UNESP - Universidade Estadual Paulista
"Júlio de Mesquita Filho"
Faculdade de Odontologia de Araraquara

Cindy Grace Pérez Pacheco

Potencial terapêutico do curcumin nanoparticulado como adjuvante no tratamento e reparo da periodontite

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Tese apresentada à Universidade Estadual Paulista (UNESP), Faculdade de Odontologia de Araraquara, como requisito para obtenção do título de Doutor em Nome do Programa de Pós-Graduação em Odontologia, Área de Concentração em Periodontia.

Orientador: Prof. Dr. Carlos Rossa Júnior

Coorientadora: Profa. Dra. Morgana Rodrigues Guimarães Stabili

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Cindy Grace Pérez Pacheco

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Comissão Julgadora

Tese para obtenção do Grau de Doutor em Odontologia

Presidente e Orientador: Prof. Dr. Carlos Rossa Júnior

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Araraquara, 01 de Abril de 2020.

DADOS CURRICULARES

CINDY GRACE PÉREZ PACHECO

NASCIMENTO	24 de Julho de 1983 – Lima – Perú
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Ao meu filho,

Gabriel Matias Hermoza Perez,

por ser meu grande amor e incentivador.

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Perez-Pacheco CG. Potencial terapêutico do curcumin nanoparticulado como adjuvante no tratamento e reparo da periodontite [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2019.

RESUMO

Curcumin é o principal composto biologicamente ativo extraído do rizoma da espécie *Curcuma longa.* Diversos estudos relatam efeitos biológicos do curcumin que são de interesse em diferentes condições clínicas, como doenças inflamatórias intestinais, artrite reumatóide, câncer, diabetes, obesidade, depressão, líquen plano e periodontite. Estudos pré-clínicos em modelos de periodontite experimental consistentemente demonstram efeito anti-inflamatório com a redução da reabsorção óssea. No entanto, existem poucas informações sobre o efeito biológico do curcumin no reparo dos tecidos periodontais, uma vez que na maioria dos estudos pré-clínicos a administração do curcumin é feita como único tratamento e de forma concomitante à indução da doença. Estudos clínicos do uso do curcumin como adjuvante do tratamento periodontal mecânico relatam, em geral, efeitos benéficos; porém há necessidade de avaliação sistemática dos resultados e da validade interna e externa dos trabalhos clínicos. Este trabalho tem o objetivo geral de investigar o potencial do curcumin aplicado localmente como adjuvante do tratamento periodontal mecânico no reparo dos tecidos periodontais. A hipótese é que a aplicação local de curcumin potencializa seus efeitos biológicos favorecendo o reparo tecidual ao evitar as dificuldades associadas às pobres propriedades farmacocinéticas da administração oral. O trabalho está organizado em três estudos: (i) estudo pré-clínico em modelo de periodontite experimental com aplicação local de curcumin como adjuvante da terapia mecânica e durante a fase de reparo; (ii) estudo clínico randomizado controlado com aplicação única do curcumin como adjuvante do tratamento mecânico; e (iii) revisão sistemática de estudos clínicos avaliando a aplicação local de curcumin como adjuvante do tratamento periodontal mecânico. Os resultados indicam que a aplicação local de curcumin promoveu o reparo periodontal no estudo pré-clínico; porém o estudo clínico não indicou benefício significativo clínico, microbiológico ou imunológico. A revisão sistemática e meta-análise indicou benefícios clínicos significativos (redução de sangramento e profundidade de sondagem, ganho de inserção) com o uso local de curcumin. A conclusão geral é que a aplicação local de curcumin tem potencial de favorecer o reparo periodontal, porém são necessários estudos adicionais para otimização do veículo, dosagem e posologia de aplicação.

Palavras chave: Curcumin. Periodontite. Nanopartículas. Ensaio clínico. Ratos.

Perez-Pacheco CG. Therapeutic potential of curcumin-loaded nanoparticles as an adjuvant to the treatment and repair of periodontitis [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2019.

ABSTRACT

Curcumin is the main biologically active compound extracted from the rhizome of the *Curcuma longa*. Several studies report biological effects of curcumin that are of interest in different clinical conditions, such as inflammatory bowel diseases, rheumatoid arthritis, cancer, diabetes, obesity, depression, lichen planus and periodontitis. Pre-clinical studies in experimental periodontitis models consistently demonstrate an anti-inflammatory effect by reducing bone resorption. However, there is limited information on the biological effect of curcumin in repairing periodontal tissues, since in most preclinical studies curcumin is administered as the sole treatment and concurrently with the induction of the disease. Clinical studies using curcumin as an adjunct to mechanical periodontal treatment report, in general, beneficial effects; however, there is a need for systematic evaluation of the results and the internal and external validity of clinical studies. This work aims to investigate the potential of locally-delivered curcumin as an adjunct to mechanical periodontal treatment in the repair of periodontal tissues. The hypothesis is that local application of curcumin enhances its biological effects, favoring tissue repair by avoiding issues associated with the poor pharmacokinetic properties of oral administration. The work is organized in three studies: (i) pre-clinical study in an experimental periodontitis model with local application of curcumin as an adjunct to mechanical therapy and during the repair phase; (ii) randomized controlled clinical study with a single application of curcumin as an adjunct to mechanical treatment; and (iii) systematic review of clinical studies assessing the local application of curcumin as an adjunct to mechanical periodontal treatment. The results indicate that the local application of curcumin promoted periodontal repair in the preclinical study; however, the clinical study did not indicate a significant clinical, microbiological or immunological benefit. The systematic review and meta-analysis indicated significant clinical benefits (reduced bleeding and probing pocket depth, and gain of clinical attachment) with the local use of curcumin. In conclusion, local application of curcumin has potential to favor periodontal repair, however, additional studies are needed to optimize the vehicle, dosage and application dosage.

Keywords: Curcumin. Periodontitis. Nanoparticles. Clinical Trial. Rats.

SUMÁRIO

1 INTRODUÇÃO

O curcumin é o componente ativo da *Curcuma longa*, uma planta herbácea da família do gengibre (Zingiberaceae) cultivada em países do sudeste e sudoeste asiático, onde é comumente utilizada como uma especiaria; mas, também, como antiinflamatório na medicina ayurvédica[1].

Em 1815, Vogel e Pelletier descreveram pela primeira vez o curcumin como "o colorante amarelo" da cúrcuma; em 1910, Milobedzka identificou a sua estrutura química; em 1913, Lampe e Milobedzka reportaram a primeira síntese de curcumin e em 1953, Srinivasan conseguiu separar e quantificar seus componentes (curcuminoides) por cromatografia[2,3].

1.1 Características Químicas e Físicas do Curcumin

O curcumin está composto de carboidratos, minerais, proteínas, gordura, óleos, umidade e curcuminóides. Os curcuminóides são: curcumin (~77%), desmetoxicurcumin (DMC, ~17%) e bis-desmetoxicurcumin (BDMC, ~3%)[4]. Estas proporções dos curcuminóides podem variar segundo a origem geográfica da planta e método de extração/purificação[5]. Cabe mencionar, que os curcuminóides têm distintos efeitos biológicos, portanto a variação na proporção desses compostos no extrato poderia ser uma das causas da grande variabilidade dos efeitos relatados na literatura. Sua nomenclatura química é 1,7-bis-(4-hidroxi-3-metoxifenil)-hepta-1,6-dien-3,5-diona, sua fórmula química C21H20O6 e seu peso molecular 8,54 pKa[6]. Este composto é insolúvel em água em ph ácido ou neutro, mas é solúvel em acetona, dimetilsulfóxido, metanol e etanol[1]. É também sensível à luz[7].

1.2 Potencial Anti-Inflamatório do Curcumin

O curcumin inibe a atividade de NF-kB, reduzindo a produção de diversos mediadores pró-inflamatórios, incluindo interleucinas (IL-1, -2, -6, -8, -12), citocinas (TNF-α, MCP-1) e enzimas (NOS, COX-2, lipoxigenase)[8]. Também afeta a proliferação, ativação e outras funções biológicas de diversas células do sistema imune-inflamatório, como linfócitos B e T, macrófagos, neutrófilos, células natural killer e células dendríticas[9].

Devido ao seu potencial terapêutico, o curcumin tem sido extensamente estudado, isto é demonstrado por 13981 artigos publicados no banco de dados do National Institutes of Health (NIH / PubMed) (www.ncbi.nlm.nih.gov) usando a palavra-chave "curcumin" em outubro de 2019. Desses 13000+ estudos, 269 são ensaios clínicos. Esta abundante literatura demonstra propriedades antimicrobianas, anticancerígenas, antivirais, antioxidantes e anti-inflamatórias[10-13] em diversas condições / ambientes de estudo, como diabetes[14-17], câncer[18-21], lúpus eritematoso[22], psoríase[23], esclerose múltipla[24], osteoporose[25], talassemia[26], enxaqueca[27], esquizofrenia[28,29], doença intestinal inflamatória[30-32], artrite reumatoide[33], osteoartitre[34,35], obesidade[36,37], síndrome metabólico[38], depressão[39, 40], líquen plano[41], estomatite[42], periodontite[43-46], entre outras.

A Administração de Alimentos e Medicamentos dos Estados Unidos declarou que o curcumin é "geralmente considerado um composto seguro"[8]. Chattopadhyay et al.[47] apontaram que a ingestão média de curcumin dos asiáticos varia de 0,5 a 1,5 g / pessoa / dia sem relato de efeitos colaterais. O curcumin foi usado em ensaios humanos sem toxicidade em doses de 1-8 g / dia[48] e até 12g / dia[12,49]. Dois estudos avaliaram a eficácia do curcumin no tratamento da artrite na melhora da inflamação pós-operatória e usaram essas doses de 1,2 a 2,1 gramas de curcumin por dia durante 2 a 6 semanas sem nenhum efeito adverso[50,51]. Mas, apesar de seus potenciais benefícios no tratamento de diversas doenças serem bem documentados, o curcumin apresenta algumas desvantagens que limitam sua aplicação terapêutica. As principais desvantagens estão relacionadas à suas propriedades farmacológicas: baixa biodisponibilidade, resultante da fraca solubilidade em meio aquoso; reduzida absorção no trato gastrointestinal, que limita a administração via oral; rápida metabolização e reduzida meia-vida plasmática[52]. Têm-se buscado modificar essas características farmacocinéticas através de diferentes abordagens, com o uso de adjuvantes como o piperine, ou através da formulação do composto em lipossomas, micelas, complexos fosfolipídicos e em nanopartículas[53-57].

A incorporação do curcumin em nanopartículas aumenta sua solubilidade e, portanto, biodisponibilidade; potencializando seus efeitos por promover a liberação controlada da droga e permitindo a utilização de doses terapêuticas reduzidas[57].

Estudo in vitro relatou que o curcumin em nanopartículas induziu a apoptose em mais de 50% de células de câncer cólon humano em doses reduzidas em comparação ao curcumin livre[56]. Estudo pré-clínico em ratos mostrou que o curcumin

veiculado em nanopartículas de PLGA apresentou biodisponibilidade plasmática 9 vezes maior em comparação ao curcumin associado com piperine, após administração via oral[58]. Zhang et al.[59] observaram melhores resultados para aplicação tópica de nanopartículas de curcumin quando comparados à administração oral de curcumin na progressão da osteoartrite e no alívio dos sintomas de dor associados à osteoartrite em um modelo de camundongo com osteoartrite pós-traumática. Outro estudo demonstrou que a nanoencapsulação aumenta a biodisponibilidade em cerca de 8,9 vezes em comparação com o "curcumin livre" (cristais de curcumin em pó preparados em 1,0% de carboximetilcelulose de sódio) em ratos[60]. Um estudo clínico demonstrou que a administração oral de curcumin em uma dispersão coloidal de nanopartículas aumentou em 27X sua concentração plasmática em comparação com a formulação em pó[54].

1.3 Potencial do Curcumin Como Agente Terapêutico em Doenças Periodontais

As doenças periodontais são doenças inflamatórias de origem infecciosa que acometem os tecidos de suporte dos dentes e que progridem a partir da gengiva, podendo levar à perda de osso e ligamento periodontal[61]. Ainda que sua prevalência e severidade apresentem uma distribuição bastante variável no mundo, estima-se que 10% da população global apresente as formas mais severas da doença[62], e que as formas mais leves estejam presentes em cerca de 35 a 60% dos indivíduos[63]. A abordagem terapêutica tradicional para seu controle é feita através da remoção mecânica de placa e cálculo das superfícies radiculares por meio de raspagem e alisamento radicular (RAR), além da manutenção de bons hábitos de higiene oral pelo paciente e visitas regulares de manutenção periodontal[64].

Devido ao seu potencial terapêutico anti-inflamatório e anti-oxidante, o curcumin tem sido proposto como adjuvante ao tratamento mecânico da periodontite. Existe vasta evidência de estudos in vitro e in vivo que documentam as propriedades anti-inflamatórias do curcumin na periodontite, incluindo: inibição de citocinas pró-inflamatórias como TNF-α, IL-6, IL- 1β[43,65-68]; redução do infiltrado inflamatório[43-46]; diminuição da ativação de NF-kB[44,45,65,69]; bem como da osteoclastogênese e reabsorção óssea inflamatória[43-46,69-71].

Estudos in vivo de periodontite experimental de nossa equipe, tem demonstrado que a administração sistêmica, por gavagem oral, inibe a produção de citocinas inflamatórias e a reabsorção óssea em ratos[43,72]. Resultados que coincidem com outros estudos[65,69]. Nenhum destes trabalhos em modelos animais relatou efeitos adversos de qualquer natureza (gastrointestinal, neurológico/comportamental, hepato ou nefrotoxicidade) nos animais tratados com curcumin.

Diversos estudos buscam identificar adjuvantes que aumentem a eficácia do tratamento periodontal convencional (remoção/desagregação mecânica do biofilme microbiano)[73]. O uso de agentes locais, colocados no interior da bolsa periodontal, é especialmente atraente por possibilitar a concentração do benefício terapêutico nas áreas efetivamente doentes, ao contrário da administração sistêmica. Na possível aplicação terapêutica em Periodontia, são de especial relevância as atividades anti-microbianas[10,11] e antiinflamatórias do curcumin. No entanto, pouco se sabe sobre os benefícios da administração tópica/local do curcumin como adjuvante ao tratamento periodontal convencional. Um estudo clínico avaliou o efeito de um gel de 1% de curcumin associado à RAR e relatou maior redução de bolsa periodontal, índice gengival e placa, e ganho de inserção clínica, sendo igualmente eficaz que 0.1% de clorexidina em gel[74]. Outro estudo clinico, mostra que uma única aplicação de gel a 2% de *C. longa* associado à RAR melhora significativamente a profundidade à sondagem[30], nível de inserção clínica[26] e índice gengival (IG) aos 30 e 45 dias[75]. Porém, um outro estudo clínico não encontrou diferenças estatisticamente significativas nos parâmetros clínicos avaliados entre os pacientes tratados com RAR associado à uma única aplicação tópica de 10mg/g do extrato de *C. longa* e os submetidos únicamente ao debridamento subgengival[76].

Não encontramos na literatura estudos que avaliem o curcumin em nanopartículas como adjuvante na doença periodontal, mas os resultados de um estudo in vivo de nosso grupo de pesquisa demonstra a completa inibição da reabsorção óssea inflamatória em modelo de doença periodontal induzida por LPS bacteriano[45].

Considerando-se as evidências da literatura indicando o potencial terapêutico do curcumin no tratamento da periodontite, propusemos avaliar os efeitos da aplicação local de uma emulsão contendo nanopartículas carregadas com esse composto natural, como adjuvante ao tratamento periodontal convencional em pacientes diagnosticados com periodontite e em modelo de periondontite experimental em ratos.

