress the immunoinflammatory response, such as IL-10 and transforming growth factor (TGF)-β. Both conditions manifest as a result of an imbalance between pro-inflammatory and anti-inflammatory cytokines.[22] Additionally, there is strong evidence for a correlation between increased levels of IL-1β and the presence of periodontal disease and rheumatoid arthritis,[36] and also showing that salivary levels of IL-1β, TNF-α, and MMP-8 levels are influenced by the disease,[183] which represent the various stages of progression of inflammatory response in both conditions.[184]

Furthermore, RA and periodontal disease share common molecular pathways within the RANK/OPG/TRAIL axis, leading to osteoclast differentiation and bone resorption.[36] When secreted by activated T cells within the inflamed joints, receptor activator of nuclear factor κ-β ligand (RANKL) is responsible for mediating the joint destruction in patients with rheumatoid arthritis.[11,23,185] Similarly, in periodontitis, periodontal bone loss is highly dependent on the existence and stimulation of osteoclasts, which are regulated by the balance between RANKL and osteoprotegerin (OGP),[12] with evidence showing a negative correlation between OPG ratio and periodontal disease.[186]

Despite the pro-inflammatory similarities between RA and periodontitis, both diseases also share comparable oxidative stress parameters even though literature on local and systemic oxidant levels in their combination is still scarce.[50,187] While hyperactive peripheral blood PMNs in periodontal patients produce higher amounts of ROS systemically and locally, neutrophil respiratory burst also occurs in the joints and synovial fluids of RA patients and accounts for an excessive production of a wide range of ROS.[188] As an example, MPO plasma concentrations, lipid (LPO) and protein peroxidation markers are found significantly higher in RA patients in comparison with healthy controls and are reported to play important roles in

the pathogenesis of RA.[188,189] Similarly, oxidative damage is accounted for periodontal destruction and progression through lipid, protein and DNA damage, as well as reduction of the physiological antioxidant defenses.[17,45] Interestingly, LPO has been implicated in several conditions interconnections, such as RA and periodontal diseases.[190] On a similar line, the oxidative stress index (OSI) for RA periodontally compromised (RA-CP) patients is reported as significantly higher in comparison with patients presenting chronic periodontal disease only, suggesting that the combined effect of RA and periodontitis significantly increased oxidative stress and its destructive consequences.[187]

In spite of the still lacking well-controlled and representative clinical studies evaluating the periodontal consequences of RA and vice-versa, in terms of their oxidative patterns, the role played by the imbalance between the increased presence of free radicals and the host's incapability to protect against peroxidation mechanisms is well described in the current literature for the two conditions separately. As two destructive chronic inflammatory conditions that raise questions whether one is the consequence or trigger of the other, it seems quite possible that novel techniques and methods of comparison between RA and periodontitis will enable clarification of the mechanisms that link their pathogenesis to not just separate entities but to a common condition with oxidative damage in its central axis.

2.5. Concluding remarks

As discussed above, chronic periodontal disease is highly prevalent in patients presenting T2DM, cigarette smoking habits, obesity and/or rheumatoid arthritis. The literature is consistent in reports on how the diseases/conditions aforementioned are not only associated with more severe periodontitis but how they likely have their effects on account of their ability to upregulate oxidative stress both locally and systemically, which results in a sustained redox imbalance that favours disease progression. As such, oxidative stress as a therapeutic target for management of periodontitis will be discussed in the following section presenting three antioxidant compounds, resveratrol, resveratrol derivative-rich Melinjo seed extract (MSE) and curcumin as adjunctive tools for periodontal treatment.

3. Oxidative Stress as a Therapeutic Target for Management of Periodontitis.

3.1. Resveratrol and MSE

Resveratrol (trans-3,40,-5-trihydroxystilbeneis), a plant-derived polyphenolic compound found in the skin of dark-colored grapes, red wine, berries, and peanuts,[191,192] is a natural compound with anti-inflammatory properties.[193] There are reports also suggesting its anti-cancer, cardioprotective and vasoprotective effects,[194] as well as improvement of T2DM control and RA treatment.[11,195] Additionally, the plant polyphenol has been described as an antioxidant itself, directly acting against ROS overproduction and in the reestablishment of the redox balance.[12] Resveratrol, which is found in two isoforms, *trans*-resveratrol and *cis*-resveratrol,[196] is composed of two phenolic rings that are connected by a double bond and, as a natural compound, has been considered as an alternative to synthetic drugs due to the virtual absence of side effects.[194] Melinjo seed extract (MSE) is one of the sources of resveratrol and presents variants of the compound, such as *trans*-resveratrol, gnetin C, gnemonoside A and D.[12] In the following section, the effects of resveratrol and resveratrol derivative-rich MSE on chronic periodontal disease and its destructive consequences will be addressed. Additionally, the main mechanisms through which resveratrol acts to prevent, control and heal periodontal tissue destruction will be discussed.

Host response modulation

There are many different mechanisms through which resveratrol acts to control, prevent and reverse the destructive progression of inflammatory conditions, such as periodontal disease. One of these mechanisms is the capacity to modulate host response facing an exacerbated inflammation setting. The stimulation of an oxidative stressed environment through the exposure of hydrogen peroxide to human gingival fibroblasts (HGFs) cultures was performed to evaluate the effects of resveratrol on the control of ROS production, mitochondrial respiratory capacity, and type 1 collagen synthesis. Resveratrol inhibited most effectively free radicals with a longer incubation period in comparison with the other two tested antioxidants and the mitochondrial respiratory modulation induced by the polyphenol was more pronounced. Most interestingly, type 1 collagen mRNA expression was significantly upregulated when resveratrol was administered.[198] Conversely, using HGFs to analyze the protective role of resveratrol in rats, the induction of different inflammatory factors, such as MMP-2 and -9, was strongly reduced in the presence of the compound even when treated with LPS.[194] Their findings agree with other reports that also suggest antioxidants may play a role in biological functions and in both soft and hard tissue turnover during periodontitis induced oxidative stress.[199,200] On the same line, Rizzo and colleagues,[191]

using extracted teeth for orthodontic purposes, stimulated human periodontal ligament cells (HPLCs) with *P. gingivalis* LPS in order to simulate periodontal infection. They treated the cultures with resveratrol in different concentrations (25, 50 and 100μM), assessing nitric oxide (NO) levels and pro-inflammatory cytokines response to the administration. The inhibition of NO production in stimulated HPLCs showed a concentration-dependent gradient. Most interestingly, secretion of IL-1β, IL-6, IL-8, IL-12 and TNF-α significantly decreased in comparison with the control group, irrespective of resveratrol concentration.[191] Conversely, Chin et al.[200,201] demonstrated a similar disease control pattern using a resveratrol derivative (THSG) and a compatible trend was observed for experimental periodontitis in diabetic rats.[202] In addition, He and colleagues[203] concluded resveratrol prevents RANKL-induced osteoclastogenesis through the inhibition of ROS.[203]In another experimental study, a significant reduction of IL-17 levels was promoted by resveratrol. A ligature-induced periodontitis model in rats was used with daily administration of resveratrol at a dose of 10mg/kg diluted in water for 30 days.[204] The microbiological analysis of the ligatures used in this study evaluated the impact of resveratrol on the bacterial load of species related to the periodontium (*P. gingivalis, T. forsythia and A. actinomycetemcomitans*). It has been demonstrated that resveratrol does not promote benefits for microbiological outcomes of an experimental model of periodontitis, which reinforces its role in modulating host response.[205] Using a similar experimental model and the same daily administration, Correa and colleagues[11] combined resveratrol with another antioxidant, curcumin, to assess their possible effects on gingival tissues cytokine levels and bone loss. The ligated and unligated sides showed significant reductions in IL-1α levels when the plant-derived anti-oxidants were administered.[11] Their results are in accordance with other reports.[12,94] Thus, modulation of cytokine levels and ROS within periodontal tissues may represent possible mechanisms by which resveratrol acts on the host response thereby leading to control of initiation and advancement of periodontal disease.[11,204]

Nuclear Factor erythroid 2 (Nrf2) - related pathway

Another important antioxidant mechanism triggered by resveratrol is the activation of the master regulator of antioxidants, nuclear factor erythroid 2-related factor 2 (Nrf2), which attenuates osteoclastogenesis,[206] modulates intracellular ROS,[207] inhibits periodontal ligament cell apoptosis[208] and is downregulated in PMNs derived from patients with chronic periodontitis.[209] Nrf2 is directly responsible for antioxidant defenses and resistance to

oxidative stress,[12,194] demonstrating a protective role.[26] The role of Nrf2 levels in periodontal disease has been investigated over the years,[26,117,210] while the positive effects of resveratrol on Nrf2 levels in different tissues[211-213] has also been investigated. However, the effects of resveratrol on the Nrf2 pathway with respect to periodontitis more specifically has yet to be addressed in detail. Along these lines though, Ikeda and colleagues[12] used immunohistochemistry to assess the effects of MSE-derived resveratrol (mainly a source of the resveratrol dimer; gnetin-c) administration on levels of Nrf2 protein in an experimental periodontitis model in rats. Higher levels of immunostaining for Nrf2 were demonstrated clearly in the tissue samples taken from the animals treated with MSE. This suggested that MSE (and by extension resveratrol and/or the resveratrol dimer) activated the Nrf2 pathway and led to downregulation of oxidative stress. Moreover, the authors also suggested that through interaction with the aryl hydrocarbon receptor (for which resveratrol is an antagonist), not only was the production of ROS reduced, but any ROS that were produced were subsequently neutralized by resveratrol or the resveratrol dimer as well as NrF2 protein-mediated reduction of 8-OHdG.[12] Their findings agree with the results reported by others using similar methodological approaches.[214] Specifically; in both studies, in addition to Nrf2 activation, the levels of 8-OHdG were found significantly reduced, both locally[12] and systemically,[214] in the presence of resveratrol. Different reports also showed that the sirtuin 1 (Sirt1)/AMP-activated protein kinase (AMPK) (Sirt1/AMPK) pathway was triggered by resveratrol.[200,214] The sirt1/AMPK pathway has important anti-inflammatory effects, modulates NF-κB activity and suppresses oxidative stress, and might represent another defense pathway induced by the administration of resveratrol or resveratrol dimer. [215-216]

The roles played by Nrf2 pathway, such as the inhibition of fibroblast apoptosis and osteoclastogenesis, as well as the scavenging of ROS, have unprecedented and beneficial clinical implications in relation to diseases that are mediated by oxidative stress and in this case periodontitis. Therefore, the effects of resveratrol may suggest that this molecule, and similar derivatives, can be utilized as a novel adjunctive tool in the management of chronic periodontitis, given the demonstrated capacity of protection against periodontitis-mediated damage and disease progression.[194]

Osteoclastogenesis, osteoblast proliferation, AhR, RANKL, and bone loss

Given that one of the major structures of the periodontium is bone, anything that has a beneficial effect on bone-homeostasis and bone cell (osteoblast) health should also be

beneficial in management of periodontitis. In relation to this, various protective effects of resveratrol on bone metabolism have been reported in the literature,[193,217] as well as in studies performed in vitro (see below).[90,94] Several studies have investigated their effects in a ligature-induced periodontitis model, an experimental model that effectively induces alveolar bone loss in rats.[214] Using sutures placed around molars, periodontal disease is induced during a specific period of time, usually ranging from 15 – 30 days, and then is either removed, which is considered "conventional treatment", or left in place in the presence or absence of the drug aimed to be tested. Usually, the contralateral teeth are used as controls in a split-mouth design.

Tamaki[214] divided eighteen male Wistar rats into three groups as described earlier. The rats were given resveratrol solutions at a dose of 10mg/kg body weight and were sacrificed after twenty days of ligature-induced periodontitis (and the ligatures were not removed, meaning that the causative factors were still present; tantamount to untreated disease). Using micro-CT scan analysis, it was demonstrated that there was decreased periodontal bone loss in the periodontitis + resveratrol (P + RESV) as compared to the group that was not treated with resveratrol (P).[214]Thus, even in the absence of "treatment", resveratrol *prevented* periodontal disease progression. Similar results were also reported by others using morphometric measurements of alveolar bone loss with standardized photographs.[11,204] In addition, resveratrol modulated the production of osteoclastogenesis-related factors as shown by significantly decreased IL-17 levels in rat gingival tissues.[204] Most interestingly, resveratrol antioxidant capacity was demonstrated by significantly reduced levels of 8-OhdG in urine in the P + RESV group as compared to the P group, likely through the activation of Nrf2/antioxidant defense pathway.[214] These findings agree with other authors.[12,210,211] Bhattarai and colleagues[194] used a similar design with a daily subcutaneous injection of resveratrol (5mg/kg concentration) combined with lipopolysaccharide (1ml/mg concentration) administrated three times per week in male Sprague-Dawley rats. Using micro-CT scans, decreased bone mineral density and bone volume found in the LPS group were restored in the presence of resveratrol which also significantly reduced alveolar bone loss ($p < 0.05$) and inhibited osteoclastogenesis in comparable levels to the controls. In addition to osteoclastogenesis inhibition, Ornstrup and colleagues[193] reported that with resveratrol, osteoblast cell differentiation was increased as demonstrated by increased levels of alkaline phosphatase (ALP) and OPG, which are both used as biomarkers for osteoblast cell differentiation, in mesenchymal stem cells, even in the presence of LPS.[193] This agreed with Tamaki et al.,[214] whereby oxidative stress parameters were also positively influenced (i.e.

reduced) by resveratrol as serum antioxidant SOD activity was found to be increased when the compound was applied.[194] Those findings suggest resveratrol may modulate host response by controlling the redox state in periodontitis.

A recent study also demonstrated[12] that not only does resveratrol appear to prevent the initiation and progression of alveolar bone loss caused by periodontitis but also has the capacity to actually *reverse* the loss of alveolar bone once the disease has been established, and that this occurs even when the triggering factors (e.g. the silk ligatures) have not been removed. This healing of bone loss or regeneration of periodontal tissue occurs even when the triggering ligatures are still in place suggesting a rather powerful effect given that by leaving in the ligatures this essentially represents the equivalent of periodontitis having not been treated from the perspective of debridement.[214] To reiterate, even with the ligatures still in place, periodontal bone regeneration was observed.[12] This finding, though in accordance with previous findings suggesting resveratrol positively affects periodontal tissues, might be considered the first report showing that resveratrol can mediate actual regeneration and healing of periodontal lesions as opposed to solely inhibiting initiation and progression of periodontal lesion formation. Again, this might relate to the fact that resveratrol has been reported to upregulate expression of proteins that induce osteodifferentiation and osteoblast cell activity such as bone morphogenetic protein (BMP)-2, BMP-7 and osteopontin (OPN). Having said this, other studies have not necessarily replicated the aforementioned findings as regards BMP-2.[192] Nonetheless, in the study by Casarin and colleagues,[197] calvarial defects were created for bone remodeling assessment in rats and for investigation of resveratrol effects on biomechanical retention of implants placed in the tibial bone. They suggested resveratrol stimulated the early phases of ossification and bone maturation as the treatment group showed higher removal torque force in comparison with the controls.[197] In another study,[218] occlusal trauma was induced on maxillary 1st molars in mice by overlaying composite resin onto their occlusal surfaces. Inhibited expression of RANKL was demonstrated in mice treated with resveratrol leading to decreased loss of bone as compared to the control group.[218]