2 PROPOSIÇÃO

A hipótese deste trabalho é que a administração local do curcumin veiculado em nanopartículas favorece o reparo do tecido periodontal inflamado. Para avaliar esta hipótese, os objetivos específicos deste estudo foram:

1- Avaliar o efeito da administração local de curcumin nanoparticulado sobre o reparo ósseo alveolar associada à doença periodontal induzida em ratos.
2- Avaliar o efeito da aplicação local do curcumin nanoparticulado na resposta clínica, microbiológica e imunológica associada ao tratamento convencional da periodontite em pacientes diagnosticados com periodontite.
3- Revisar a literatura de forma sistemática, utilizando a pergunta focada: em pacientes saudáveis com periodontite, o uso local/tópico do curcumin como adjuvante favorece a resposta clínica à raspagem e alisamento radicular?

3 PUBLICAÇÕES

A avaliação do efeito terapêutico do curcumin nanoparticulado no tratamento da doença periodontal foi realizada em diferentes níveis de evidências científica:

Publicação 1: Pérez-Pacheco CG, Constantino AC, Paschoal DF, Favoreto AS, Fernandes NAR, Primo FL, Tedesco AC, Guimarães-Stabili MR, Rossa C Jr. *Curcumin-loaded nanoparticles enhance periodontal repair*. Artigo a ser submetido ao Journal of Periodontology.

Publicação 2: Pérez-Pacheco CG, Fernandes NAR, Primo FL, Tedesco AC, Feres M, Guimarães-Stabili MR, Rossa C Jr*. Local application of curcumin-loaded nanoparticles as an adjunct to scaling and root planing in periodontitis: Randomized, placebo-controlled, double-blind split-mouth clinical trial.* Artigo submetido ao Journal of Clinical Periodontology.

Publicação 3: Pérez-Pacheco CG, Diaz KC, Wambier LS, Guimarães-Stabili MR, Rossa C Jr. *Clinical efficacy of the local administration of curcumin in the non-surgical treatment in periodontitis: A systematic review and meta-analysis.* Artigo a ser submetido ao Journal of Periodontal Reseach.

3.1 PUBLICAÇÃO 1*

i. Title: Curcumin-loaded nanoparticles enhance periodontal repair

ii. Authors:

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iv. 3389 words, 5 figures and 50 references

v. Short title: Curcumin enhances periodontal repair

vi. Curcumin promotes healing of both mineralized and non-mineralized tissues in the periodontal microenvironment.

* O artigo segue as normas do periódico *Journal of Periodontology* para o qual será submetido.

vii. Abstract

Background: The anti-inflammatory properties of curcumin makes this compound promising for enhancing resolution of chronic inflammatory conditions. This study aims to evaluate the effect of nanoparticle-encapsulated curcumin on the repair of alveolar bone in a murine model of experimental periodontitis.

Methods: Periodontitis was induced, bilaterally, by placement of ligatures around the lower first molars of rats. After 15 days of disease induction, ligatures were removed ('treatment') and animals were allocated in three groups (n=8/group): (i) 0.05mg/ml curcumin-loaded nanoparticles (N-Curc), (ii) empty nanoparticles (vehicle) and (iii) sterile saline (Ctrl). Experimental treatments were administered locally on days 0 (removal of ligatures), 3, 5, 7, 9 and 11. Animals were euthanized at 14 days, and hemi-mandibles were harvested. Bone repair was assessed by microcomputed tomography. Histological sections were stained with hematoxylin/eosin (H/E) for the assessment of cellular infiltrate, extracellular matrix (ECM), osteoblasts and osteocytes. Expression of osteopontin (OPN) and Runt-related transcription factor 2 (Runx-2) was studied by immunohistochemistry.

Results: Locally-delivered curcumin-loaded nanoparticles increased soft tissue repair, demonstrated by the increase in the relative amount of extracellular matrix. Bone repair was also enhanced, as indicated by significant increases in mineralized tissue fraction (18% and 15% increases in comparison with Ctrl and vehicle, respectively) and in the number of osteoblasts and osteocytes, which was accompanied by a significant increase of Runx2 expression.

Conclusion: Curcumin-loaded nanoparticles applied locally after removal of disease-inducing stimulus promoted periodontal tissue repair.

Keywords: Periodontitis, curcumin, turmeric, repair, rats

viii. Main text

Introduction

Periodontitis is an infectious chronic inflammatory disease triggered by a dysbiosis between microorganisms of the dental biofilm and the host response that causes destruction of tooth-supporting tissues[1]. Worldwide, severe forms of periodontitis affect an estimated 740 million people[2].

Periodontal therapy (PT) aims to correct dysbiosis by reducing the microbial burden through mechanical removal (scaling and root planning, SRP, and by improving the efficacy of self-performed oral hygiene). Adjuvant/complementary treatment approaches are intensely studied to enhance the therapeutic results[3,4]. Antibacterial and anti-inflammatory drugs have been studied as an adjunctive to PT, even though the magnitude of their clinical benefits in the long-term is questionable[5]. A particular concern associated with the use of antibiotics as adjuvants to PT is microbial resistance and various other undesired side effects, which may occur with systemic[6] and local[7] administration. This justifies, alongside with its antimicrobial and anti-inflammatory effects[8], the interest in the study of natural compounds as adjuvant treatment to PT.

Curcumin (diferuloylmethane) is a polyphenol extracted of the rhizomes of *Curcuma longa* herbal plant and is considered a safe natural compound by the FDA. Due to its anti-inflammatory[9,10], antioxidant[11] and antimicrobial[12] properties, it has been intensely studied in chronic inflammatory conditions such as periodontitis.

The chronic inflammation in the microenvironment of periodontitis is characterized by accumulation of inflammatory cells and their secreted products, including proteolytic enzymes[13]. Data from in vitro and in vivo studies indicate that curcumin mitigate the response of immune cells, such as macrophages, to antigens associated with periodontitis[14,15], thus inhibiting the degradation of periodontal tissues[16]. Curcumin reduces expression of TNF-α and IL-1β by *Porphyromonas gingivalis* LPS-stimulated macrophages in a dose-dependent manner[14].

Most pre-clinical studies investigating curcumin in periodontitis use a preventive experimental design in which curcumin is administered simultaneously with disease induction and report on attenuation of inflammatory bone resorption [16-18]. This is mostly due to decreased inflammation and reduced activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)[17,19].

However, there are fewer studies assessing the repair-promoting effects of curcumin using experimental designs focusing on its therapeutic effects. Among the repair-promoting biological activities of curcumin, resolution of inflammation, increasing proliferation of stromal cells and production of extracellular matrix and enhancing bone repair are some biological activities reported after local[20] and systemic[21] administration in pre-clinical studies.

Considering its promising biologic features in the repair process, we investigate the effect of nanoparticle-encapsulated curcumin on the repair of alveolar bone in an experimental periodontitis model.

Methods

Curcumin-loaded nanoparticles

Purified curcumin was commercially obtained (Sigma-Aldrich Co. cat# C1386, Lot# 081M1611V) and curcumin nanoparticles containing 0.05mg/mL were prepared by Dr. Antonio Claudio Tedesco (Department of Chemistry, University of Sao Paulo at Ribeirao Preto). A 1:1 ratio of poly-lactic and polyglycolic acids (obtained from Sigma-Aldrich Co.) were loaded with 0.05 mg/mL of curcumin (ethanol solution). The combination was vigorously stirred (10,000 - 15,000 rpm) to obtain water in oil (w / o) emulsification. The organic solvent was removed from the solution by stirring at room temperature and evaporation under reduced pressure. The particles were centrifuged (4–10 ° C; 1100–4600 x g.) at intervals of 10 to 20 minutes. The preparation was washed three times in distilled water, suspended in 1mL PBS and stored at 4° C for up to 30 days. This formulation and its concentration was previously used in another in vivo study from our research group[17].

In vivo model of periodontal repair

The experimental protocol was approved by the Ethical Committee for Animal Use (CEUA) of the School of Dentistry at Araraquara − UNESP (process n° 37/2017) and performed in accordance with the guidelines from the National Council for the Control of Animal Experimentation (CONCEA). This study was reported following the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

A total of 24 male adult Wistar (*Rattus norvegicus albinus, Wistar*) rats weighing between 200 and 250 g were used in this study. Animals were kept in polypropylene cages (4 animals/cage) with controlled temperature (21 ± 1C) and humidity (65–70%) and a 12 h light–dark cycle in the animal facility. The rats were fed standard rat chow

(Labina/Purina) and water ad libitum. The sample size was based on previous studies in which the primary outcome was the change in bone volume in experimental periodontitis model in rats [16,21].

Animals were randomly distributed in the following three experimental groups (n=8 animals/group): (i) 0.05mg/ml curcumin-loaded nanoparticles (N-Curc), (ii) empty nanoparticles (Vehicle) and (iii) physiological saline (Ctrl). To induce periodontitis, animals were anesthetized by intramuscular administration of ketamine (Dopalen, Sespo Ind. and Com. Ltd., São Paulo, Brazil) and xylazine (Anasedan, Sespo Ind. And Com. Ltd., São Paulo, Brazil) at 0.08ml/100g and 0.04ml/100g body weight, respectively. Cotton threads (n° 24; Coats Corrente, São Paulo, SP, Brazil) were inserted in the subgingival region and tied around the lower first molars bilaterally. After 15 days, animals were anesthetized by inhalation (Fluovac, Harvard Apparatus Holliston, Massachusetts, USA) using isoflurane, ligatures were removed, the tooth surfaces cleaned, and the ligature sites of both lower first molars immediately treated according to the experimental groups. A volume of 30 µl of 0.05mg/ml curcumin-loaded nanoparticles or empty nanoparticles or physiological saline was locally applied into gingival sulcus around the first molars with the aid of a 1 mL sterile disposable syringe and 13 x 0,38 mm gauge blunted needle (INJEX, Ourinhos, SP, Brazil). Experimental treatments were administered in day 0 (day of ligatures removal), 3, 5, 7, 9 and 11, in a total of six applications (Figure 1). Animals were euthanized by dissociative anesthesia 72 h after the last treatment.

Hemi-mandibles were resected, fixed in 4% paraformaldehyde for 24 hours at 4 °C and stored in 70% ethanol for micro computed tomography (µCT) scanning. After scanning, twelve hemi-mandibles (n = 4 / experimental condition) were decalcified in EDTA 10%, pH 7.2 during 6-8 weeks. Specimens were reduced to include the first and second molars with their alveolar process and soft tissues and embedded in paraffin for subsequent sectioning.

Alveolar bone repair

Specimens were scanned in 18 µm slices, using pre-optimized settings (50 kV, 500 µA, 0.5 mm aluminum attenuation filter and frame averaging of 3) on a SkyScan 1176 microtomograph (Bruker, Kontich, Belgium). Imagens were submitted to three-dimensional reconstruction with SkyScan NRecon® software version 1.6.6.0 (Bruker, Kontich, Belgium) and rotated in a standardized three-dimensional orientation using the Data Viewer software version 1.4.4 64-bit (Bruker, Kontich, Belgium).

Subsequently, a region of interest (ROI) was determined using bidimensional images in the sagittal plane with CTAnalyser (CTAn) software version 1.12.4.0 (Bruker, Kontich, Belgium), to analyze the interproximal region between the first and second molars.

A polygonal interproximal ROI was positioned on all the reconstructed tridimensional scans according to the following anatomical landmarks: cementoenamel junction (CEJ) from the distal root of the first molar (mesial-upper limit) and CEJ from the mesial root of the second-molar (distal-upper limit) to the apex of these roots (mesial and distal lower limits of the ROI) (Figure 2A). This ROI was extended in the buccal-lingual (frontal) plane through 100 slices (1,8 mm) beginning on the first slice in which it was possible to see the buccal aspect of the distal root from the first molar.

A standardized threshold (255-65) was set to differentiate non-mineralized and mineralized tissues in order to quantify the bone volume fraction (BF / TV), thickness and separation of trabeculae on the ROI, using CTAn software.

Stereometric analysis

Serial sections of 4 μm were obtained in the sagittal plane and stained with Hematoxylin and Eosin (H/E). Photomicrographs of four sections (two from the buccal aspect and two from the lingual aspect) from specimens of four different animals in each experimental group were captured at 200x magnification using an optical microscope (Diastar-Cambridge Instruments) coupled to a color digital camera (Leica Application Suite 3.8, Wetzlar, Germany). There was an interval of 240 μm in the buccal-lingual (frontal plane) between these sections.

Stereometric analysis determined the proportion of cells (inflammatory cells, fibroblasts, etc.) and extracellular matrix (ECM) using a grid of 239.4 mm^2 with 80 points symmetrically distributed on standardized 200X magnification images. This grid was positioned on the images considering the following landmarks: base of the junctional epithelium between the first and second molar (upper limit) and the root surface of the first molar (lateral limit).

Histomorphometric analysis of osteoblasts and osteocytes was performed on 200X magnification images. The area of interest was defined as the alveolar crest between the first and second molars, from the most coronal aspect of the bone crest to the 200 μm apically to this limit. Osteoblasts were determined as large cuboidal cells along the alveolar bone surface and were counted and normalized by the perimeter of the bone surface (mm). Osteocytes, defined as cells entrapped within the mineralized bone

matrix, were counted in the alveolar bone process and normalized by the bone area (mm^2).

All the analyses were performed in a total of 16 sections per group using the ImageJ software (v. 1.51 s, National Institutes of Health, USA – http://imagej.nih.gov/ij). Each analysis was conducted by two examiners independently, who were calibrated (CI: 0.96) and blind to the experimental groups coding.

Immunohistochemistry

Osteopontin (OPN) was selected as a marker of bone formation/maturation, as it is considered a potent inhibitor of mineralization[22] which is associated with chronic inflammatory diseases, such as rheumatoid arthritis[23], due to its upregulation observed in inflammatory cells[24]. Runt-related transcription factor 2 (Runx2) was selected due to its critical role in osteoblastic differentiation and in the proliferation of osteoblast progenitors[25], essential processes for bone development and bone regeneration.

Immunohistochemical analyses of Runx2 (Abcam #76956) and OPN (Abcam #8448) expression were performed by biotin–streptavidin–HRP-DAB method (Envision FLEX kit, Dako, #K800021-2) according to manufacturer's instructions. A total of 3 semi-serial section obtained in the sagittal (4 μm thickness, spanning a distance of 320 μm) of four specimens from different animals in each experimental group were analyzed. Briefly, sections were de-paraffinized and rehydrated in decreasing concentrations of ethanol. After blocking of endogenous peroxidase activity, antigen retrieval was performed in high pH buffer, non-specific binding was blocked with 2% BSA in 1% PBS (OPN) or with a serum-free blocking buffer (Runx2) (protein block serum-free, ready-to-use, Dako, #X0909). Sections were incubated with the primary antibodies (1:100 dilution for OPN and Runx2) overnight (16 hours) at 4°C. Primary antibodies were omitted as negative controls. Detection of primary antibodies and visualization were done using secondary antibodies conjugated to peroxidase and the reaction developed with DAB substrate. Sections were counterstained with Carrazi's hematoxylin and mounted with Permount. Photomicrographs (200X magnification) were taken in the same area of interest of the histomorphometric analysis with (Leica Application Suite 3.8, Wetzlar, Germany). Percentage of positive staining in the area of interest was assessed by a trained examiner that was blind to the experimental conditions using ImageJ software and the plugin IHC profile (v. 1.51 s, National Institutes of Health, USA – http://imagej.nih.gov/ij).

Data analyses

Data obtained was analyzed using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). Statistical analyses aimed to compare the results according to the different experimental conditions. After analyzing normal distribution by Shapiro-Wilk test, data were analyzed by ANOVA One-Way parametric test for independent samples or Kruskal-Wallis non-parametric test, and Tukey or Dunn's post hoc test, respectively.

Results

There were no adverse effects and no animal was lost before euthanasia. Two ligatures were lost before 14 days in two different animals in the N-Curc group.