Using a chick periosteal osteogenesis (CPO) model and the rat bone marrow stromal cell model (RBMC), Singh[90] demonstrated that TCDD (dioxin), a prototypical AhR ligand and agonist, that is analogous to aryl hydrocarbons found in cigarette smoke, inhibited osteodifferentiation and therefore bone formation *in vitro*. Using these model systems, different concentrations of resveratrol were added to the cultures treated with TCDD, demonstrating that the negative effects of TCDD could be blocked with the former agent. This

was demonstrated by assessing the levels of biomarkers for osteogenesis biomarkers, such as ALP, OPN and bone sialoprotein (BSP) in the presence and absence of TCDD. As expected, TCDD-mediated inhibition of osteodifferentiation was reversed by resveratrol in both *in vitro* model systems. And since the model systems relied on cells derived from wholly unrelated species, the findings observed with resveratrol and TCDD (as well as other smoke related hydrocarbons studied in other experiments) could be given significant credence as representing generally expected biological effects.[90] On the same lines, Andreou and colleagues[94] used a rat bone marrow cell (RBMC) osteogenesis model to test another AhR ligand (benzo[a]pyrene (BaP); this aryl hydrocarbon actually being a component of cigarette smoke) in combination with LPS derived from *P. gingivalis* on osteogenesis also with and without resveratrol. In this case, osteogenesis was assessed using several approaches including enzymatic, molecular and electrophoretic methods as well as for the formation of bone nodules that stain red when using Alizarin Red. The additive inhibitory effects of BaP + LPS on nodule bone formation were confirmed. And it was also demonstrated, depending on how concentrated was the LPS exposure the deleterious effects of BaP + LPS were attenuated partially or completely by the addition of resveratrol. The authors concluded that in addition to the fact that resveratrol antagonizes AhR activation it also demonstrated direct anti-inflammatory effects.[94]

Another study performed in male Wistar rats assessed the effects of resveratrol in combination with smoke inhalation (SMK + RESV) on the repair of critical-sized bone defects in calvarial bone.[192] Histomorphometric analysis showed no statistically significant difference between the SMK + RESV group and controls. These findings could fit with the notion that the effects of resveratrol, as shown in the periosteal osteogenesis model, could be most profound during osteodifferentiation. Hence, if osteodifferentiation is not a requirement of healing, resveratrol effects would be limited. This said, it was still demonstrated that mRNA levels of RANKL/OPG were significantly lower in the resveratrol + smoke inhalation group as compared to those exposed to smoking inhalation and placebo ($p = 0.017$).[192] Their results agree with the findings from another recent study that used a similar model and showed upregulated levels of SIRT1 and SOD activity, as well as reduced alveolar bone loss and NADPH oxidase levels in the group exposed to daily cigarette smoke inhalation and resveratrol administration.[219] The authors suggest resveratrol might be an additional tool in periodontal treatment, especially in smokers. The same group showed reduction in periodontitis and rheumatoid arthritis progression using a rat model.[219] Periodontitis was ligature-induced, and RA was induced by immunizations through injections in the tail, fist,

paw, knee joint, and subcutaneously. Their findings suggest resveratrol modulates serum levels of rheumatoid factor (RF) and anti-citrullinated protein antibody, which may give resveratrol effects on disease severity and progression.[219] Orally, morphometric analysis showed a significant higher bone loss in the placebo group in comparison with the two other drugs tested (resveratrol and ibuprofen). Interestingly, resveratrol presented no difference in bone loss when compared with ibuprofen. These findings indicate resveratrol might be used to modulate periodontal destruction and articular damage with no related side effects. Human studies testing the effects of resveratrol on periodontal disease are still lacking. Even still, in a randomized double-blind, placebo-controlled, clinical trial, T2DM patients presenting chronic periodontitis were divided into intervention (480mg/day of resveratrol for 4 weeks) and control (placebo) groups. Non-surgical periodontal therapy was performed in both groups. The mean serum levels of fasting insulin and insulin resistance were found significantly lower in the intervention group *versus* control group, as well as mean pocket depth (2.35 ± 0.6 and 3.38 ± 0.5, for intervention and control groups, respectively).[195] The authors suggest resveratrol supplementation might improve insulin resistance and control periodontal disease activity. As systemic resveratrol presents poor oral bioavailability, Kassem and colleagues[220] developed resveratrol-containing microbeads for local treatment of periodontitis. The formula presented by this research group showed strong mucoadhesion and a slow rate of resveratrol release. The authors indicated these microbeads as candidates for a locally adjunctive treatment modality for higher intrasulcular drug concentration and no systemic side effects.[220] Even though there is consistent data showing no side effects with systemically administered resveratrol.[194]

3.2. Curcumin

Curcumin, a plant-derived compound isolated from dried rhizomes of *Curcuma longa*,[221] is usually used as dietary spice. As the major active compound present in the roots of the turmeric plant, natural curcumin is an extended pseudosymmetric polyphenol (diferuloylmethane) composed of the mixture of three curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin),[222] and has been increasingly investigated due to its potent anti-inflammatory, anti-microbial, anti-cancer, and most importantly its antioxidant properties.[223] Curcumin administration has also been associated with beneficial effects on different tissues, such as skin,[224] lungs,[225] and liver[226] with as yet few to no side effects.[227]

Therefore, experimental periodontitis models have been used to assess the effects of curcumin on periodontal tissues, as they effectively develop periodontal destruction as has been done with the other antioxidant resveratrol.[228] Using the experimental ligature induced periodontitis model in rats as discussed above, has also been induced/modified by the addition of LPS injections or induction of diabetes (or more accurately hyperglycaemia). Because natural curcumin presents relatively poor pharmacological properties, such as poor bioavailability, high insolubility in water, and short half-life in plasma,[229] several studies have compared natural curcumin with their chemically modified analogues or 'Chemically Modified Curcumins' (CMCs), which present better chemical characteristics.[222] As such, studies have been done to study the effects of curcumin or CMCs in relation to whether these compounds might also alter the progression and/or initiation of periodontal disease.

Inhibition of NF-κB activation pathway and host modulation

Activation of the transcription factor nuclear factor kappa B (NF-κB) is associated with a hyper-inflammatory state and expression of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α,[229] osteoclastogenesis markers, such as RANKL[227], MMP activity[230] and ROS overproduction.[231] Even though there are other signaling pathways curcumin depends on,[223]inhibition of the NF-kB pathway activation is considered one of the main mechanisms through which the natural compound acts to prevent and control matrix-degrading enzymes activity,[229] RANKL-mediated bone resorption,[227] and exacerbated free radicals release,[230] all of which have a role to play in periodontal disease destruction.

Modulation of the immune response through the reduction of pro-inflammatory cytokines was reported in an LPS-induced experimental periodontitis study in rats given daily doses of natural curcumin in two concentrations (30 and 100mg/kg) via oral gavage. Stereometric analysis showed significant reduction in inflammatory infiltrate, as increased collagen content was observed in rats given both curcumin concentrations.[232] On a similar line, Hu and colleagues[233] stimulated human gingival fibroblasts (HGFs) with *P. gingivalis* LPS and treated the cultures with curcumin. Pre-treatment with curcumin resulted in NF-κB pathway inhibition and, consequently, downregulation of tissue-destructive mediators through the attenuation of LPS-stimulated cycloxygenase-2 (COX-2) expression.[233] Brandão and colleagues[231] showed a positive, but a lack of a dose-dependent effects of CMCs on bone resorption, osteoclastogenesis and TNF-α.[231] However, the results from Hu showed dose-

dependent effects on HGFs[233], which agrees with the findings from other research groups that showed curcumin produced a marked, dose-dependent inhibition of NF-κB.[223,229] Along the same line, two additional recent studies also illustrated a positive effect of curcumin on NF-κB inhibition, considering it the main mechanism by which curcumin acts in tissue healing.[11,232]

Elburki and colleagues[229] tested the effects of curcumin administration on LPS-induced periodontitis (repeated injections from LPS *Escherichia coli*) and diabetes-associated periodontitis (intravenous tail injections of streptozotocin).[229] Parenthetically, it should be emphasized though that the induction of hyperglycaemia by way of injection of streptozotocin does not actually produce a model for type 2 diabetes, although the latter term; diabetes, will be used for the sake of convenience from time to time. Oral administration of CMC 2.24 was performed in hyperglycaemic animals. Significant reduction in bone loss was observed in both LPS-induced and diabetes-associated periodontitis models, 22.3% and 24.4%, respectively, with the administration of the curcumin analog. Additionally, marked reductions in IL-1β (50%), IL-6 (50%), and TNF-α (70%) levels were recorded in the LPS-induced group, IL-1β "normal" levels were achieved in the diabetes-associated periodontitis group, and inhibited NF-κB activation levels were comparable to controls in both models after CMC2.24 administration. The authors concluded that CMC 2.24 controls both locally induced and systemically modified periodontal disease.[221,229] Conversely, the combination of LPS-induced and diabetes-associated periodontitis was positively influenced by CMC 2.24 in terms of IL-1β and IL-6 levels, MMP-2, -8, and -9, and bone loss levels. Even though CMCs did not induce significant effects on connective tissue turnover, the authors suggested they might present beneficial effects on breakdown of collagen and probably bone, thereby having the potential for use in the treatment of periodontal disease.[229]

Alveolar bone loss prevention and osteoclastogenesis inhibition

Utilizing an LPS-induced periodontitis model in rats, natural curcumin and CMC 2.24 were compared for osteoclast-mediated bone resorption, apoptosis, and inflammation. Both curcumin compounds showed improved inflammation modulation results, as a significant reduction of inflammatory cell infiltrate was observed. Interestingly, CMC 2.24, but not natural curcumin, reduced alveolar bone loss (using microcomputer tomograph analysis) and osteoclastogenesis.[222] Similar results were found in another study using ligature-induced

periodontitis in rats that were administered natural curcumin. Even though curcumin administration was not associated with alveolar bone loss prevention through microcomputer tomograph analysis, the compound suppressed inflammation, increased collagen content and fibroblastic cells proliferation, and effectively inhibited IL-6 and TNF-α gene expression in periodontal tissues.[223] Thus, the beneficial effects of natural curcumin seem to be limited to host response modulation, as the pro-inflammatory cytokine profile demonstrates a benefit from the administration, whereas "clinical" results, represented by alveolar bone levels, did not show improvement in a ligature-induced periodontitis model. These results are partially contradicted by Bakir,[234] who tested the "prophylactic/preventive" effects of curcumin. Intragastric gavage curcumin administration was tested in ligature-induced periodontitis rats and alveolar bone loss was compared with periodontally healthy and compromised groups. The combination curcumin + periodontitis presented significantly lower alveolar bone loss than non-treated periodontitis ($p < 0.0125$). However, curcumin was not capable of decreasing alveolar bone loss in comparable levels to the healthy controls.[234] This difference might have resulted from differences in the measurement technique between studies (standardized photographs *versus* micro-CT scans).

Different studies present heterogeneous methods, such as discrepancies in drug presentations, concentrations, type of administration and data interpretation; thus, comparisons should be taken carefully. As such, a recent study on the effects of curcumin and piperine, a pepper derivative with putative positive effects on curcumin bioavailability, on experimental periodontitis in rats was conducted.[232] Besides the beneficial effects on NF-□B inhibition, cellular infiltrate, and collagen content, curcumin treated sites showed significantly increased bone neoformation using micro-CT analysis, irrespectively of the combination with piperine. The authors concluded curcumin augmented alveolar bone repair.[232] Similarly, curcumin and piperine combined suppressed osteoclastogenesis in vitro in periodontal ligament cells.[227] Conversely, another possible synergistic effect was tested (curcumin and resveratrol combined) by other authors. In a ligature-induced periodontitis model in rats, morphometric measurements of alveolar bone loss showed no statistical difference between curcumin, resveratrol and both drugs combined, even without "periodontal treatment", represented by ligature removal.[11] Given the protective role played by resveratrol in bone metabolism, as previously described, similar positive results presented by curcumin undoubtedly indicate the compound as a promising alternative for periodontitis management. Even though three different human studies have reported on positive additive effects of a 0.2% loaded curcumin strip,[235] a 1% curcumin gel[236] and the combination of curcumin with

1% ornidazole gel[237] on periodontal parameters after scaling and root planning, the sample size and study design raise questions about the validity and reliability of the results. Thus, the current literature is still lacking well controlled human trials on the subject.

Finally, Zambrano and colleagues[238] tested the viability and biological effect of local administration of curcumin in a nanoparticle formula (nanocurcurmin). Three microliters of nanocurcumin were administrated twice a week in rats given LPS (periodontitis) or PBS (control) injections. After nanocurcumin treatment, a marked reduction in NF-κB activation levels was observed. In addition, the number of osteoclasts in sections from the hemi-maxillae treated with LPS/nanocurcumin did not differ from the PBS-injected group (control). Furthermore, the bone volume/tissue volume ratio showed no statistical difference when LPS-injected/nanocurcumin was compared with PBS-injected group. Thus, bone resorption was attenuated by curcumin administration. Finally, the experimental periodontitis model effectively increased PMN counting that was markedly diminished after local curcumin administration twice a week. No statistical difference was found in PMN counting between LPS/nanocurcumin and controls. The authors refer to topical administration of nanocurcumin as a non-invasive, effective adjunctive tool in the conventional treatment of periodontitis, with virtual absence of side effects.[238] Recently, Chauhan and colleagues[238] developed a curcumin loaded biodegradable crosslinked gelatin film for curcumin delivery into the periodontal pockets. The film was able to effectively release curcumin up to 7 days.[238] Similarly, mucoadhesive films containing curcumin-loaded nanoparticles showed 80% of swelling in oral cavity.[239] Swelling is an important characteristic as water absorption creates a network within the drug delivery system, entraps the drug of interest and slowly release it.[240] It is possible that the only trustworthy human trial available is the one conducted by Nasra and colleagues[241] with a sample size of twenty subjects. They inserted a 2% curcumin-containing gel into the periodontal pockets of periodontitis patients combined with non-surgical periodontal therapy. The control group comprised periodontitis patients conventionally treated. Both groups showed significant reductions in PD, BoP and PI ($p<0.05$). The experimental groups showed higher yet nonsignificant reductions in the assessed parameters attributed to the controlled curcumin release for prolonged duration.[241]

3.3. Final Conclusions

Resveratrol and curcumin, as well as their derivatives, represent an important step in the development of new drugs that act in conjunction with conventional techniques for

periodontal disease treatment. The studies surveyed have suggested the two natural compounds as possible candidates in alternative periodontal therapies. Initially, both compounds prevented an exacerbated inflammatory setting that is characteristic of chronic periodontal disease. Moreover, they also yielded effects on the control of established periodontal infection. Furthermore, in the case of resveratrol, the polyphenol was described as capable of reversing the destructive effects of periodontitis. Thus, even though additional pharmacological tests are still to be conducted for both agents, the evolution of the methods for their delivery (systemic and even local) should produce new paradigms in the management of periodontal diseases that transcend infection control alone (i.e. by surgical or non-surgical debridement) in the near future. Current evidence indicates that oxidation plays a significant role in many human diseases, including periodontitis. Antioxidants and upregulation of NrF2-associated antioxidant and detoxification enzymes enhance cytoprotective effects by decreasing inflammation downstream of oxidative tissue damage.[28,209] Accordingly, therapies that increase antioxidants and/or antioxidant activity may be viable additions to current approaches related to both the prevention and treatment of periodontitis, as well as other diseases of oxidative stress.

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DISCUSSÃO - ARTIGO 2

O estabelecimento de um ambiente sistêmico caracterizado pelo estresse oxidativo pode causar uma condição pró-inflamatória exagerada que é o centro de uma ampla gama de distúrbios metabólicosqueapresentam padrões destrutivos como sua consequência.[46] Esses distúrbios multifatoriais são complexos (que incluem diabetes, obesidade e artrite reumatóide)[47-51]e compartilham as espécies reativas de oxigênio e seu subsequente desequilíbrio antioxidante como uma característica comum para seu desenvolvimento e progressão.[52,53] Assim, estabelecer uma conexão entre esse grupo de doenças, sua correlação com a doença periodontal grave e suas semelhanças pró-inflamatórias é de grande valor.