Curcumin-loaded nanoparticles enhance alveolar bone repair

Data from 46 hemi-mandibles were analyzed (16 for Ctrl and for the Vehicle groups and 14 for N-Curc). The bone volume in the ROI in the N-Curc samples was significantly greater in comparison with both control and vehicle groups (59.53% ± 7.97 vs. 50.44% ± 8.99; p=0.008; and 59.53% ± 7.97 vs. 51.79% ± 6.72; p=0.03, respectively) (Figure 2B), indicating that local application of curcumin promoted alveolar bone repair. In addition, there was a statistically significant increase of trabecular thickness in N-Curc group in comparison with the Ctrl group (0.35μm ± 0.04 vs. 0.30μm ± 0.57; p=0.02) (Figure 2C). No differences were observed for trabeculae separation among the experimental groups (Figure 2D). Representative three-dimensional images of each experimental group are shown in figure 2E.

Reduced cellular infiltration, increased extracellular matrix, osteoblast-like and osteocytes in sites treated with curcumin-loaded nanoparticles

Cellular infiltration in the connective tissue between the first and second molars was not significantly different; however, there is a trend of reduced cellular infiltration in tissues of the N-Curc group (Figure 3A), suggesting an enhanced resolution of inflammation.

This interpretation is supported by the significant increase in the proportion of extracellular matrix in the N-Curc group in comparison with both control and vehicle groups (75.47% ± 7.68 vs. 60.94% ± 11.88; p=004 and vs. 50.7 %± 15.07; p<0.0001, respectively) (Figure 3B). Considering that collagen fibers are the main component of the extracellular matrix in the periodontium, it is possible that this result indicates a potential of curcumin in favoring the tissue repair by enhanced resolution of

inflammation coupled with an anabolic effect on stromal cells. Representative H/E-stained images of the experimental groups are shown in figures 3C-E.

Since μCT analysis indicated an enhanced bone formation in N-Curc-treated animals, we assessed the number of osteoblasts and osteocytes in a defined bone surface perimeter and bone area, respectively. The number of osteoblasts was significantly increased in the N-Curc group (27.68 osteoblasts/mm ± 5.19) in comparison with control and vehicle groups (27.68 ± 5.19 osteoblasts/mm vs. 19.86 ± 4.17; and vs. 20.14 ± 3.24 osteoblasts/mm, respectively - $p<0.0001$) (Figure 4A). The density of osteocytes in the interproximal alveolar bone of N-Curc group was also significantly greater than in both control and vehicle groups (1.16 ± 0.18 osteocytes/mm^2 vs. 0.90 ± 0.10 and vs. 0.86 osteocytes/mm^2 ± 0.15, respectively - $p<0.0001$) (Figure 4B). Representative H/E-stained images of the region of interest in each experimental group are shown in figure 4C-E. Collectively, these finds are supportive of an anabolic effect in N-Curc-treated animals, promoting bone formation and resolution of inflammatory process, with restoration of the non-mineralized extracellular matrix.

Curcumin-loaded nanoparticles reduce osteopontin and increase Runx2 in periodontal repair

Runx2 protein expression was significantly increased in N-Curc animals (43.16% ± 11.86) in comparison with Ctrl group (vs. 26.78% ± 11.57; $p=0.02$, 17% lower than in the N-Curc group). In comparison with the vehicle group (27.1% ± 8.04; $p=0.07$, 16% lower than in N-Curc group), Runx2 was also increased, but without reaching statistical significance (Figure 5A). These findings correlate with the higher number of osteoblasts and osteocytes in the N-Curc group, further suggesting an anabolic effect of nanoencapsulated curcumin on alveolar bone.

Expression of OPN was not significantly different among the experimental groups, however the percentage of positively-stained area in the N-Curc group (27.8% ± 15.35) was in comparison with Ctrl (34.06% ± 17.24) and vehicle (41.14% ± 24.77) groups. This trend of reduced OPN correlates with the trend of reduced cellular infiltrate and may reflect improvement in the resolution of inflammation (as inflammatory cells are a major source of OPN) and in maturation of bone tissue (as OPN is an inhibitor of mineralization) (Figure 5B).

Discussion

Topical administration of curcumin-loaded PLA/PGLA nanoparticles significantly enhanced soft tissue and bone repair. Soft tissue repair was verified by increased amount of extracellular matrix, and bone repair by increased volume of mineralized tissue, as well as by the greater expression of Runx2 and augmented numbers of osteoblasts and osteocytes.

Our findings agree with a previous study[21] describing attenuation of cellular infiltrate and increased extracellular matrix density in animals treated with curcumin in experimental periodontitis, but also with studies using a preventive model[26-28]. This reduced inflammation may be explained by curcumin-induced inhibition of NF-κB activation, reported by several studies[17,18,29,30]. The essential role of NF-kB pathway for innate immunity, inflammation[31] and expression of multiple inflammatory mediators (e.g., IL-1, IL-2, IL-6, IL-8, TNF) and chemokines[32] puts this pathway in center stage when assessing the effects of curcumin in inflammatory settings. However, there is evidence that curcumin also affects other signaling pathways and biological mechanisms involved in inflammation and immune response, including mitogen-activated protein kinases (MAPKs)[33], Wnt/β-catenin[34], PI3K/Akt[35], JAK/STAT[36], ERK[37], SOCS[36], inflammassomes[38] and microRNAs[39]. This highlights the pleiotropic effects of curcumin and suggests that its biological effects may influence the inflammation associated with healing/repair in resolution processes. The possibility of a pro-healing/repair effect of curcumin, however, is less explored. It is tempting to speculate that the increased ECM observed in the curcumin-treated animals may be due to various mechanisms, which shall be explored in subsequent studies: (i) reduced production of MMPs and gelatinases; (ii) reduced infiltration by MMP/gelatinase-producing cells due to inhibition of chemokine and other biological mediators with chemoattracting effects, such as reduced OPN expression which may attract inflammatory cells[40]; (iii) increase in healing/repair-promoting mediators; and (iv) increased proliferation and differentiation of stromal matrix-producing cells, such as the increased expression of osteoblast differentiation marker Runx2.

In fact, our data indicates that local application of curcumin in the sulcus enhanced proliferation of osteoblast progenitors/committed cells, as well as their differentiation and bone matrix production, based on morphometric analysis showing increased numbers of osteoblasts and osteocytes and increased expression of Runx2. Recently, we have reported on enhanced bone repair (5 days of healing), analyzed by uCT, with

a daily systemic administration of 400mg/kg curcumin after 15 days of ligatures-induced periodontitis, using a healing/repair model[21]. Interestingly, another study described that single local irrigation of 40uM curcumin for sixty seconds significantly increased late bone repair (15 and 30 days of healing), assessed by the level of alveolar bone, and decreased the number of TRAP+ cells[20].

Pre-clinical studies demonstrate that curcumin can promote osteoblastic differentiation by upregulation of Runx2: Increased alkaline phosphatase (ALP) activity and osteoblast-specific mRNA expression of Runx2 and osteocalcin (OC) were observed when bone marrow-derived stem cells of rats (rBMSCs) were cultured in osteogenic medium and treated with curcumin[41]. Similarly, curcumin induced osteoblastic differentiation of mouse embryonic mesenchymal stem cells by augmenting gene expression of distal-less homeobox 5 (Dlx5), Runx2, ALP, and OC[42]. Curcumin-loaded scaffold enhanced bone formation in bone defects of rats with type 3 diabetes by increasing proliferation, migration, and osteogenic differentiation of mesenchymal stem cells[43]. Curcumin also promoted healing in a femoral bone fracture model in rats[44], an effect associated with reduced differentiation and maturation of osteoclasts, which indicates that curcumin may also affect coupled bone turnover and suggests curcumin as an interesting alternative for bone tissue engineering[45,46]. In vitro, liposomal-encapsulated curcumin incorporated into a three-dimensional printed scaffold promoted osteoblast cell viability and proliferation on human fetal osteoblast cells[45].

Although most of the available evidence reports on anti-inflammatory, antioxidant and anti-microbial properties of curcumin, there is relatively scarcity of information on its repair/healing-promoting effects. Clinical studies assessing curcumin as an adjunct to periodontal treatment are not conclusive[47,48]. Interestingly, all of these clinical studies use curcumin in association with mechanical periodontal treatment and thus in a healing/repair setting. This highlights the need for improved understanding of the effects of curcumin on healing/repair processes. This may imply in different concentrations and administration regimens. Also, considering the poor bioavailability of systemically-administered curcumin and the site-specific nature of periodontitis, future studies may investigate structural modifications or association with other substances to enhance biological activities and/or pharmacokinetic properties; as well as alternative vehicles for local delivery (such as encapsulation in controlled-release systems, polymeric particles, lipid nanoparticles and liposomes) [49,50].

We assessed the potential of curcumin associated to the periodontal treatment in six applications of 0.05mg/ml curcumin-loaded nanoparticles to ensure proper levels that promote healing. Importantly, the PLA/PGLA nanoparticles used as vehicle had no significant influence on the outcomes assessed. The concentration of curcumin loaded onto the nanoparticles was determined after evaluating the stability of the nanoparticles loaded with different concentrations of curcumin. It is important to acknowledge the limitations associated with the use of a single concentration, which is related with an intrinsic limitation of the nanoparticles used in this study as vehicle. Also, subsequent studies should assess longer follow-up periods and investigate possible biological mechanisms relevant for the repair-promoting effect.

In summary, to the best of our knowledge this is the first study to assess nanoparticle-encapsulated curcumin on the repair of periodontal tissues in a model of experimental periodontitis repair. Our results suggest that local delivery of nanoparticle-encapsulated curcumin promotes healing of both mineralized and non-mineralized tissues in the periodontal microenvironment. These results should be considered in the context of a preliminary, inception-generating study on the potential of locally-applied curcumin as an adjunct to conventional therapy in periodontitis.

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x. Conflict of interest

The authors declare no conflicts of interest related with the study.

xi. References

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xii. Figure legends

Figure 1. Study timeline representing the ligatures-induced periodontitis model, administration regimen of curcumin-loaded nanoparticles and euthanasia period.

Figure 2. Local administration of curcumin-loaded nanoparticles promoted bone repair. **A.** Region of interest (ROI) in the interproximal region was delimited by CEJ from first-molar distal root, CEJ from the second-molar mesial root, apex of the first-molar distal root and the apex of the second-molar mesial root. **B.** Quantification of the ratio of mineralized tissue (BV/TV), trabecular thickness **(C)** and trabecular separation **(D)** in the standardized ROI. **E.** Sagittal view of representative images of three-dimensional reconstructions of the hemi-mandibles in each experimental condition. Data from animals treated with six local applications of curcumin nanoparticles (N-Curc: 8 animals/14 hemi-mandibles), empty nanoparticles (Vehicle: 8 animals/16 hemi-

mandibles) or physiological saline (Ctrl: 8 animals/16 hemi-mandibles) in the sulcus of both first mandibular molars (after removing ligatures) during 14 days.

Figure 3. Curcumin reduced cellular density **(A)** and significantly increased extracellular matrix **(B). C, D & E.** Representative photomicrographs (200x) of the histological aspect of the gingival tissues, according to the topically applied compound. Animals were treated with six local applications of curcumin nanoparticles (N-Curc), empty nanoparticles (Vehicle) or physiological saline (Ctrl) in the sulcus of both first mandibular molars (after removing ligatures) during 14 days. Stereometric analysis was performed in the region between first and second molar, and determined the proportion of cells (inflammatory cells, fibroblasts, etc.) and extracellular matrix (ECM) using a grid of 239.4 mm^2 with 80 points symmetrically distributed on standardized 200X magnification images. Four sections from four right hemi-mandibles (4 animals), totalizing 16 HE-stained sections per group were analyzed in an interval of 240 μm in the buccal-lingual orientation using ImageJ software. **1M:** Distal root of first mandibular molar, **2M:** mesial root of second mandibular molar, **black arrow:** Cells, *****: ECM.

Figure 4. Curcumin markedly increased number of osteoblasts per millimeter of bone surface **(A),** and quantity of osteocytes in a defined area of the alveolar crest **(B).** Data from 16 HE-stained sections (4 from 4 animals) per group were analyzed. **C, D & E.** Representative photomicrographs (200x) of the region in the morphometric analysis, according to the groups. Animals were treated with six local applications of curcumin nanoparticles (N-Curc), empty nanoparticles (Vehicle) or physiological saline (Ctrl) in the sulcus of both first mandibular molars (after removing ligatures) during 14 days. Histomorphometric analysis was performed in the alveolar crest between the first and second molars, from the most coronal aspect of the bone crest to the 200 μm apically to this limit. Four sections from four right hemi-mandibles (4 animals), totalizing 16 HE-stained sections per group were analyzed in an interval of 240 μm in the buccal-lingual orientation using ImageJ software. **Black solid arrow:** osteoblast, **black dash arrow**: osteocyte, *****: bone.

Figure 5. Curcumin promoted osteoblastic differentiation by upregulation of the protein expression of Runx2 **(A)** and reduced osteopontin (OPN) **(B).** Animals were treated with six local applications of curcumin nanoparticles (N-Curc), empty nanoparticles (Vehicle) or physiological saline (Ctrl) in the sulcus of both first mandibular molars (after removing ligatures) during 14 days. Twelve immunohistochemistry (IHC)-stained

sections (three sections from four different animals) per group, spanning a distance of 320 µm, were analyzed in order to determine the percentage of positive chromogen staining in each experimental group. Representative images (200x) of IHC-stained sections for Runx2 **(C, D & E)** and OPN **(F, G & H)** were assessed using ImageJ software.

Figure 1

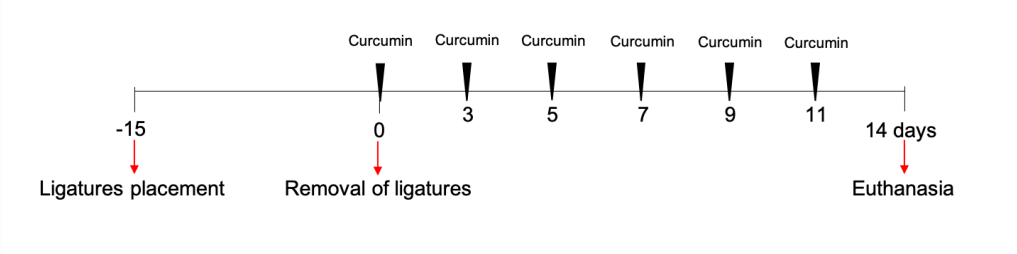


Figure 2

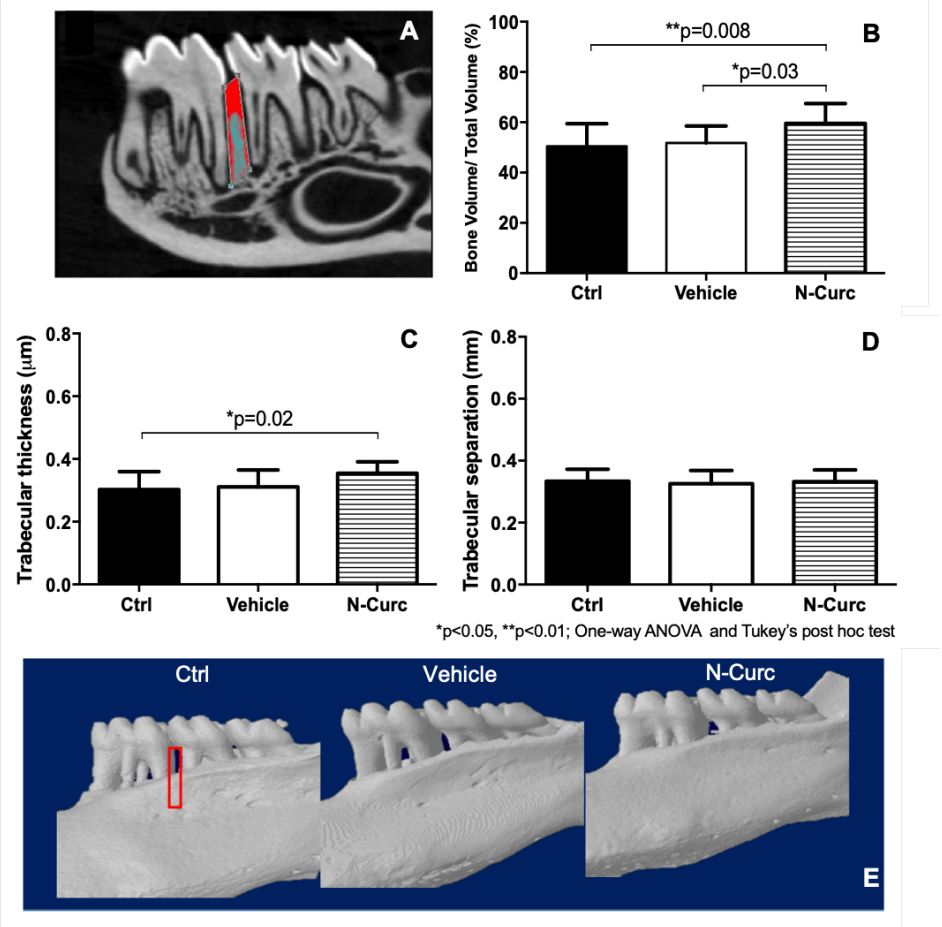


Figure 3

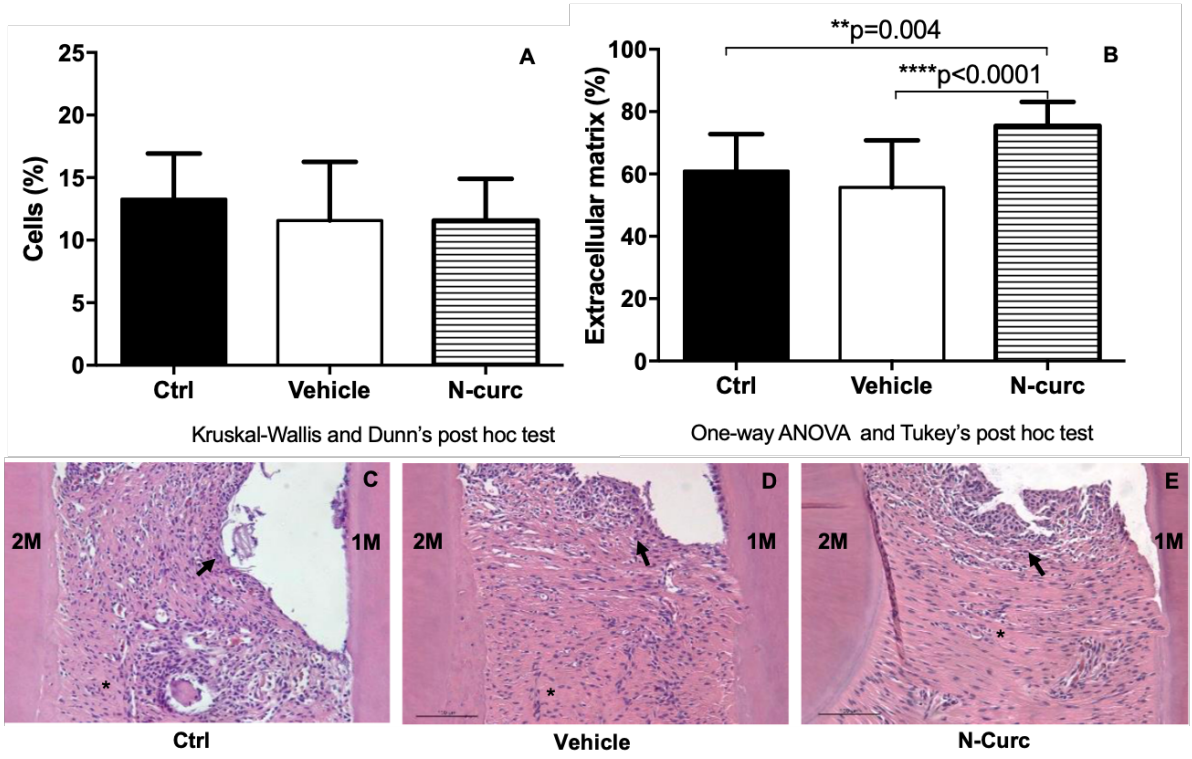


Figure 4

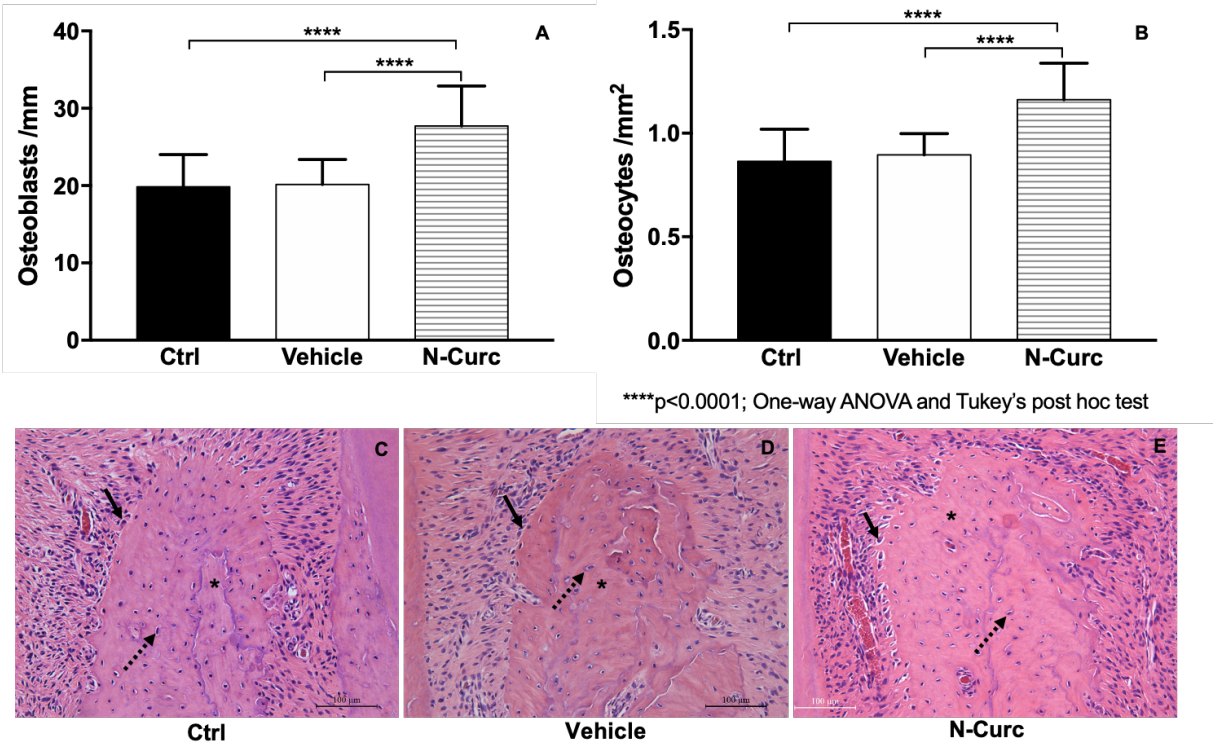
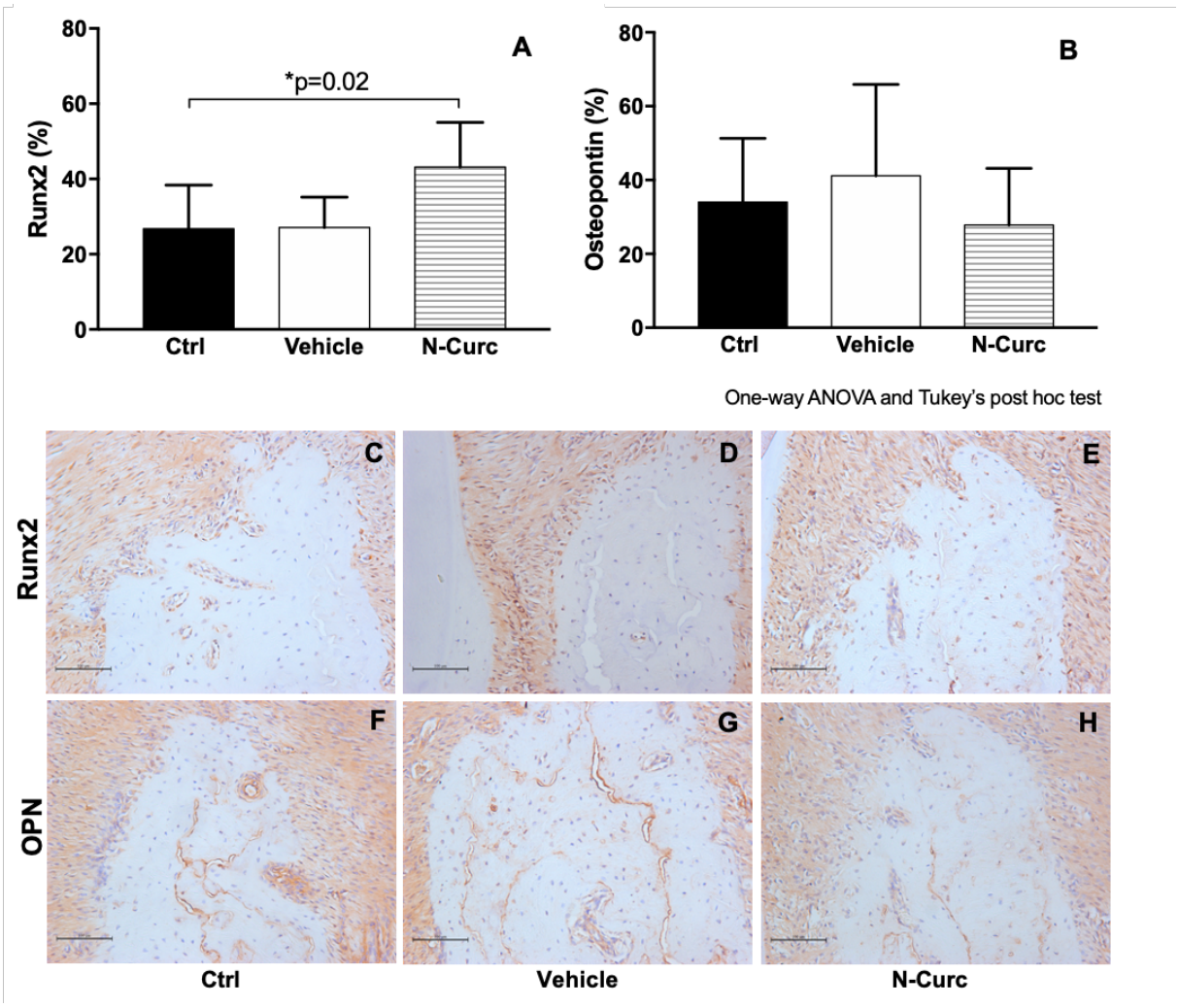


Figure 5


A
*p=0.02
Runx2 (%)
80
60
40
20
0
Ctrl
Vehicle
N-Curc
B
Osteopontin (%)
80
60
40
20
0
Ctrl
Vehicle
N-Curc
One-way ANOVA and Tukey's post hoc test
Runx2
OPN
C
D
E
F
G
H
Ctrl
Vehicle
N-Curc

3.2 PUBLICAÇÃO 2*

i. Title: Local application of curcumin-loaded nanoparticles as an adjunct to scaling and root planing in periodontitis: Randomized, placebo-controlled, double-blind split-mouth clinical trial

ii. Short title: Curcumin-loaded nanoparticles in periodontitis

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v. Conflict of interest

The authors declare no conflicts of interest related with the study.

vi. Source of funding

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vii. Abstract and keywords

Aim: Assess a single local application of curcumin-loaded nanoparticles as an adjunct to scaling and root planing (SRP) in nonsurgical periodontal treatment (NPT).

Material and Method: Twenty healthy subjects with periodontitis received SRP+PLGA/PLA nanoparticles loaded with 50 ug of curcumin (N-Curc) or SRP+empty nanoparticles. Probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP) were monitored at baseline, 30, 90 and 180 days. IL-1α, IL-6, TNFα and IL-10 in the gingival crevicular fluid (GCF) were assessed by ELISA and counts of 40 bacterial species were determined by DNA hybridization at baseline, 3, 7 and 15 days post-therapy.

Results: PPD, CAL and BOP were similarly and significantly improved in both experimental groups. There was no difference in GCF cytokine levels between experimental groups; although IL-6 was decreased at 3 days only in the N-Curc group. NPT reduced counts of red complex bacterial species in both groups. *Veillonella Parvula* counts increased significantly only in N-Curc group at 7 days, whereas

Aggregatibacter actinomycetemcomitans counts increased significantly only in the control group from day 3 to day 15.

Conclusion: We conclude that a single local administration of nanoencapsulated curcumin in periodontally diseased sites had no additive benefits to NPT.

Keywords: Curcumin, Periodontitis, Dental Scaling

viii. Clinical Relevance

Scientific rationale for the study

Pre-clinical studies indicate therapeutic potential of curcumin in periodontitis, due to antimicrobial and anti-inflammatory properties. Because of poor bioavailability, nanoparticles were used as vehicle for local application. There are few clinical reports with conflicting results and there is scarcity of properly controlled clinical studies assessing curcumin as an adjunct in the treatment of periodontitis.

Principal findings

A single local application of curcumin-loaded nanoparticles associated with nonsurgical periodontal therapy did not improve the results.

Practical implications

The results of this study do not support the use of curcumin as an adjunct to nonsurgical periodontal therapy.

ix. Main text

1. Introduction

Periodontitis is a chronic inflammatory condition with a multifactorial etiology characterized by destruction of tooth-supporting tissues (Papapanou et al., 2018). Periodontitis was considered the 6th most prevalent chronic inflammatory condition worldwide in 2010 (Kassebaum et al., 2014).

Disease is initiated by a dysbiosis between host and microorganisms of the dental biofilm (Kinane, Demuth, Gorr, Hajishengallis, & Martin, 2007; Slots, 2017). The standard therapy is the mechanical disruption of the microbial biofilm and reduction of bacterial load by scaling and root planning (SRP) combined with implementation of adequate self-performed oral hygiene. Nonetheless, not all diseased sites respond adequately to SRP (Heitz-Mayfield, Trombelli, Heitz, Needleman, & Moles, 2002) and various adjunct therapies have been studied (Akram et al., 2017; Golub et al., 2016; Ikram, Hassan, Raffat, Mirza, & Akram, 2018; Ma et al., 2018; Martins et al., 2017; Preshaw, 2018; Ren, McGrath, Jin, Zhang, & Yang, 2017; Shinwari, Tanwir, Hyder, & Bin Saeed, 2014).

Some evidence indicate that systemic administration of antibacterial or anti-inflammatory drugs can improve the results of SRP alone, but magnitude and duration of these effects is questionable (John, Michalowicz, Kotsakis, & Chu, 2017; Manresa, Sanz-Miralles, Twigg, & Bravo, 2018). Moreover, systemic medication may cause adverse effects (Zandbergen, Slot, Cobb, & Van der Weijden, 2013). Phytotherapeutics are an interesting cost-effective alternative with potent biological properties and reduced adverse effects.

Curcumin is the major polyphenol extracted from the rhizomes of the plant *Curcuma longa (*Family*: Zingiberaceae),* used as a culinary spice and in traditional eastern medicine. The Food and Drug Administration of the United States consider curcumin “a safe compound” (Gupta, Patchva, Koh, & Aggarwal, 2012). Its anti-microbial, anti-carcinogenic, antiviral, antioxidant and anti-inflammatory properties have been demonstrated in diverse conditions and experimental approaches (Aggarwal, Yuan, Li, & Gupta, 2013; Amalraj et al., 2017; Asteriou et al., 2018; Epstein, Docena, MacDonald, & Sanderson, 2010; Hanai & Sugimoto, 2009; Kuriakose et al., 2016; Momtazi-Borojeni et al., 2018; Panahi et al., 2017), including periodontitis (Guimaraes

et al., 2011; Guimaraes et al., 2012; Izui et al., 2016; Shahzad et al., 2015; Zambrano et al., 2018; Zhou et al., 2013).