Diabetes Mellitus tipo 2 (DM tipo 2)

Dentro do amplo espectro de condições secundárias relacionadas a Diabetes Mellitus tipo 2, a doença periodontal crônica é considerada a sexta complicação diabética, desempenhando um papel bidirecional com o distúrbio metabólico crônico.[54] A relação entre níveis glicêmicos alterados e periodontite é amplamente estabelecida na literatura.[44,55,56] Ambas são doenças crônicas e inflamatórias que compartilham fatores de risco comuns e interagem mutuamente entre si, apresentando aumento do estresse oxidativo e liberação exacerbada de mediadores pró-inflamatórios.[56,57]Parâmetros como a hemoglobina glicada (HbA1C) correlacionam-se positivamente com biomarcadores inflamatórios orais e atividade polimorfonuclear, progressão da doença e probabilidade de desenvolver periodontite.[58-63] De um lado, os mecanismos de defesa imunológica dos pacientes sistemicamente afetados por DM tipo 2 não conseguem agir contra a agressão microbiana, particularmente devido a um perfil bacteriano mais patogênico nesses sujeitos, colapsando os tecidos circundantes dos dentes.[60] Por outro lado, os parâmetros da doença periodontal, como profundidade de sondagem (PD), perda de inserção clínica (CAL), sangramento à sondagem (BoP) e índice de sangramento gengival (ISG) são afetados negativamente pela hiperglicemia crônica e pela disfunção das células ß.[64] Rovai et al.,[65] em revisão sistemática da literatura, demonstrou que o tratamento periodontal não-cirúrgico de pacientes com DM tipo 1 e DM tipo 2 melhorou significativamente o CAL e reduziu os níveis de doença periodontal.[65] Além disso, a relação mútua entre periodontite e DM tipo 2 é destacada em diferentes relatórios concluindo que o tratamento periodontal resulta em uma resposta positiva em níveis glicêmicos previamente

aumentados e em níveis de estresse oxidativo sistêmico em pacientes diabéticos, concluindo quea resistência à insulina e a função de células ß alteradas podem predizer a progressão e severidade da doença periodontal crônica.[59-63]

Um grupo representativo de mecanismos estimuladores do estresse oxidativo e marcadores antioxidantes foi analisado e proposto como desempenhando papéis importantes na patogênese do DM tipo 2 e em sua interação com a doença periodontal. Avaliando produtos finais de oxidação de proteínas, de DNA, ou delipídios, marcadores antioxidantes ou mecanismos antioxidantes enzimáticos, através da utilização diferentes métodos de análise, a literatura corrente tem estabelecida uma ligação consistente entre ambas as desordens em termos de produção de espécies reativas de oxigênio e seus caminhos oxidativos.[59,61-66]

Tabagismo

Embora tenham sido atribuídos diferentes mecanismos pelos quais o fumo afeta a progressão da periodontite, uma teoria definitiva que explica esse processo ainda não está clara. Um dos mecanismos sugere uma mudança na composição da microbiota para uma altamente patogênica dentro dos tecidos periodontais. O tabagismo também tem sido relacionado a uma alteração na migração de neutrófilos e disfunção de sua quimiotaxia, levando a uma resposta imunológica deficiente frente à ameaça microbiana. Além disso, o hábito de fumar pode levar a uma mudança na atividade dos neutrófilos para um estado mais hiperativo, o que aumenta a liberação de citocinas pró-inflamatórias e a produção de espécies reativas de oxigênio através do *burst* respiratório mitocondrial, seguida pela destruição dos tecidos gengivais.[67-69]

A literatura ainda é controversa em relação ao perfil de biomarcadores do fluido crevicular gengival (GCF) de fumantes que apresentam doença periodontal. Enquanto a maioria dos estudos relata um efeito depressivo do tabagismo na expressão de citocinas pró-inflamatórias, outros não relatam diferenças significativas e até um aumento no perfil de citocinas em fumantes.[70] Ao avaliar o GCF, Tymkiw e colegas[70] encontraram diminuição das citocinas pró-inflamatórias (IL -1β, IL-6) e perfis de quimiocinas.[70] Da mesma forma, os fumantes não apresentaram diferença estatisticamente significativa na expressão de IL-1β, IL-6 e TNF-α no fluido de sulco peri-implantar quando comparados aos não fumantes.[71] Este pode ser o reflexo dos efeitos imunossupressores do tabagismo que, por sua vez, podem aumentar a suscetibilidade à destruiçãoperi-implantar. Embora TNF-α e a IL-1β sejam

secretados por mecanismos semelhantes, a IL-1β não parece ser influenciada pelo tabagismo em pacientes com periodontite.[72]Esses achados são contraditos pelos resultados de Liu et al.[73] Adicionalmente, a interleucina 1β (IL-1β) estimula a reabsorção óssea, inibe a formação óssea e é considerada ainda mais potente do que o TNF-α em termos de efeitos no metabolismo ósseo. Da mesma forma, uma análise de regressão linear múltipla mostrou correlações significativas entre os níveis de citocinas no fluido creviculardе tabagistas. Além disso, Giannopoulou e colegas analisaram amostras de GCF e observaram associações entre o tabagismo e as quantidades totais de IL-6 e IL-8, mas não com os níveis de IL-1β.[74] Esses achados estão de acordo com estudos subsequentes.[75]

Os níveis da enzima superóxido dismutase(SOD) de fumantes e não fumantes apresentando doença periodontal foram comparados por Tonguç et al.[76] Os autores relataram alterações insignificantes nos níveis sanguíneos de SOD, mas níveis significativamente mais altos de SOD gengival. Achados semelhantes foram encontrados por outros autores.[77,78] No entanto, em um estudo observacional recente avaliando os efeitos do tratamento periodontal sobre biomarcadores oxidativos, uma interação significativa entre o status do tabagismo e os níveis de SOD salivar no início e após o tratamento foi relatada. Fumantes tiveram reduções significativamente menores nos níveis de SOD após o tratamento em comparação com os não-fumantes e ex-fumantes. Os autores sugeriram que o tabagismo influencia a homeostase oxidativa e altera os níveis de antioxidantes em favor das espécies reativas de oxigênio.[79] Seus achados concordam com outros estudos.[79,80]

Em ambos os relatos, os níveis de SOD foram encontrados significativamente menores em fumantes quando comparados a não fumantes e, o mais interessante, os níveis antioxidantes de fumantes pesados diferiam dos fumantes leves, levando os autores a inferir que o consumo de tabaco influencia os níveis de SOD de maneira dose-dependente.[79,80] O mesmo padrão é relatado por outros grupos usando amostras de sangue e saliva.[80] Discrepâncias semelhantes na literatura são compartilhadas por outros marcadores antioxidantes,[82-84] mas as evidências atuais disponíveis parecem confirmar uma redução significativa da capacidade total anti-oxidativa (TAOC) na combinação de doença periodontal e tabagismo.[85] Além disso, foi comprovado que o tabagismo afeta a função neutrófila, que estimula a liberação de espécies reativas de oxigênio e, consequentemente,a mediação do estresse oxidativo. Com a capacidade de proteção diminuída em fumantes, é plausível que o uso de compostos antioxidantes que são capazes de agir contra a superprodução de espécies reativas de oxigênio dentro deste cenário deva ser abordado.

Obesidade

Existe uma forte associação entre as medidas de gordura corporal e a periodontite.[86] Um grupo analisou apenas estudos longitudinais e experimentais e concluiu que, especialmente em estudos longitudinais (com *follow-up*> 20 anos), sobrepeso, obesidade, ganho de peso e aumento de peso podem ser fatores de risco para o desenvolvimento de doença periodontal.[87] Além disso, uma revisão sistemática com meta-análise delineou o perfil de indivíduos com IMC elevado como mais propensos a apresentar maior media de perda clinica de inserção.[88] Um padrão semelhante de associação também é encontrado entre altos níveis de triglicerídeos séricos e lipoproteína dealta densidade (HDL) com bolsas periodontais aprofundadas em pacientes obesos.[89] Em termos de parâmetros clínicos periodontais, existem diversos ensaios clínicos e estudos comparativos relacionando diferentes níveis de doença periodontal com IMC elevado. Pacientes obesos também são descritos como apresentando maiores índices de índice de sangramento gengival (ISG) quando comparados a pacientes não obesos com doença periodontal. Buduneli et al.[90] encontraram valores significativamente mais elevados de profundidade sondagem (PD) e perda de inserção clínica(CAL) nos indivíduos obesos ($p < 0,05$) e uma tendência para uma correlação positiva entre IMC e CAL.[90] Além disso, um estudo de coorte com mais de mil participantes no Brasil identificou um risco maior de desfechos periodontais desfavoráveis, representados por sangramento à sondagem e perda de inserção clínica, em pacientes obesos (RR: 1,45).[91] Esses resultados estão de acordo com estudos anteriores.[92,93]

A obesidade é considerada um fator modificador da doença periodontal através da promoção de um estado mais pró-inflamatório, que pode afetar sua suscetibilidade a bactérias patogênicas e favorecer uma mudança para o desenvolvimento da periodontite.[94]O fator de necrose tumoral-α (TNF-α) é considerado o principal candidato conectando ambas as condições.[95] Um modelo proposto ligando a obesidade à periodontite descreve o aumento da secreção de citocinas pró-inflamatórias, especialmente o TNF-α, que inibe a sinalização da insulina, causando resistência à mesma e o desenvolvimento do DM tipo 2, estado pró-inflamatório, *priming* de tecidos periodontais, resposta exagerada à colonização microbiana, e, finalmente, destruição dos tecidos periodontais.[96] Além disso, Lundin e colegas[97] encontraram uma associação positiva entre os níveis de TNF-αem fluido crevicular gengival e alto IMC em indivíduos periodontalmente saudáveis, sugerindo que este citocina específica

pode originar-se de outro tecido em vez do periodonto e pode afetar estruturas diferentes do que apenas o tecido adiposo.[97]Interessantemente, um estudo mostrou que o aumento da expressão de TNF-α foi detectado em amostras de GCF de crianças obesas antes do diagnóstico de periodontite.[98] Por outro lado, Saxlin e cols.[99] sugeriram que não os níveis séricos de TNF-α, mas de IL-6poderiam mediar a conexão entre o peso corporal e a bolsa periodontal profunda, principalmente devido à expressão da proteína C-reativa.[99] Seus achados são contraditos por diferentes autores que sugerem que a IL-6 pode atuar como um coadjuvante e não como papel principal.[100] No entanto, embora os mecanismos subjacentes que relacionam a obesidade à periodontite permaneçam incertos e sua relação seja considerada bidirecional, o IMC elevado é um fator de risco significativo para a doença periodontal, sugerindo que os indivíduos obesos têm 35% de chance aumentada de desenvolver periodontite, sendo que o estresse oxidativo crônico pode ser o elo comum entre as duas condições.[101,102]

A presença de diversos marcadores em amostras periodontais, mesmo na ausência de infecção periodontal, poderia ser interpretada como um indicativo de uma conexão íntima bidirecional entre obesidade e periodontite. De um lado, essas adipocinas secretadas em excesso na corrente sanguínea em pacientes obesos ajudam no estabelecimento de um estado inflamatório, causando superprodução de produtos finais oxidativos nos tecidos periodontais. Concomitantemente, a infecção periodontal libera uma ampla gama de citocinas pró-inflamatórias, contribuindo para a manifestação de outras doenças crônicas, como a obesidade.[103]Portanto, indivíduos obesos são estatisticamente mais suscetíveis a desenvolver doença periodontal através de um estado inflamatório contínuo e de um ambiente hiper-oxidativo, influenciando negativamente a resposta imune frente a patógenos periodontais. Uma vez iniciada a destruição dos tecidos periodontais, uma ampla gama de citocinas pró-inflamatórias é liberada na corrente sanguínea, contribuindo para a expansão de ambas condições inflamatórias.

Artrite reumatóide (AR)

A artrite reumatóide(AR) e a periodontite apresentam várias semelhanças patogênicas. Elas incluem desregulação da resposta imune do hospedeiro, levando à inflamação do tecido mole com subsequente destruição do tecido duro e fatores de risco compartilhados, como tabagismo e excesso de peso corporal ou obesidade.[104] Além disso, estudos iniciais indicam que pacientes com AR podem ter uma maior incidência de doença periodontal e vice-versa,

sendo que existe a possibilidade de ambas as condições resultarem de patologias subjacentes comuns, resultando em uma forte associação entre ambas.[104] Além disso, ambas as condições têm aspectos comuns em termos do padrão de destruição de tecidos moles e duros. Enquanto a artrite reumatóide é responsável pela inflamação do líquido sinovial e destruição das articulações, a periodontite causa inflamação dos tecidos periodontais e perda óssea.[105]

Pacientes com AR foram comparados com pacientes sem AR para a avaliação de seu estado periodontal. A perda de inserção clínica e a profundidade de sondagem foram as medidas periodontais mais avaliadas para esta comparaçãocom relatos de pacientes com AR apresentando profundidade de sondagem significativamente mais profunda e 4,28 mais chances de apresentar periodontite.[105] Kaur e colegas,[104] em revisão sistemática da literatura com 10 estudos fizeram essa comparação. Setenta por cento deles mostraram uma diferença estatística entre a CAL em pacientes com apenas AR e AR com doença periodontal.[104] As análises também indicaram um aumento da perda dentária associada a pacientes com AR quando comparados com seus pares sistemicamente saudáveis. Alguns dos estudos incluídos nesta revisão mostraram não apenas uma diferença estatisticamente significativa, mas os pacientes com AR apresentando duas vezes mais CAL e quase o dobro de chance de mostrar CAL> 5 mm em comparação com pacientes não sem AR.[106-109] Para destacar ainda mais sua relação, estudos experimentais avaliando os efeitos do tratamento periodontal sobre marcadores bioquímicos para AR mostraram uma melhora estatisticamente significante para velocidade de hemossedimentação (VHS), proteína C-reativa e DAS28 (marcador de atividade de AR) em pacientes com doentes.[110,111] Seus achados estão de acordo com outro estudo e uma revisão sistemática.[112] Embora alguns desses estudos tivessem amostras relativamente pequenas, eles podem representar uma tendência indicativa significativa em termos de uma possível relação de periodontite e AR, assim como ferramentas adicionais de tratamento da doença degenerativa.

Apesar das semelhanças pró-inflamatórias entre AR e periodontite, ambas as doenças também compartilham parâmetros de estresse oxidativo comparáveis, embora a literatura sobre níveis de oxidantes locais e sistêmicos em sua combinação ainda seja escassa.[113,114] Adicionalmente, apesar da falta de estudos clínicos bem controlados e representativos que avaliem as consequências periodontais da AR e vice-versa, em termos de seus padrões oxidativos, o papel desempenhado pelo desequilíbrio entre o aumento da presença de radicais livres e a incapacidade do hospedeiro de proteção contra esses mecanismos destrutivos é bem descrito na literatura atual para ambas condições. Como duas entidades inflamatórias crônicas destrutivas que levantam questões sobre se uma é a consequência ou a provocadora da outra,

parece bastante possível que novas técnicas e métodos de comparação entre AR e periodontite possibilitem o esclarecimento dos mecanismos que ligam sua patogênese não apenas a entidades separadas, mas a uma condição comum com dano oxidativo em seu eixo central.

Resveratrol

A modulação dos níveis de citocinas e espécies reativas de oxigênio nos tecidos periodontais pode representar possíveis mecanismos pelos quais resveratrol atua na resposta do hospedeiro, levando ao controle da iniciação e do avanço da doença periodontal.Os papéis desempenhados pela via Nrf2, como a inibição da apoptose de fibroblastos e osteoclastogênese, bem como a eliminação de espécies reativas de oxigênio, têm implicações clínicas inéditas e benéficas em relação a doenças mediadas por estresse oxidativo e, neste caso, periodontite. Portanto, os efeitos do composto natural resveratrol podem sugerir que essa molécula, e derivados similares, possa ser utilizada como uma nova ferramenta adjunta no tratamento da periodontite crônica, dada a capacidade demonstrada de proteção contra danos mediados por progressão da doença periodontal.