In periodontitis, studies report antimicrobial (Najafi et al., 2016; Pourhajibagher et al., 2018; Shahzad et al., 2015) and anti-inflammatory properties, including reduction of: pro-inflammatory cytokines (Elburki et al., 2014; Guimaraes et al., 2011; Zhou et al., 2013); inflammatory infiltrate (Guimaraes et al., 2011; Guimaraes et al., 2012; Zambrano et al., 2018); nuclear factor-κB activation (Correa et al., 2017; Guimaraes et al., 2012; Zambrano et al., 2018); osteoclastogenesis and inflammatory bone resorption (Correa et al., 2017; Curylofo-Zotti et al., 2018; de Almeida Brandao et al., 2019; Elburki et al., 2014; Guimaraes et al., 2011; Guimaraes et al., 2012; Zhou et al., 2013). Meanwhile, some clinical trials indicate that local (Anitha et al., 2015; Bhatia et al., 2014; Gottumukkala, Koneru, Mannem, & Mandalapu, 2013; Hugar, Patil, Metgud, Nanjwade, & Hugar, 2016; H. Kaur, Grover, Malhotra, & Gupta, 2019; Nasra, Khiri, Hazzah, & Abdallah, 2017; Ravishankar et al., 2017; Ricci Donato et al., 2017; Singh, Sridhar, Shrihatti, & Mandloy, 2018) and systemic (S. Kaur, Sharma, Sarangal, Kaur, & Prashar, 2017) administration of curcumin can enhance the results of SRP in periodontal treatment.

Local delivery allows higher concentrations of the active compounds in the diseased site and avoids possible adverse effects of systemic administration.

Despite its pleiotropic biological effects and safety, curcumin has poor bioavailability with low absorption rate in the gastrointestinal tract, and short half-life in the plasma (Sharma et al., 2001). Nanoencapsulation has been shown to increase bioavailability by approximately 9-fold in comparison with ‘free curcumin’ in inert vehicles in rats (Peng et al., 2018) .

To the best of our knowledge, there is only one in vivo study assessing nanoencapsulated curcumin in the treatment of experimental periodontitis (Zambrano et al., 2018). This study assesses the effect of single topical application of nanoencapsulated curcumin as an adjunct to conventional non-surgical periodontal treatment.

2. Material and Methods

This double-blind split-mouth randomized clinical trial was approved by the Institutional Human Research Ethics Committee (CAAE 61307416.9.0000.5416) and registered in the Brazilian clinical trial registry – ReBEC (RBR-5ynbgw). CONSORT guidelines were followed (http://www.consort-statement.org/) for reporting the study.

2.1. Curcumin nanoparticles

Purified curcumin was commercially obtained (Sigma-Aldrich Co. cat# C1386, Lot# 081M1611V) and PGLA/PLA nanoparticles loaded with 0.05 mg/mL of curcumin were prepared by Dr. Antonio Claudio Tedesco (Department of Chemistry, University of Sao Paulo at Ribeirao Preto) as described by Zambrano et al. (Zambrano et al., 2018).

2.2. Subject population

Patients seeking periodontal care at the Periodontal Clinic of School of Dentistry at Araraquara (UNESP) between March 2017 and March 2018 were screened. Subjects deemed eligible were invited to participate and an informed written consent was obtained before enrollment.

2.3. Inclusion and exclusion criteria

Inclusion criteria: (i) patients diagnosed with generalized periodontitis with stage III and Grade A (Papapanou et al., 2018), presenting two non-adjacent sites with probing pocket depth (PPD) ≥ 5 mm and bleeding on probing (BoP) in two different quadrants, and bone loss confirmed by radiographs; (ii) ≥ 30 years of age; (iii) at least 15 remaining teeth; (iv) availability for the period of the study.

Exclusion criteria: (i) medical condition requiring prophylactic antibiotics or use of medications that may affect periodontal tissues; (ii) use of antibiotics in the last 6 months; (iii) use of steroid or non-steroidal anti-inflammatory drugs for 7 or more days in the past 3 months; (iv) pregnancy or nursing; (v) current smoker or smoker over the past 5 years; (vi) subgingival scaling in the last year.

2.4. Sample size calculation

The sample size was based on the changes in PPD in selected sites with PPD ≥ 5mm (5-8mm) as the primary outcome. Considering as significant a difference of 1 mm on the mean PPD between groups, with a 95% confidence interval and intragroup standard deviation of 1 mm, 16 subjects would be necessary in order to provide 80% of power. However, considering possible drop-outs during follow-up, 20 subjects were recruited.

2.5. Treatment protocol

Extraction of hopeless teeth and removal of retentive factors were performed before SRP. All subjects received the same kit of oral hygiene products and standardized instructions on proper self-performed oral hygiene.

After all periodontal clinical parameters were recorded, 4 sites (two sites in two different quadrants) with PPD 5-8mm and BoP were selected to receive nanoencapsulated

curcumin or empty nanoparticles topical application after SRP. Teeth with poorly-adapted prostheses, endodontic or caries lesions were excluded. When there were more than two eligible sites per quadrant, selection was prioritized according to the accessibility.

A random sequence of 20 numbers was generated using an online algorithm (http://random.org). By convention, even numbers would be associated with 'treatment A' in the sites located in the quadrant identified by the lowest number according to the FDI numbering system. If the random number was odd, then 'treatment B' was assigned to the sites in the lowest numbered quadrant. Sequentially numbered, sealed and non-transparent envelopes, containing the random number, were used for concealment of treatment allocation and were opened only immediately prior to the application time of the compounds, after completing the SRP.

Gingival crevicular fluid (GCF) and samples of subgingival biofilm (SB) were collected from all 4 selected sites. One trained periodontist, blind to the allocated sites, performed a single-session, full-mouth SRP using manual curettes (Hu-Friedy, Chicago, IL, USA) and an ultrasonic device (ProfiNeo, Dabi Atlante, São Paulo, SP, Brazil) under local anesthesia. After SRP, another trained operator applied the treatments according to the randomization. A volume of 100 µL of 0.05mg/mL nanoencapsulated curcumin (N-Curc) or of empty nanoparticles (control) were applied into the gingival sulcus of the selected sites with a 1 mL sterile disposable syringe and 13 x 0.38 mm gauge blunt needle (INJEX, Ourinhos, SP, Brazil). Patients were blind to the treatment allocation, and were advised not to brush the treated areas and to avoid interproximal cleaning for 1 day. Figure 1 outlines treatment protocol.

2.6. Clinical parameters

Assessment of clinical parameters was performed at baseline, 1, 3 and 6 months. All clinical assessments were done by a single trained and calibrated examiner (C.P.P), who was blind to the treatment allocation.

Periodontal clinical parameters assessed in six sites per teeth with a UNC periodontal probe (PUNC # 15, Hu-Friedy, Chicago, IL, USA): plaque index (O'Leary, 1972), gingival bleeding index (GBI) (Ainamo & Bay, 1975), BoP, PPD, gingival recession (GR) and CAL. Data of the selected sites were extracted for analysis.

2.7. GCF sampling

GCF samples were obtained from the selected sites at baseline, 3, 7 and 15 days with a standardized collection paper strip (Periopaper, Oraflow Inc., Smithtown, NY, USA) gently inserted to the bottom of the sulcus for 30s. Strips contaminated with blood were discarded.

Each sample was weighted on a calibrated device and then converted into volume units (μL) based in a standard curve (Periotron 8000, Oraflow Inc. Plainview, NY). Paper strips were placed in sterile microcentrifuge tubes containing 250μL of phosphate-buffered saline and stored at -80°C. Concentrations of IL-1α, IL-6, IL-10 and TNF-α were determined in a total of 320 samples by ELISA (PeproTech, Rocky Hill, NJ, USA).

2.8. Microbiological analysis

SB samples from the selected sites were collected with sterile mini-Gracey curettes and placed in sterile microcentrifuge tubes containing 0.2mL of a buffer solution (10mM Tris.Cl, 1mM EDTA, pH 7.6). Immediately, 0.1 mL of 0.5M NaOH were added and homogenized for 30 seconds. Samples were stored at -20ºC. A total of 320 samples were evaluated for the prevalence of 40 bacterial species by the checkerboard DNA-DNA hybridization technique (Socransky et al., 1994).

2.9. Outcome variables

Differences in average PPD six months after periodontal treatment were determined as the primary outcome. Secondary outcomes were CAL, GR, BoP, cytokine concentration, and microbiological data.

2.10. Data Analyses

Significance level was set at 5%. Data was tabulated and assessed for normal distribution using the D'Agostino-Pearson normality test. Parametric or non-parametric tests were used based on data distribution.

Mean differences in PPD, CAL and GR were analyzed with a linear mixed effects model (LMM) containing fixed effects for group, month, and group*month. BoP differences were tested independently for each month by likelihood ratio chi-square test.

PI and GBI comparisons were evaluated by one-way ANOVA for repeated measures, Tukey's post hoc test. Differences in cytokine concentration and bacterial counts were analyzed by Wilcoxon test, and differences in the same group by Friedman and Dunn test.

3. Results

Twenty patients (6 females and 14 males) with a mean age of 48.98 ±6.46 (range 37 to 62 years), were enrolled. Four subjects failed to follow up and no adverse effects were reported. Figure 2 represents the study flow chart.

3.1. Clinical results

Clinical data from 16 patients (64 sites) were analyzed. All clinical parameters were significantly improved, and there were no significant differences between the control and N-Curc sites (Table 1). The significant improvements in PPD, CAL, GR and BOP were observed at 1, 3 and 6 months after treatment. Table 3 shows significant improvements of full-mouth PI and GBI at all time-points ($p<0.05$).

3.2. Cytokine expression

Figure 3 shows the average of cytokine levels in the GCF of all 20 participants, according to the experimental treatment and period. IL-6 was reduced in N-Curc sites, whereas in control sites there was a slight increase at 15 days. IL-1α levels increased significantly in the control sites compared to N-Curc sites at 3- and 15-days. IL-10 was significantly reduced in control sites at 3 days, although IL-10 levels fluctuation was similar in both groups in the remaining periods. TNF-α was increased at 3 and 7 days in control and N-Curc and stabilized at 15 days, albeit there was a trend to lower concentrations in N-Curc sites.

3.3. Composition of subgingival microbiota

Data from all 20 participants were analyzed. Shifts in the subgingival microbial composition of control and N-Curc sites were similar, including Actinomyces species, yellow, orange and red complexes (Figure 4). However, in curcumin-treated sites there was a decrease in the counts of *Porphyromonas gingivalis* at 15 days, and purple complex species (*Veillonella parvula)* were significantly increased in N-Curc sites at 7 days; whereas in control sites increase in the counts of this microorganism only reached statistical significance at 15 days. Moreover, only control sites had a significant recolonization/regrowth of *Aggregatibacter actinomycetemcomitans* (green complex) between 3 and 15 days.

Figure 5 shows the mean bacterial counts grouped in color-coded complexes at baseline and 15 days. In both control and N-Curc sites, counts of bacteria in the yellow

and purple complexes increased significantly at 15 days, whereas counts of bacteria in the orange and red complexes were significantly reduced. There was a 4% increase in *Actinomyces spp.* proportion in control sites at 15 days compared with a 10% increase in N-Curc sites. In addition, the counts of 'other' bacteria (not classified in color-coded complexes) increased significantly only in control sites (Figures 4 and 5).

4. Discussion

To the best of our knowledge this is the first randomized, split-mouth, placebo-controlled double-blind clinical study to assess the effects of a single local application of 0.05mg/mL nanoparticle-encapsulated curcumin as an adjuvant to non-surgical periodontal treatment. There were no significant differences between the placebo-treated sites and curcumin-treated sites. However, a slightly better response was observed in all clinical parameters (PPD, CAL GR and BOP), as well as a greater reduction of inflammatory cytokines (IL-6 and TNF-α). Moreover, in curcumin-treated sites there was a significant increase in the counts of "beneficial bacteria"(*V. parvula)* at 7 days and a non-significant decrease in the counts of *P. gingivalis* at 15 days. Importantly, similarly to other clinical studies (Gottumukkala et al., 2013; Hugar et al., 2016; H. Kaur et al., 2019; Nasra et al., 2017; Singh et al., 2018) there were no adverse effects.

We assessed clinical parameters, bacterial counts and inflammatory cytokine levels based on the biological effects associated with the use of curcumin described in previous pre-clinical (Correa et al., 2017; Curylofo-Zotti et al., 2018; de Almeida Brandao et al., 2019; Elburki et al., 2014; Guimaraes et al., 2011; Guimaraes et al., 2012; Guimaraes-Stabili et al., 2019; Najafi et al., 2016; Pourhajibagher et al., 2018; Shahzad et al., 2015; Xiao, Yu, Xie, Liu, & Li, 2018; Zambrano et al., 2018; Zhou et al., 2013) and clinical studies (Gottumukkala et al., 2013; Hugar et al., 2016; Kudva, Tabasum, & Gupta, 2012) related with periodontitis.

A preclinical study using local application of the same formulation of curcumin-loaded nanoparticles verified significant reductions of the inflammatory infiltrate and osteoclast counts; as well as attenuation of activated p38 MAPK and NF-kB and a decrease of inflammatory bone resorption (Zambrano et al., 2018). This supports the biological activity of the curcumin concentration and vehicle formulation used in this clinical study.

Local application allows site-specific delivery, bypassing the need for absorption in the GI tract and reduces the possibility of adverse effects. However, differently from the pre-clinical study (Zambrano et al., 2018), curcumin nanoparticles were topically applied into the gingival sulcus. It is conceivable that differences in the microenvironmental conditions (e.g., flow of the gingival crevicular fluid, pH, inactivation by proteases in the GCF, limited access to inflammatory cells within the gingival connective tissue) account for changes in the concentration and in the biological activity of curcumin. Importantly, in this clinical study curcumin nanoparticles were applied only once.

A number of clinical studies on curcumin as an adjuvant to non-surgical periodontal therapy report mixed results: some studies indicate significant improvements in clinical (Hugar et al., 2016; Jaswal, Dhawan, Grover, & Malhotra, 2014; Nagasri et al., 2015; Nasra et al., 2017; Raghava et al., 2019; Singh et al., 2018), immunological (Elavarasu et al., 2016) or microbiological parameters (Gottumukkala et al., 2013; Sreedhar et al., 2015); whereas other studies report no benefit in comparison with SRP alone (Anuradha et al., 2015; Behal, Mali, Gilda, & Paradkar, 2011; H. Kaur et al., 2019).

In this study, all clinical parameters improved markedly and significantly in control sites, indicating a high efficacy of the treatment protocol. Curcumin-treated sites had discrete (and not statistically significant) improvements on PPD and CAL in comparison with control sites. This lack of significance may be due to the high efficacy of treatmemt protocol in systemically healthy, non-smoking patients.

Vehicle, concentration and mode of application of curcumin influence its efficacy. Different concentrations of curcumin (10mg/mL to 50mg/mL) have been used in collagen sponges (Anitha et al., 2015); gel formulations both with (Anuradha et al., 2015; Behal et al., 2011; Hugar et al., 2016; Ravishankar et al., 2017) and without surgical dressing (Elavarasu et al., 2016; Jaswal et al., 2014; Nagasri et al., 2015; Sreedhar et al., 2015); and as a solution used in subgingival irrigation (Gottumukkala et al., 2013; Jalaluddin et al., 2019). Another split-mouth randomized clinical trial compared SRP + chlorhexidine gluconate chip, SRP + 5% turmeric chip and SRP alone (Singh et al., 2018) and reported no differences in PPD, GI, PI and relative attachment level (RAL) after 1 month, followed by a similar significant improvement in

PPD and RAL in sites treated with chlorhexidine or turmeric chips at 3 months (Singh et al., 2018).