Singh[115] demonstrou que o TCDD (dioxina), um ligante e agonista protótipo de AhR, que é análogo aos hidrocarbonetos encontrados na fumaça do cigarro, inibiu a osteodiferenciação e, portanto, a formação óssea *in vitro*. Diferentes concentrações de resveratrol foram adicionadas às culturas tratadas com TCDD, demonstrando que os efeitos negativos da TCDD poderiam ser bloqueados com o agente anterior. Isso foi demonstrado pela avaliação dos níveis de biomarcadores de osteogênese, tais como ALP, OPN e sialoproteína óssea (BSP) na presença e ausência de TCDD. Como esperado, a inibição da osteodiferenciação mediada por TCDD foi revertida pelo resveratrol em ambos os sistemas modelo *in vitro*.[115]Na mesma linha, Andreou e colegas[116] usaram um modelo de osteogênese de células da medula óssea de rato para testar outro ligante de AhR (benzo [a] pireno (BaP), este hidrocarboneto realmente sendo um componente da fumaça do cigarro) em combinação com lipopolissacarídeos (LPS) derivados de *P.gingivalis*na osteogênese com e sem resveratrol. Neste caso, a osteogênese foi avaliada usando várias abordagens, incluindo métodos enzimáticos, moleculares e eletroforéticos, bem como para a formação de nódulos ósseos que mancham-se de vermelho ao usar o vermelho de alizarina. Os efeitos inibitórios aditivos de BaP + LPS na formação óssea dos nódulos foram confirmados, sendo também demonstrado, dependendo de quão concentrada foi a exposição ao LPS, que os efeitos deletérios de BaP + LPS foram atenuados parcial ou completamente pela adição de

resveratrol. Os autores concluíram que, além do fato de resveratrol antagonizar a ativação do AhR, também demonstraram efeitos diretos anti-inflamatórios.[117]

Curcumina

Modelos experimentais de periodontite têm sido utilizados para avaliar os efeitos da curcumina nos tecidos periodontais, assim como tem sido feito com resveratrol, pois eles efetivamente desenvolvem destruição periodontal.[118] Usando o modelo experimental de periodontite induzida por ligadura em ratos, a doença é modificada pela adição de injeções de LPS ou indução de diabetes (ou, mais precisamente, indução de hiperglicemia). Como a curcumina natural apresenta propriedades farmacológicas relativamente fracas, como baixa biodisponibilidade, alta insolubilidade em água e curta meia-vida no plasma,[119] vários estudos compararam a curcumina natural com seus análogos modificados quimicamente(CMCs), que apresentam melhores características químicas. Como tal, estudos têm sido feitos para estudar os efeitos da curcumina ou CMCs em relação para avaliar se esses compostos também podem alterar a progressão e/ou o início da doença periodontal.

A ativação do fator de transcrição fator nuclear κ-B (NF-κB) está associada ao estado hiperinflamatório e à expressão de citocinas pró-inflamatórias, como IL-1β, IL-6 e TNF-α,[119] marcadores de osteoclastogênese, como a RANKL,[117] a atividade de MMP[120] e a superprodução de espécies reativas de oxigênio. Embora haja outras vias de sinalização nas quais a curcumina dependa,[121] a inibição da ativação da via NF-κB é considerada um dos principais mecanismos pelos quais o composto natural atua para prevenir e controlar a atividade de enzimas degradantes,[119] reabsorção óssea mediada por RANKL,[117] e a liberação exacerbada de radicais livres,[120] todos os quais desempenham um papel na destruição caracterizada pela doença periodontal.

Nessa linha, a administração oral de CMC 2.24 foi realizada em animais hiperglicêmicos. Uma redução significativa na perda óssea foi observada em modelos de periodontite induzida por LPS e associada a diabetes, 22,3% e 24,4%, respectivamente, com a administração do análogo de curcumina. Adicionalmente, reduções acentuadas nos níveis de IL-1β (50%), IL-6 (50%) e TNF-α (70%) foram registradas no grupo induzido por LPS. Níveis IL-1β “normais” foram alcançadosno grupo com periodontite associada ao diabetese os níveis de ativação do NF-κB inibidos foram comparáveis aos controles em ambos os modelos após a administração do CMC2.24. Os autores concluíram que a CMC 2.24 controla

a doença periodontal localmente induzida e modificada sistemicamente.[111,119] Por outro lado, a combinação de periodontite associada a LPS e diabetes foi positivamente influenciada pela CMC 2.24 em termos de níveis de IL-1 e IL-6, MMP -2, -8 e -9 e níveis de perda óssea. Embora as CMCs não tenham induzido efeitos significativos no *turnover* do tecido conjuntivo, os autores sugeriram que poderiam apresentar efeitos benéficos na destruição do colágeno e provavelmente do osso, tendo, portanto, potencial para uso no tratamento da doença periodontal.[119]

Diferentes estudos apresentam métodos heterogêneos, como discrepâncias nas apresentações das drogas, concentrações, tipos de administração e interpretação dos dados. Assim, as comparações devem ser tomadas com cuidado. Como tal, um estudo recente sobre os efeitos da curcumina e piperina, um derivado de pimenta com possíveis efeitos positivos sobre a biodisponibilidade da curcumina, na periodontite experimental em ratos foi conduzido.[119] Além dos efeitos benéficos sobre a inibição do NF-κB, infiltrado celular e colágeno conteúdo, os sítios tratados com curcumina mostraram neoformação óssea significativamente aumentada usando análise de micro-CT, independentemente da combinação com piperina. Os autores concluíram que a reparação óssea alveolar foi aumentada pela curcumina.[121] Em um modelo de periodontite induzida por ligadura em ratos, as medidas morfométricas da perda óssea alveolar não mostraram diferença estatística entre a curcumina, o resveratrol e as duas drogas combinadas, mesmo sem tratamento periodontal, representado pela remoção da ligadura.[58] Diante do papel protetor do resveratrol em ratos através do seu papel no metabolismo ósseo, como descrito anteriormente, resultados positivos semelhantes apresentados pela curcumina indiscutivelmente indicam o composto como uma alternativa promissora para o tratamento da periodontite.

Apesar de três estudos humanos diferentes terem relatado efeitos aditivos positivos de uma tira de curcumina com concentração de 0,2%, um gel de curcumina a 1% e a combinação de curcumina com ornidazol a 1% nos parâmetros periodontais após o escalonamento e planejamento radicular, o tamanho da amostra e o modelo do estudo levantam questões sobre a validade e confiabilidade dos resultados. Assim, a literatura atual ainda carece de ensaios humanos bem controlados sobre o assunto.

Conclusões

Portanto, o resveratrol e a curcumina, assim como seus derivados, representam um importante passo no desenvolvimento de novos fármacos que atuam em conjunto com as

técnicas convencionais de tratamento da doença periodontal. Os estudos pesquisados sugerem os dois compostos naturais como possíveis candidatos em terapias periodontais alternativas. Inicialmente, ambos os compostos impediram um cenário inflamatório exacerbado que é característico da doença periodontal crônica. Além disso, eles também produziram efeitos sobre o controle da infecção periodontal estabelecida. Além disso, no caso do resveratrol, o polifenol foi descrito como capaz de reverter os efeitos destrutivos da periodontite. Assim, embora testes farmacológicos adicionais ainda devam ser realizados para ambos os agentes, a evolução dos métodos para sua entrega (sistêmica e até local) deve produzir novos paradigmas no manejo das doenças periodontais que transcendam o controle da infecção isoladamente (por raspagem e alisamento radiculares) em um futuro próximo. A evidência atual indica que a oxidação desempenha um papel significativo em muitas doenças humanas, incluindo a periodontite. Os antioxidantes e a regulação positiva das enzimas antioxidantes e desintoxicantes associadas a NrF2 aumentam os efeitos citoprotetores ao diminuir a inflamação derivada dos danos oxidativos teciduais.[28]Consequentemente, as terapias que aumentam a atividade antioxidante podem ser adições viáveis às abordagens atuais relacionadas à prevenção e tratamento de periodontite, bem como outras doenças de cunhooxidativo.

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ANEXOS

ANEXO A – Carta de aprovação do CEP/PUCRS (PLATAFORMA BRASIL). Artigo 1.