Dose and frequency of application may have influenced the efficacy of curcumin in this study. A single application clinically convenient, but it may reduce biological effects in comparison with repeated applications. In fact, a study in which multiple irrigations with a 1 % curcumin solution improved PPD, BoP, redness and PI at 30 days in comparison with saline-irrigated sites, and differences in PPD and PI were still significant at 6 months (Gottumukkala et al., 2013). On the other hand, a local single administration of 2% of curcumin gel + SRP significantly improved PPD, CAL and GI at 30 and 45 days in comparison with SRP-alone (Jaswal et al., 2014).

Even with a single application, there was a non-statistically significant trend towards lower concentrations of IL-6 and TNF-α in N-Curc sites. It is tempting to speculate that the magnitude of these trends in cytokine expression observed in this study could be increased with repeated applications that might enhance the biological effects of curcumin.

Both control and N-Curc sites showed reduction of red complex species count, but a significant increase in the amount of "beneficial bacteria" (*Veilonella parvula)* was observed at 7 days only in N-Curc sites. *V. parvula* belong to the purple complex and is considered a beneficial bacteria in the periodontium (Roberts & Darveau, 2002). A significant regrowth of *Aggregatibacter actinomycetemcomitans* between 3 to 15 days was observed only in control sites. There is evidence that curcumin can decrease survival and virulence of *A. actinomycetemcomitans* (Pourhajibagher et al., 2018). *A. actinomycetemcomitans* and black pigmented bacteroids were reduced in sites treated with SRP + 2% curcumin gel (Sreedhar et al., 2015). A single application of 2% turmeric gel did not improve clinical parameters over control group, but reduced red complex species (Behal et al., 2011) .

Clinical evidence on the benefits of locally-delivered curcumin as an adjunct to SRP in coflicting. This can be explained by the heterogeneity of experimental designs and the biases associated (e.g. sample size, baseline severity of periodontal involvement and inflammation, inclusion and exclusion criteria, selection of treated sites by clinical criteria, lack of proper randomization, lack of examiner blinding and lack of use of a

placebo control). In addition, not all studies report the geographical origin of curcumin, as well as the extraction / purification methods, which may influence the abundance of curcumin present in the purified extract and, consequently, its biological effect (Hayakawa, Minaniya, Ito, Yamamoto, & Fukuda, 2011; Sandeep et al., 2017).

We conclude that single local administration of 0.05mg/mL nanoencapsulated curcumin as an adjunct to SRP in periodontally diseased sites had no significant additional benefits. The non-significant positive trends in clinical, immunological and microbial aspects associated with this single application, justify additional prospective clinical studies to assess the benefits associated with multiple applications of curcumin-loaded nanoparticles, as well as diferent concentrations of curcumin. Moreover, considering the efficacy of SRP, the potential additive effect of curcumin should also be assessed in non-responsive sites, which may be associated with an altered host response (e.g., smoking, diabetes) and/or with an increased prevalence of periodontopathic bacteria, resulting in a more severe dysbiosis.

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xii. Tables and figure legends

Table 1. Clinical parameters in test (N-Curc) and control (Ctrl) groups at baseline, 30, 60 and 180 days after SRP plus topical application, and the results of intra and intergroup comparisons.

Parameter	Period	Treatment Group		Between Group *p*-value
		Ctrl	N-Curc	
		mean ± std	mean ± std * sig	
PPD (mm)	Baseline	5,31 ± 0,54	5,50 ± 0,80	0.24
	1m	2,84 ± 0,72****	3,06 ± 0,91****	0.61
	3m	2,91 ± 0,69****	2,81 ± 0,78****	0.50
	6m	2,84 ± 1,02****	2,72 ± 1,63****	0.31
PPD reduction (mm) Δ PPD	1m-BL	-2,47 ± 0,84****	-2,44 ± 0,91****	0.35
	3m-BL	-2,41 ± 0,76****	-2,69 ± 1,03****	0.48
	6m-BL	-2,47 ± 1.05****	-2,78 ± 1.07****	0.38
CAL (mm)	Baseline	5,53 ± 0,95	5,94 ± 0,91	0.15
	1m	3,91 ± 1,45****	4,09 ± 1,35****	0.50
	3m	3,78 ± 1,21****	3,75 ± 1,48****	0.91
	6m	3,72 ± 1,25****	3,72 ± 1,57****	1.00
CA gain (mm) Δ CA	1m-BL	1,63 ± 1,18****	1,84 ± 0,99****	0.60
	3m-BL	1,75 ± 0,95****	2,19 ± 1,20****	0.18
	6m-BL	1,81 ± 0.97****	2,22 ± 1.34****	0.22
GR (mm)	Baseline	0,19 ± 0.90	0,44 ± 0,76	0.28
	1m	1,00 ± 1.39****	0,97 ± 1,03***	0.89
	3m	0,84 ± 1.22***	0,94 ± 1,22**	0.68
	6m	0,94 ± 1.24****	0,91 ± 1,06**	0.89
BOP (%)	Baseline	100	100	ns
	1m	0****	12.5****	0.11
	3m	18.75 ****	3.13****	0.11
	6m	12.5****	9.38****	0.69

p-value within group compared to baseline *** p<0.05, **p<0.01,*** p<0.001,****p<0.0001**

Clinical parameters were analyzed with a linear mixed effects model (LMM) containing fixed effects for group, month, and group*month. Probing pocket depth (PPD), Clinical attachment level (CAL), Clinical attachment (CA), Gingival recession (GR), Bleeding on probing (BOP).

Table 2. Mean and standard deviation of full-mouth Plaque Index and Gingival Bleeding Index in the different periods.

	Period	Positive surfaces (%) ± std deviation
Plaque Index	BL	61,23 ± 20,75
	1 m	12,15 ± 8,30 ***
	3 m	13,54 ± 6,16 ****
	6 m	11,46 ± 4,29 ****
Gingival Bleeding Index	BL	20,39 ± 11,67
	1 m	2,10 ± 2,02 ****
	3 m	2,89 ± 2,58 ****
	6 m	3,06 ± 2,92 ****

One-way ANOVA for repeated measures, Tukey's post-hoc test. (*) indicates significant differences from the baseline (BL).***p<0.001, ****p<0.0001

Figure 1. Study timeline. OHI: Oral Hygiene Instructions; GCF: Gingival Crevicular Fluid; SBC: Subgingival biofilm collection; SRP: Scaling Root Planing

Figure 2. Study Flowchart

Figure 3. Levels of inflammatory mediators in gingival crevicular fluid from selected sites treated with vehicle (Ctrl) or curcumin-loaded nanoparticles (N-Cur). Assays were performed according to the manufacturer's instructions and the results for each sample were normalized by the total sample volume collected. Graphs represent data from all participants at different times. Friedman and Dunn tests were applied to assess intragroup differences, and Wilcoxon test for differences between groups; */#p<0.05, **/## p<0.01, ***/####p<0.001. (*) shows differences in control group and (#) in the test group.

Figure 4. Mean bacterial counts at baseline, 3, 7 and 15 days post-therapy and mean changes from baseline to 15 days post-therapy of the 40 bacterial species evaluated. The species were ordered according to the microbial complexes described by Socransky et al. (1998). The significance of differences in the mean counts of the bacterial species from baseline to the different time-points was assessed by the Friedman and Dunn tests. Mean changes of the bacterial species from baseline to 15

days between the experimental groups was assessed by Wilcoxon test ($p<0.05$). (*) shows significant difference when compared baseline to 3 days; (+)7 days; (#)15 days; (⏃) 3-15days

Figure 5. Pie charts of the mean proportion of each microbial complex at baseline and 15 days post-therapy for control and nanocurcumin groups. The colors represent the different complexes described by Socransky et al (1998). The blue color represents species that did not fall into any complex, and Actinomyces species are represented in gray. The significance of differences between baseline and 15 days post-therapying the same groups and between the groups was assessed using Wilcoxon Test (*$p<0.05$, **$p<0.01$ and ***$p<0.001$).

x. Figures

Figure 1

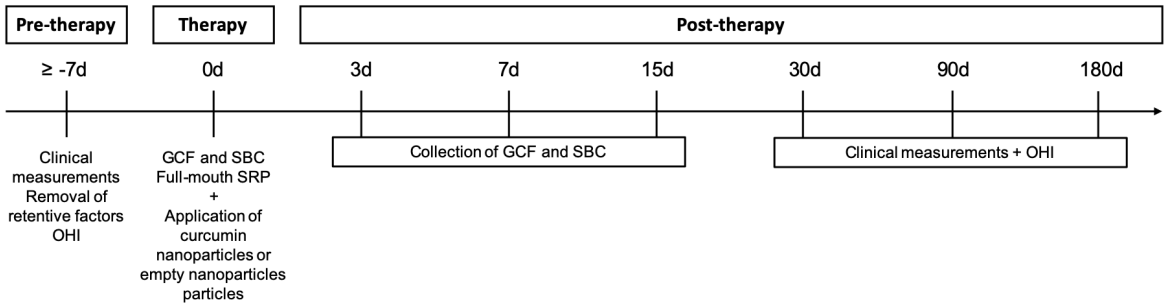


Figure 2

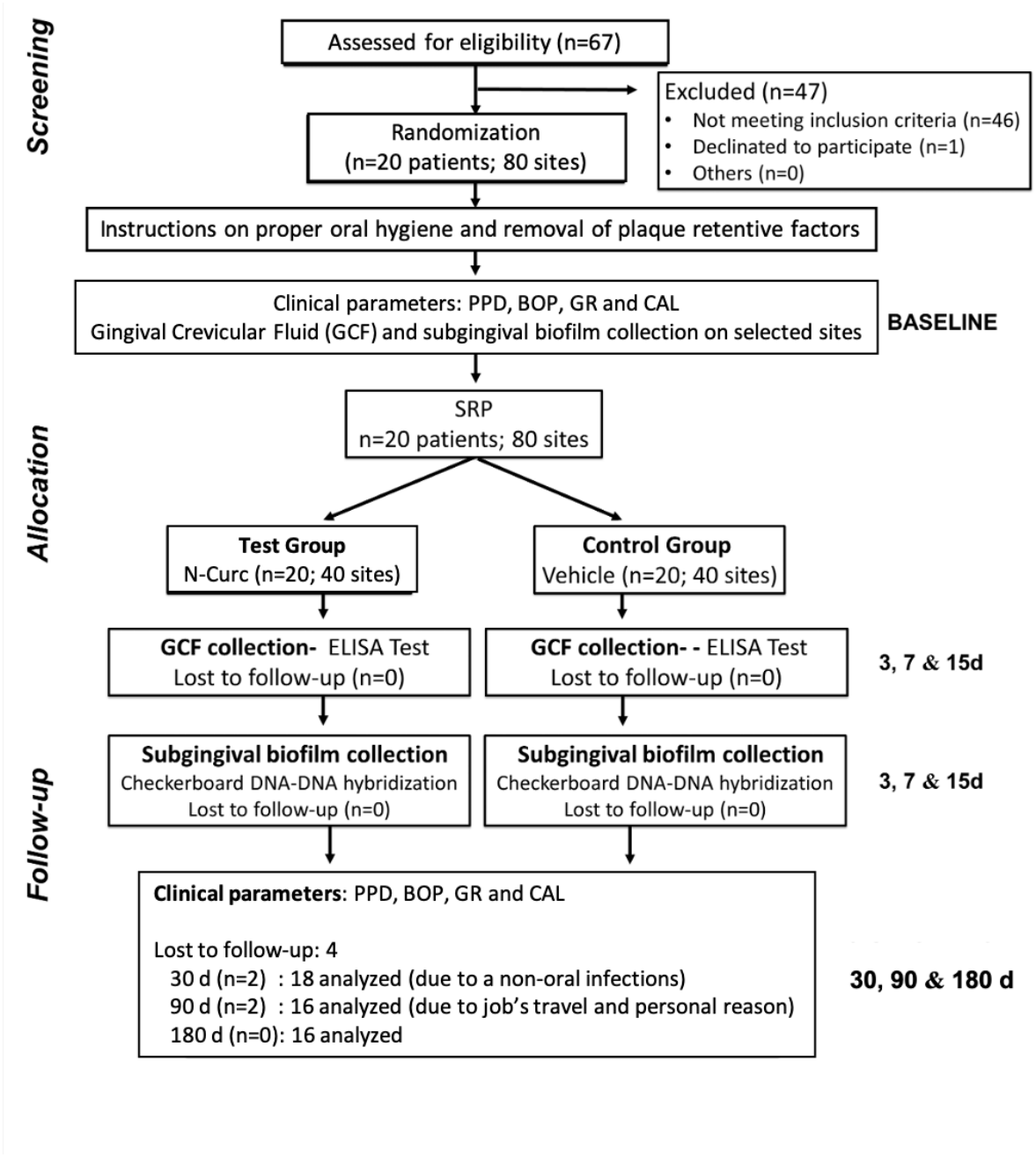

Screening
Assessed for eligibility (n=67)
Excluded (n=47)
• Not meeting inclusion criteria (n=46)
• Declinated to participate (n=1)
• Others (n=0)
Randomization
(n=20 patients; 80 sites)
Instructions on proper oral hygiene and removal of plaque retentive factors
Clinical parameters: PPD, BOP, GR and CAL
Gingival Crevicular Fluid (GCF) and subgingival biofilm collection on selected sites
BASELINE
Allocation
SRP
n=20 patients; 80 sites
Test Group
N-Curc (n=20; 40 sites)
Control Group
Vehicle (n=20; 40 sites)
Follow-up
GCF collection- ELISA Test
Lost to follow-up (n=0)
GCF collection- - ELISA Test
Lost to follow-up (n=0)
3, 7 & 15d
Subgingival biofilm collection
Checkerboard DNA-DNA hybridization
Lost to follow-up (n=0)
Subgingival biofilm collection
Checkerboard DNA-DNA hybridization
Lost to follow-up (n=0)
3, 7 & 15d
Clinical parameters: PPD, BOP, GR and CAL
Lost to follow-up: 4
30 d (n=2) : 18 analyzed (due to a non-oral infections)
90 d (n=2) : 16 analyzed (due to job's travel and personal reason)
180 d (n=0): 16 analyzed
30, 90 & 180 d

Figure 3

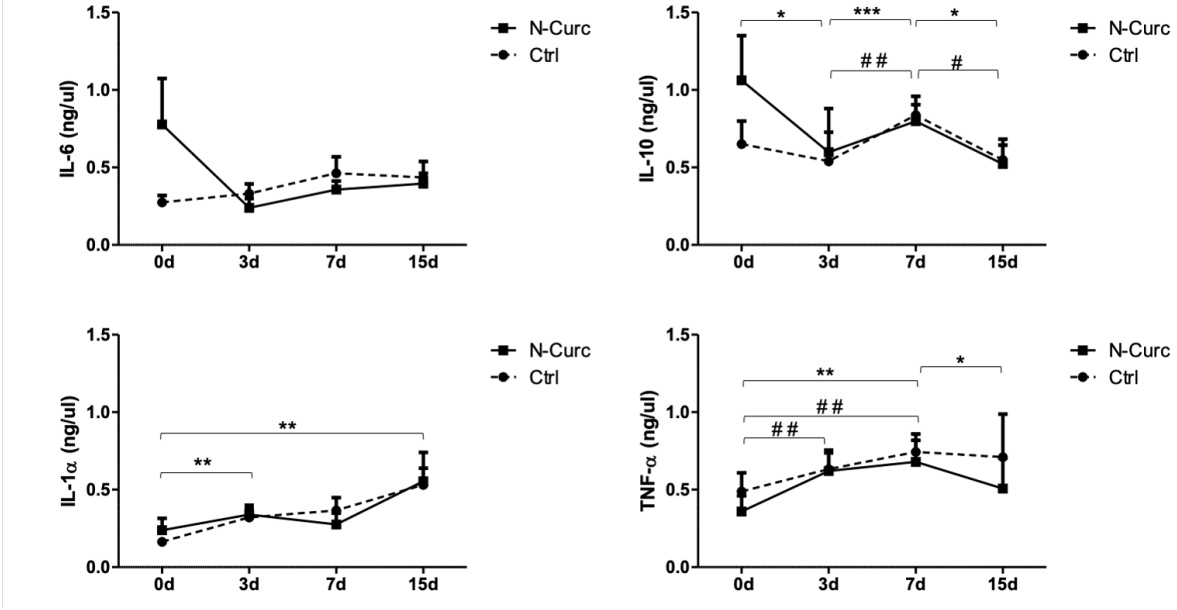
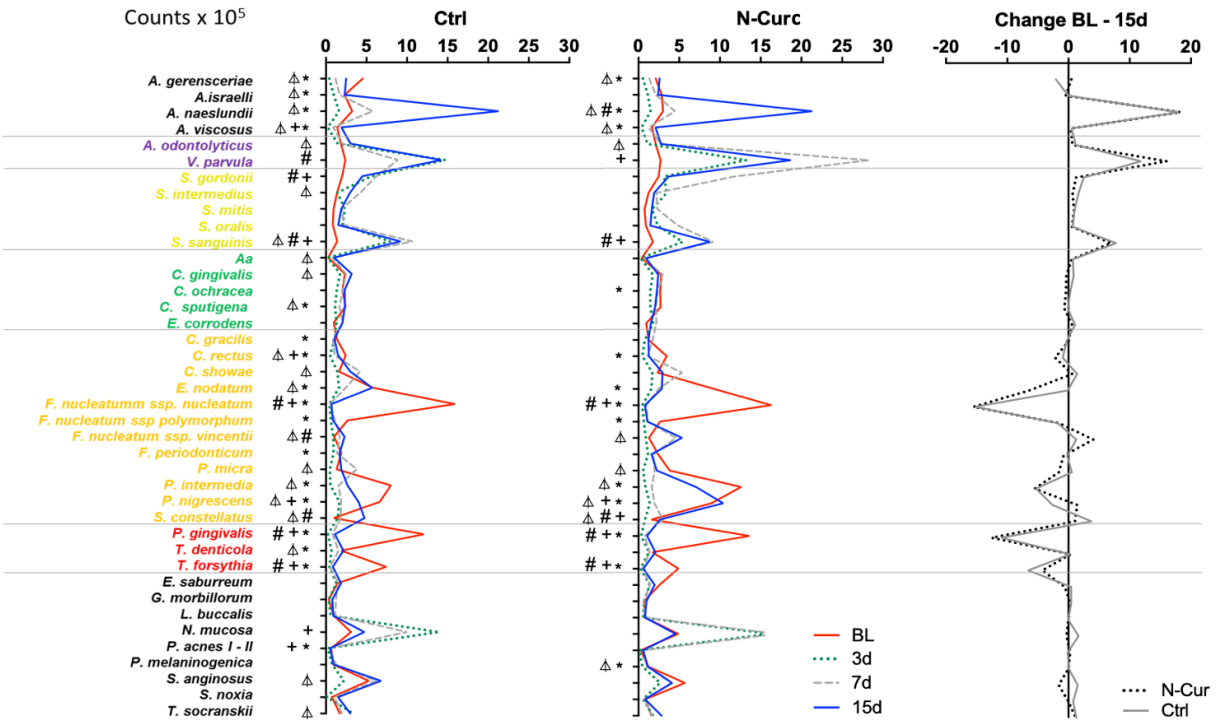
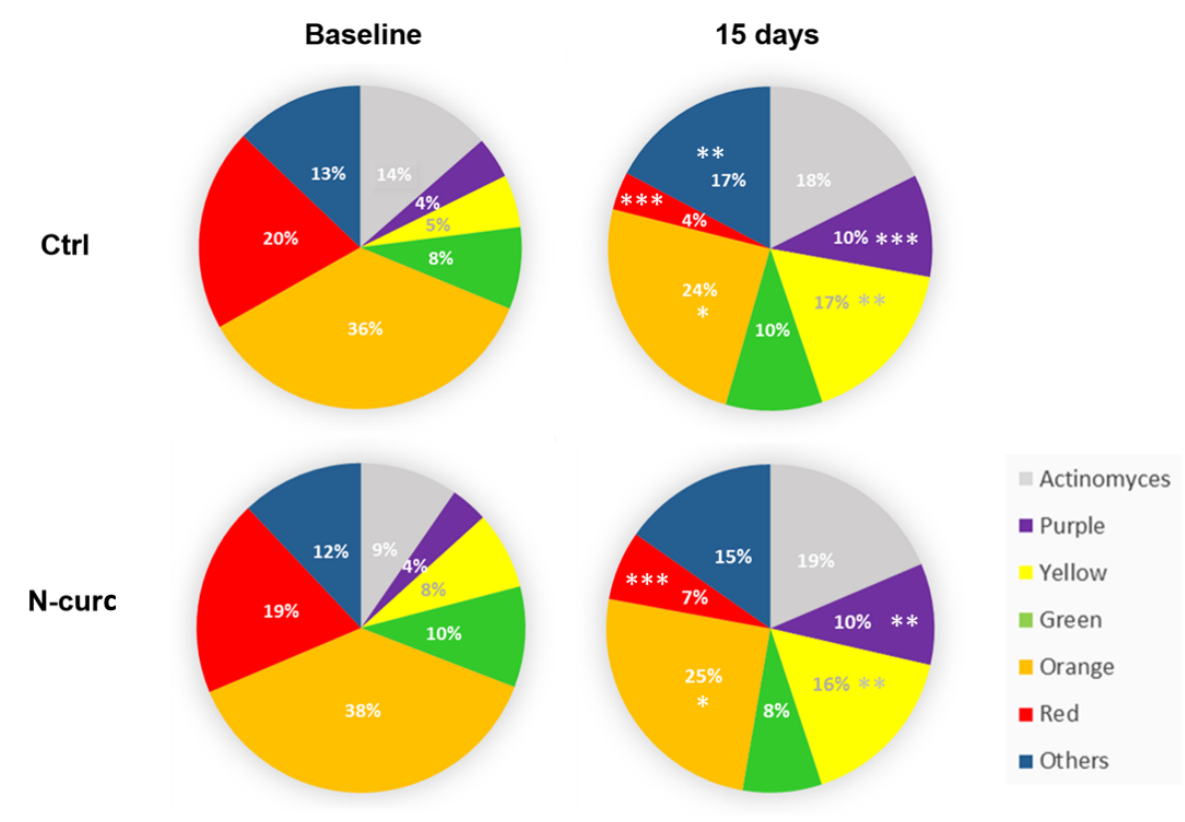

IL-6 (ng/ul)
IL-10 (ng/ul)
IL-1α (ng/ul)
TNF-α (ng/ul)
N-Curc
Ctrl
0d
3d
7d
15d
0.0
0.5
1.0
1.5
*
***
**
##
#

Figure 4


Counts x 10⁵
Ctrl
N-Curc
Change BL - 15d
0 5 10 15 20 25 30
-20 -10 0 10 20
A. gerensceriae
A.israelli
A. naeslundii
A. viscosus
A. odontolyticus
V. parvula
S. gordonii
S. intermedius
S. mitis
S. oralis
S. sanguinis
Aa
C. gingivalis
C. ochracea
C. sputigena
E. corrodens
C. gracilis
C. rectus
C. showae
E. nodatum
F. nucleatumm ssp. nucleatum
F. nucleatum ssp polymorphum
F. nucleatum ssp. vincentii
F. periodonticum
P. micra
P. intermedia
P. nigrescens
S. constellatus
P. gingivalis
T. denticola
T. forsythia
E. saburreum
G. morbillorum
L. buccalis
N. mucosa
P. acnes I - II
P. melaninogenica
S. anginosus
S. noxia
T. socranskii
BL
3d
7d
15d
N-Cur
Ctrl

.

Figure 5


Baseline
15 days
Ctrl
N-curc
13%
14%
4%
5%
20%
8%
36%
**
17%
18%
***
4%
10% ***
24%
*
17% **
10%
12%
9%
4%
8%
19%
10%
38%
15%
19%
***
7%
10% **
25%
*
16% **
8%
Actinomyces
Purple
Yellow
Green
Orange
Red
Others

3.3 PUBLICAÇÃO 3*

Title: Clinical efficacy of the local administration of curcumin in the non-surgical treatment in periodontitis: A systematic review and meta-analysis

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Keywords: Curcumin, periodontitis, systematic review, meta-analysis.

* O artigo segue as normas do periódico *Journal of Periodontal Research* para o qual será submetido.

Abstract:

Background and objective: Curcumin has anti-inflammatory and antimicrobial properties, which justify the interest in treating periodontitis, a dysbiotic condition characterized by chronic inflammation. This systematic review aims to assess the potential clinical benefits of using local applications of curcumin as adjuvants to mechanical periodontal treatment.

Material and Methods: A comprehensive search was carried out in the MEDLINE via PubMeb, Scopus, Web of Science, LILACS, BBO and Cochrane Library without restrictions and publication dates and language. In addition, the gray literature was also researched. The risk of bias tool from the Cochrane Collaboration was used for quality assessment of the studies.

Results: A total of eleven studies remained in the systematic review, one study was considered as “high” risk of bias, seven studies as “unclear” and three studies were “low” risk of bias. Nine studies presented similar data to be included in the meta-analysis**.** There are statistically significant differences indicating a beneficial effect of local application of curcumin, including: greater decrease in SBI at 30 days (MD= -0.39; 95% CI [-0.65; -0.13], p=0.003). At 90-day follow-up, local application of curcumin resulted in significant reductions in SBI (MD= -0.21; 95% CI [-0.36; -0.06], p=0.008), PPD (MD= -0.42 mm; 95% CI [-0.83; -0.01], p=0.04), and CAL (MD= -0.71 mm; 95% CI [-1.08; -0.33], p=0.0002). The significant benefit of locally-applied curcumin was sustained at 180-day follow-up for PPD (MD= -0.53 mm; 95% CI [-0.92; -0.13], p=0.008) and CAL (MD= -0.77 mm; 95% CI [-1.30; -0.25], p=0.004). No side-effects were reported. Heterogeneity among the studies ranged from low to high, which coupled with study quality issues advise caution in interpreting these results.

Conclusions: Locally-delivered curcumin as an adjunctive to non-surgical periodontal therapy results in statistically significant benefits in clinical outcomes, without adverse effects.

Main text:

1. Introduction

Periodontitis is a highly prevalent[1] chronic multifactorial inflammatory condition triggered by a dysbiosis of the dental biofilm that is associated with destruction of tooth-supporting tissues[2], which ultimately may cause tooth loss, impairing oral health-related quality of life[3].

Conventional non-surgical periodontal therapy aims to disrupt and remove microbial biofilm by scaling and root planing (SRP) complemented by implementation of an adequate regimen of self-performed oral hygiene. SRP is the gold standard in the treatment of periodontitis because of its cost-effectiveness supported by prospective clinical studies demonstrating clinical attachment gain and reductions in probing pocket depth[4] and gingival inflammation[5-9]. Notwithstanding, some periodontally-infected sites do not respond to SRP, and additional therapeutic approaches may be implemented[10]. Local[11] and systemic[12] administration of antibiotics or anti-inflammatories have been extensively investigated as adjuncts to SRP. However, these drugs may cause adverse effects and require patient compliance[12]. Some natural compounds have become a suitable alternative to antibiotics and anti-inflammatories because of their biological effects associated with lack of adverse effects. In recent decades there has been a growing interest in the local delivery of phytochemicals as adjuncts to SRP [13-19], and some of these formulations are currently commercially available (e.g. Parondontax®, GlaxoSmithKline, Middlesex, UK)[20].

Curcumin is the major biologically-active component from root extracts of the plant *Curcuma longa*. This herbaceous plant, commonly known as turmeric, is used to treat various conditions in traditional medicine in China and India. The alcoholic extract of the rhizomes of *Curcuma longa* contains three curcuminoids (curcumin or diferuloylmethane, desmethoxycurcumin, and bisdemethoxycurcumin)[21], and curcumin is the most abundant (77%)[22].

Several publications have shown its antioxidant, anti-carcinogenic, antiviral, anti-inflammatory, anti-microbial and wound-repairing properties and they have been reported in diverse diseases such as diabetes [23], cancer [24], lupus erythematosus [25], psoriasis[26], multiple sclerosis[27], osteoporosis[28], thalassemia[29], migraine[30], schizophrenia[31,32], inflammatory bowel disease[33-35], rheumatoid arthritis[36], osteoarthritis[37], obesity[38], metabolic syndrome[39], depression[40,41], lichen planus[42], stomatitis[43], gingivitis[44,45] and periodontitis[16,46-48].

Some of the anti-inflammatory properties of curcumin that can be of relevance in the treatment of periodontitis include: inhibition of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β[49,50] and matrix metalloproteinase-9 (MMP-9)[51]; reduction of inflammatory infiltrate[49,52,53]; decreasing activation of NF-kB[49,52,54]; as well as of reduction of osteoclastogenesis and inflammatory bone resorption[49,52,53,55,56].

There are also reports indicating that curcumin has antimicrobial properties, impairing biofilm formation and maturation, by reducing the prevalence of periodontal pathogens (*Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum* and *Porphyromonas gingivalis*)[57]. In vitro curcumin (at 25 ug/mL) reduced viability of *Aggregatibacter actinomycetemcomitans* [58].

These biological properties observed in vitro and in pre-clinical studies prompted clinical studies assessing the efficacy of curcumin as adjunct to periodontal treatment. These clinical studies report its antibacterial effect[47,59] and improvement of clinical parameters[60-62]; however, there is great variability in the study design, concentration of curcumin used, administration route, vehicle used, methods and outcomes of interest. As a consequence, the literature on curcumin is often times confusing with the report of conflicting results [63,64,65].

This systematic review intends to summarize the available information from randomized clinical trials (RCTs) assessing local application of turmeric or curcumin associated to SRP in periodontitis. This systematic review aims to answer the following research question: In healthy patients with periodontitis, does the local application of curcumin as an adjuvant to scaling and root planning improves the clinical outcomes?

2. Materials and Methods

2.1. Protocol and Registration

This study protocol was submitted for registration at the International Prospective Register of Systematic Reviews database (PROSPERO/Registration number: CRD42020158512) and the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were followed[66].

2.2. Information sources and search strategy

The controlled vocabulary (MeSH terms) and free keywords in the search strategy were defined based on the following PICO categories:

- Population (P): Systemically healthy adults with periodontitis
- Intervention (I): SRP plus local application of curcumin/turmeric (in any vehicle, concentration, and administration regimen)

- Comparisons (C): SRP plus local application of a placebo or positive control (SRP alone)
- Outcomes (O): Primary outcome: Changes in PPD. Secondary outcomes: Changes in clinical attachment level (CAL) and bleeding on probing (BOP)
- Study design (S): RCTs with at least 30 days of follow-up

Search strategy was applied in the following electronic databases: MEDLINE via PubMed, Scopus, Web of Science, the Latin American and Caribbean Health Sciences Literature database (LILACS), the Brazilian Library in Dentistry (BBO) and the Cochrane Library. Search strategy developed for PubMed was modified for the other databases to identify eligible studies (Table 1). In addition, manual search was made from the reference list of the primary studies and in periodontology-related journals including *Periodontology 2000, Journal of Clinical Periodontology, Journal of Dental Research and Journal of Periodontology*. There were no restrictions of publication language or publication date.

Abstracts of the annual meeting of the International Association for Dental Research (IADR) between 1990 and February 6th, 2020 were searched. Also, grey literature was explored using the database System for Information on Grey Literature in Europe. Dissertations and theses were searched using the ProQuest Dissertations and Theses Full Text databases and the 'Periodicos' theses database of CAPES Foundation in Brazil.

To identify ongoing trials, the following clinical trials registries were searched: Current Controlled Trials (www.controlled-trials.com), International Clinical Trials Registry Platform (http:// apps.who.int/trialsearch/), the ClinicalTrials.gov (www.clinicaltrials.gov), Rebec (www.rebec.gov.br) and EU Clinical Trials Register (https:// www.clinicaltrialsregister.eu).