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL - PUC/RS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: USO DE PILARES ANATÔMICOS DEFINITIVOS APÓS FUNÇÃO IMEDIATA COM INSTALAÇÃO DE PROVISÓRIO NA ZONA ESTÉTICA: UM ESTUDO PROSPECTIVO.
Pesquisador: Marcio Lima Grossi
Área Temática:
Versão: 3
CAAE: 60895316.1.0000.5336
Instituição Proponente: UNIAO BRASILEIRA DE EDUCACAO E ASSISTENCIA
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.899.120

Apresentação do Projeto:
O pesquisador principal do estudo encaminhou ao CEP-PUCRS resposta as pendências emitidas por esse CEP em 20/12/2016 no parecer n° 1.878.772.

Objetivo da Pesquisa:
O pesquisador principal do estudo encaminhou ao CEP-PUCRS resposta as pendências emitidas por esse CEP em 20/12/2016 no parecer n° 1.878.772.

Avaliação dos Riscos e Benefícios:
O pesquisador principal do estudo encaminhou ao CEP-PUCRS resposta as pendências emitidas por esse CEP em 20/12/2016 no parecer n° 1.878.772.

Comentários e Considerações sobre a Pesquisa:
O pesquisador principal do estudo encaminhou ao CEP-PUCRS resposta as pendências emitidas por esse CEP em 20/12/2016 no parecer n° 1.878.772.

Considerações sobre os Termos de apresentação obrigatória:
Todos os termos foram apresentados.

Conclusões ou Pendências e Lista de Inadequações:
Todas as pendências foram atendidas adequadamente.

Endereço: Av.Ipiranga, 6681, prédio 50, sala 703
Bairro: Partenon **CEP:** 90.619-900
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3320-3345 **Fax:** (51)3320-3345 **E-mail:** cep@pucrs.br

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL - PUC/RS

Considerações Finais a critério do CEP:

O CEP-PUCRS, de acordo com suas atribuições definidas na Resolução CNS n° 466 de 2012 e da Norma Operacional n° 001 de 2013 do CNS, manifesta-se pela aprovação do projeto de pesquisa proposto.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_798232.pdf	22/01/2017 18:47:09		Aceito
Outros	CartaRespostaPendencia220117.pdf	22/01/2017 18:40:44	Marcio Lima Grossi	Aceito
Outros	LattesPesquisadores.pdf	22/01/2017 18:40:16	Marcio Lima Grossi	Aceito
Outros	TCUDassinadotodos.pdf	22/01/2017 18:39:12	Marcio Lima Grossi	Aceito
Outros	CartaAoRevisor.pdf	07/12/2016 20:53:16	Marcio Lima Grossi	Aceito
Outros	AutorizacaoUsoArquivosDependenciasLaboratorio.pdf	07/12/2016 20:51:05	Marcio Lima Grossi	Aceito
Folha de Rosto	FolhadeRostoProjetoFabio.pdf	05/10/2016 08:55:19	Marcio Lima Grossi	Aceito
Outros	Autorizacaodeusodearquivodados.pdf	25/09/2016 10:53:21	Marcio Lima Grossi	Aceito
Outros	Ataqualificacaoodoutorado.pdf	25/09/2016 10:51:36	Marcio Lima Grossi	Aceito
Cronograma	Cronograma.pdf	25/09/2016 10:50:53	Marcio Lima Grossi	Aceito
Orçamento	OrcamentoAssinado.pdf	25/09/2016 10:38:59	Marcio Lima Grossi	Aceito
Declaração de Instituição e Infraestrutura	CartaAprovacaoComissaoCientificaOdontologia.pdf	25/09/2016 10:33:41	Marcio Lima Grossi	Aceito
Projeto Detalhado / Brochura Investigador	DocumentoUnificadodoProjetodePesquisa.pdf	25/09/2016 10:32:53	Marcio Lima Grossi	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Endereço: Av.Ipiranga, 6681, prédio 50, sala 703
Bairro: Partenon **CEP:** 90.619-900
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3320-3345 **Fax:** (51)3320-3345 **E-mail:** cep@pucrs.br

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL - PUC/RS

Continuação do Parecer: 1.899.120

PORTO ALEGRE, 26 de Janeiro de 2017

Assinado por:
Denise Cantarelli Machado
(Coordenador)

Endereço: Av.Ipiranga, 6681, prédio 50, sala 703
Bairro: Partenon **CEP:** 90.619-900
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3320-3345 **Fax:** (51)3320-3345 **E-mail:** cep@pucrs.br

ANEXO B – E-mail de convite para publicação no periódico Periodontology 2000. Artigo 2.

From: Scannapieco, Frank <fas1@buffalo.edu>
Sent: Wednesday, January 17, 2018 9:55 AM
To: Howard Tenenbaum
Subject: Invitation to author a paper for Periodontology 2000

Dear Dr. Tenenbaum,

I am organizing an issue of Periodontology 2000 on "Prevention of Periodontal Disease". In considering a logical list of topics to be included, I think it necessary to include an article that broadly addresses "Oral agents that alter oxidative stress to prevent periodontal disease"

As I know you are very knowledgable on this topic, I would be happy if you would agree to accept my invitation to write such a manuscript, with the understanding that you would be welcome to customize the title and subject as you feel appropriate. Also, you are welcome to invite colleagues to co-author, or mentor a student or post-doc to serve as the primary author.

Please be aware that the deadline for a first draft is January of 2019.

I attach the tentative Table of Contents, FYI. Note that Wim Teughels (wim.teughels@kuleuven.be) is authoring a chapter on oral rinses that will include natural products. Please feel free to contact him or any other of the authors to minimize duplication of content.

I very much appreciate that you are considering this invitation. I would be happy to discuss this with you further if you wish.

Sincerely,

Frank

—

Frank A. Scannapieco, D.M.D., Ph.D.
Professor and Chair
Department of Oral Biology
Associate Dean for Faculty and Professional Development
School of Dental Medicine
University at Buffalo
The State University of New York
Foster Hall
Buffalo, New York 14214

Voice:716-829-3373 (Laboratory)
716-829-2854 (Office)
FAX: 716-829-3942
e-mail:fas1@buffalo.edu

ANEXO C – E-mail de submissão para publicação no periódico Periodontology 2000. Artigo 2.

Fábio Sá Carneiro 30 January 2019 20:38

Periodontology 2000 paper submission - Howard Tenenbaum's group Details

To: Frank Scannapieco, Cc: Howard Tenenbaum, Howard Tenenbaum, Dr Howard Tenenbaum

Dear Dr Scannapieco,

Our group is very pleased to participate in this Periodontology 2000 issue.

You will find attached the MS Word document of our article entitled "Periodontitis is an Inflammatory Disease with Oxidative Stress: we should treat it that way" as part of the Periodontology 2000: Prevention of Periodontal Disease volume.

If there are any questions, suggestions or corrections on our paper, please do not hesitate to contact me, Dr Tenenbaum (copied on this email) or any co-authors as you wish.

My best regards,

Fábio Sá Carneiro Sczepanik

DDS, MSc, PhD Student Prosthodontics Program (PUCRS, Brazil).
PhD Visiting Researcher Periodontics Program (University of Toronto and Matrix Dynamics Group).

Periodontology 2000 -...p.docx

ANEXO D – E-mail de confirmação de recebimento para publicação no periódico Periodontology 2000. Artigo 2.

Scannapieco, Frank 30 January 2019 22:58

Re: Periodontology 2000 paper submission - Howard Tenenbaum's group Details

To: Fábio Sá Carneiro, Cc: Howard Tenenbaum, Howard Tenenbaum, Dr Howard Tenenbaum

Siri found new contact info in this email: Frank Scannapieco fas1@buffalo.edu add to Contacts...

Dear Fabio, Howard, et al.,

Thank you all for your efforts and timely submission. I look forward to read the manuscript, and I will be back in touch in due course.

Yours,

Frank

—
Frank A. Scannapieco, D.M.D., Ph.D.
Professor and Chair
Department of Oral Biology
Associate Dean for Faculty and Professional Development
School of Dental Medicine
University at Buffalo
The State University of New York
Foster Hall
Buffalo, New York 14214

Voice:716-829-3373 (Laboratory)
716-829-2854 (Office)
FAX: 716-829-3942
e-mail:fas1@buffalo.edu

See More from Fábio Sá Carneiro