2.3. Eligibility criteria

Inclusion Criteria

In this study, we included randomized clinical trials (RCTs) that assess healthy patients older than 18 years old diagnosed with periodontitis that received SRP alone/placebo and/or SRP plus locally delivered curcumin, either in a full-mouth experimental design or in specific selected sites in single or repeated sessions. RCTs with parallel or split-mouth design, with at least 30-day follow-up and records of clinical parameters were selected.

Exclusion criteria

Trials including (i) patients with systemic diseases; (ii) smoker patients; (iii) assessing baseline PPD values < 4mm; (iv) using systemic administration of curcumin; and (v) less than 30 days of follow-up.

2.4. Study selection and Data collection process

After retrieving papers according with the search strategy, a reference manager software (Endnote, Thomson Reuters) was used to remove duplicate references before study selection. Studies were selected in two phases. The first screened title and abstract of the retrieved papers according to the inclusion/exclusion criteria. Two reviewers (C.P.P. and K.D.C.) selected the studies independently. If the analysis of the title and abstract prevented a decision for inclusion or exclusion of the paper, the full text version was analyzed. In case of disagreement between the reviewers, a third reviewer (L.M.W.) was consulted to solve the conflict. The second phase was the analysis of the full text of the selected papers. Each paper received a study ID (author + year of publication). A table with relevant information about the study design, participants, interventions, and outcomes was constructed independently by the two reviewers (Table 2).

2.5. Risk of bias in individual study

Quality assessments of the included trials were performed by two independent reviewers (C.P.P. and K.D.C.), following the recommendations described in the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0 (http://handbook.cochrane.org)[67]. If the quality assessment of the two reviewers disagree, a third reviewer (L.M.W.) analyzed both quality assessments to resolve the conflict. There are six domains in the quality assessment: adequate sequence generation, allocation concealment, blinding of participants and personnel, blinding of the outcome examiners, completeness of outcome data and selective outcome reporting.

For each domain, the reviewers recorded “+” (low risk of bias), “-” (high risk of bias), or “?” (unclear - either lack of information or uncertainty about the potential for bias). An assessment of “low” risk in these domains indicated that the study had “low” risk of bias. If one domain was classified as “unclear” or “high” risk of bias, the study was considered as “unclear” or “high” risk of bias, respectively. If there was any disagreement between the reviewers in the assessment of these domains, it was solved through discussion or by consulting a third reviewer (L.M.W.).

2.6. Summary measures and synthesis of results

Only studies classified as “low” or “unclear” risk of bias were included in the meta-analysis. Meta-analyses of the changes in probing pocket depth, clinical attachment level and bleeding index (Sulcus Bleeding Index) between the baseline and 30-day, 90-day and180-day follow-up, were performed with a statistical software (Review Manager software, version 5.3, The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark). When the differences between baseline and follow-up values were not reported, they were calculated using these published values. Variance of the difference was calculated using the formula: Var(X-Y)=Var(X)+Var(Y)-2Cov(X,Y) where Var(X-Y) is the variance of the difference, Var(X) is the variance of the mean baseline value, Var(Y) is the variance of the mean follow-up value and Cov(X,Y)=rho x (sqrt(VarX) x sqrt(VarY)). Correlation coefficient [68] was assumed as 0.5 for all studies.

For comparisons, outcomes data were pooled, expressed and analyzed using weighted mean differences and 95% confidence intervals (CI). The study-specific estimates were pooled using random effect model. Statistical heterogeneity among trials was assessed with I^2 ($I^2 \leq 25\%$: low; I^2= 26-50%: moderate; $I^2 \geq 75\%$: high heterogeneity).

Forest Plots were created to illustrate the effects in the meta-analysis of the global estimation.

3. Results

All published studies[16,59,60,62-65,69-71] did not contain all the information needed, but e-mails were sent to corresponding authors. In addition, one unpublished study[72] was retrieved and its principal investigator was also contacted. Three[62,65,72] of the corresponding authors replied.

3.1. Study selection

After initial screening of electronic databases, 361 studies were found. In addition, one clinical trial was retrieved from the Brazilian clinical trial registry (Rebec - www.rebec.gov.br). After removal of duplicates, 209 studies were identified. After title and abstract screening, 194 studies were excluded, and the remaining 15 studies were checked for eligibility by full-text assessment. Four studies were excluded because: (i) it was not a RCT (Benly et al. 2016 [73]), follow-up period was less than 30 days (Elavarasu et al. 2016 [74]), (iii) incomplete data (Kudva et al. 2012 [48]), (iv) baseline PPD mean < 2mm, in spite of the ‘materials and methods’ section described a different

criterion (Jalaluddin et al. 2019 [75]). The remaining 11 studies were selected as eligible (Fig.1).

3.2. Characteristics of included articles

Table 2 summarizes features of the eleven selected studies. Eight studies used a split-mouth design[16,59,62-64,69,71,72], and three used a parallel design[60,65,70]. All studies were unicenter clinical trials and were performed in a University/Institute. Only two studies (18%) were not conducted in India.

Characteristics of the disease definition and selection criteria are showed in Appendix 1, A1.1. Disease was defined as “chronic periodontitis” in ten studies[16,59,60,62-65,69-71] and as “periodontitis” in one study[72]. Extension was reported in five studies, as ”localized”[60,62], “generalized”[72] and as “localized or generalized”[63,64]. Five studies mentioned disease severity as “mild to moderate”[62], as “moderate-severe”[60,65], as “severe”[16] and as “stage III”[72]. Eight studies[16,59,62,65,69-72] had no periodontal therapy in the previous 6 months as criterium for inclusion; and the remaining three studies did not describe this inclusion criteria. Nine studies[16,59,62-65,69,71,72] reported assessing the outcomes in selected sites or teeth based on clinical and radiological features, whereas two studies[60,70] did not report if full-mouth or partial-mouth measures were analyzed.

Sample size ranged from 10 [16] to 40 [62] participants, with ages ranging from 20 to 65 years old and a gender distribution of varying between 46.67% and 70% of males (Table 2).

Eight studies[16,59,62-64,69,71,72] assessed 1 site/tooth and two studies[60,65] assessed 4 to 6 sites/tooth Three studies[59,65,72] described that a single examiner assessed the clinical parameters, and eight studies[16,60,62-64,69-71] did not specify how many examiners were involved . Intra-examiner calibration was reported by only one clinical trial[72] and six studies[59,62,64,65,69,71] used custom acrylic stents to ensure reproducible measures. Furthermore, microbiological and immunological outcomes were assessed in five[59,63,69,71,72] and two[65,72] studies, respectively. Only one clinical trial assessed both microbiological and immunological outcomes simultaneously[72] (Appendix 1, Table A1.2).

As a part of the nonsurgical periodontal therapy, nine studies[59,62-65,69-72] provided oral hygiene instructions (OHI) in association with the SRP, and two studies did not mention OHI (Table 2).

All studies performed mechanical debridement before the local/topical application of curcumin. Ten RCTs applied the topical curcumin immediately after debridement, and

one parallel clinical trial mentioned that participants were instructed to apply the curcumin gel at home[70].

In nine RCTs, there were only two study groups (SRP alone or placebo / SRP + curcumin), while one had three groups (SRP alone/ SRP + curcumin/ SRP + chlorhexidine)[62] and one had four groups (SRP alone/ SRP + curcumin/ SRP + curcumin + Photodynamic Therapy[PDT] -single application/ SRP + curcumin + PDT - multiple application)[59]. Ten RCTs had SRP alone as main control group, only one study had a vehicle control (placebo)[72] (Table 2).

Regarding the product tested in experimental groups; five studies[16,59,64,65,71] employed a commercially available formulation of curcuma oral gel (Curenext - Abbot Health Care, Mumbai, India) and the remaining studies assessed: 20mg/ml turmeric gel[63], 50mg/ml turmeric chip[62], 0.05mg/ml curcumin-loaded nanoparticles[72], 1mg/ml curcumin gel[69], 20mg/ml curcumin gel[60] and one study[70] did not mention the concentration of the product (Appendix 1. Table A1.3). Only five studies[16,63-65,69] used periodontal dressing immediately after local application of turmeric or curcumin (Table 2).

Most studies used a single application of curcumin or turmeric, and multiple applications were performed in three studies. The number of applications was not reported in one study[70], one study report 4 applications[69] and one study report 3 applications[60] (Table 2).

Eight studies informed absence of adverse effects and three RCTs had no mention of assessment of adverse effects (Table 2).

3.3. Assessment of the risk of bias

The assessment of the risk of bias of the selected studies is presented in Fig. 2. Some studies randomized the treatments by coin toss[16,64,65,70,71] or by generating a computer-based random number sequence[62,72], whereas others did not report the method[59,60,63,69]. Interestingly, most of the trials did not reported the allocation concealment method, only two[62,72] did. Regard of blinding, four studies[59,62,65,72] described that participants, personnel and examiner were blind, and one[70] reported that participants were instructed to apply the compound. From the eleven studies, three trials were considered to be at “low”[62,65,72] risk of bias, seven[16,59,60,63,64,69,71] at “unclear”, and one at “high”[70] risk in the Cochrane risk of bias tool.

3.4. Meta-analysis

The meta-analyses were performed on studies classified as being at “unclear” or “low” risk of bias. One study[16] reported baseline and follow-up mean values of PPD and CAL that do not match with the mean differences also presented. Another study[70] was considered at high risk of bias. Therefore, two studies were excluded from quantitative analysis, remaining nine[59,60,62-65,69,71,72] studies in the meta-analysis.

Probing Pocket Depth

PPD reduction after 30 days was assessed by pooling data from eight studies[60,62-65,69,71,72], and the meta-analysis indicated a non-significant mean difference (MD) of -0.52 mm (95%CI [-1.63; 0.63]; I^2=100%; p=0.37) favoring to the test (SRP + curcumin/turmeric) over the control (SRP alone/SRP + placebo) groups (Fig 3.A). At 90 days, data from five comparisons[59,62,65,69,72] between test and control groups were pooled and the meta-analysis indicated a statistically significant benefit (p=0.04) for test groups (MD= -0.42 mm; 95% CI [-0.83; -0.01]), albeit with high heterogeneity among studies (I^2=85%) (Fig 3.B). A sensitivity analysis was made to detect the influence of any particular study in this heterogeneity, after removing a particular RCT[62] the heterogeneity was eliminated (I^2=0%) keeping the significance favoring the test groups (MD= -0.23 mm; 95% CI [-0.41; -0.05]). At 180 day-follow up reduction of PPD was still significant (p=0.008) and favoring the test groups (MD= -0.53 mm; 95% CI [-0.92; -0.13], without heterogeneity (I^2=0%) between the two studies[69,72] included in this analysis (Fig 3.C).

Clinical Attachment Level

Data from seven studies[62-65,69,71,72] were pooled for meta-analysis of CAL gain at 30 days follow-up, there were no significant differences (p=0.34) between the control and test groups (MD= -0.31 mm; 95%CI [-0.94; 0.32]; I^2=100%) (Fig 4.A). After excluding three RCTs[62,69,71] based on sensitivity analysis, no heterogeneity was observed (I^2=0%) and a significant (p<0.00001) reduction of CAL was observed for test groups (MD= -0.22 mm; 95%CI [-0.29; -0.16]).

For the 90-day follow-up period, data from five studies[59,62,65,69,72] were combined and meta-analysis indicated a statistically significant reduction of CAL (p=0.0002) for test groups (MD= -0.71 mm; 95% CI [-1.08; -0.33]), with moderate heterogeneity (I^2=63%) (Fig 4.B). A sensitivity analysis detected the marked influence of one study[59]; and after its removal heterogeneity was eliminated without affecting the significance

(p<0.00001) of the mean difference in CAL favoring the test groups (MD= -0.94 mm; 95%CI [-1.14; -0.74]).

Changes in CAL at 180-day follow-up, data from two comparisons[69,72] between test and control groups were combined, and the meta-analysis indicated significant reduction in CAL (p=0.004) in the test groups (MD= -0.77 mm; 95% CI [-1.30; -0.25]), with low heterogeneity (I^2=21%) (Fig 4.C).

Sulcus Bleeding Index

Data from three studies[63,65,69] comparing changes on SBI (30 days – baseline) between test and control groups were combined and the meta-analysis indicated a statistically significant overall reduction in SBI in the test groups (MD= -0.39; 95% CI [-0.65; -0.13], p=0.003), with moderate heterogeneity (I^2=65%) (Fig 5.A). A sensitivity analysis detected the influence of one study[59] and after its removal, heterogeneity was eliminated without affecting the significance (p=0.01) of the mean difference in SBI favoring the test group (MD= -0.25; 95%CI [-0.44; -0.05]).

At 90-day follow up, data from three comparisons[59,65,69] between test and control groups were combined and the meta-analysis indicated significant reductions in SBI (p=0.008) for test groups (MD= -0.21; 95% CI [-0.36; -0.06]), with low heterogeneity (I^2=5%) (Fig 5.B).

4. Discussion

To the best of our knowledge, this is the first meta-analysis assessing clinical studies on the efficacy of locally-applied turmeric/curcumin associated with SRP in the treatment of periodontitis. Our data show statistically significant reductions PPD reductions (-0.42mm and -0.53mm) and CAL gains (-0.71mm and -0.77mm) at 90- and 180-days of follow up, respectively. Locally-applied curcumin formulations also decreased gingival inflammation, as indicated by significant reduction of gingival bleeding, assessed by SBI at 30- (-0.39) and 90-day (-0.21) of follow-up. Importantly, no study reported adverse effects associated with the local application of curcumin formulations, supporting the safety in the clinical use of this compound.

Only one systematic review[76] of clinical studies assessing of curcumin as adjuvant to non-surgical periodontal therapy has been published without providing a meta-analysis, which limits the possibility of comparing our results. Unlike our review, this study also selected RCTs comparing curcumin to other local antimicrobial agents, and nine studies were included in total. Of those studies, four[60,63,69,71] were considered in our review, and the other five did not meet the inclusion criteria, as they did not have

a group that was only treated with RAR with or without placebo. These other studies compared the effect of local curcumin application with the use of chlorhexidine[61,77,78], metronidazole[79], and ordinazole[80].

Our data is similar to a previous meta-analysis[81] that indicated that locally delivered phytotherapeutics used as an adjuvant to SRP improve clinical outcomes compared to SRP alone, with significant reduction in PPD (0.65mm) and gain of CAL (0.79mm) at 3-month follow-up. These clinical benefits may be related with anti-inflammatory, antimicrobial or a combination of these effects. In fact, a meta-analysis[11] indicate that SRP associated with locally-delivered antimicrobials promote statistically significant reduction in PPD (0.365 mm) and gain of CAL (0.263 mm) at 6-9 month follow up in comparison with SRP alone or SRP+placebo.

In spite of the statistically significant clinical benefits observed at 90- and 180-day follow up, the overall magnitude of the improvement in PPD and CAL was small (<1mm); and considering relatively poor quality of most of the studies reviewed, the clinical relevance of this improvement is questionable. The presentation of the data in the studies reviewed preclude the assessment of clinical improvement by cut-off values (e.g., proportion of sites presenting $\geq$ 2 mm of improvement), which is a limitation of this study.

Heterogeneity among the reviewed studies may derive from several factors, such as the experimental designs, inclusion and exclusion criteria, use of proper randomization and blinding methods, adequate sample size, the use of a placebo / vehicle control, varying doses, different formulations/vehicles, frequency of application and even the geographic origin of the plant and method of preparation of the extract. Given the numerous possible sources of heterogeneity in the reviewed studies, we repeated the analysis after removing the study contributing the most to the heterogeneity score and, invariably, the direction of the result and statistical significance were maintained. We interpret this finding as supportive to a significant, albeit of small magnitude, beneficial effect of local delivery of curcumin formulation as adjuvant to non-surgical periodontal therapy.

The split-mouth design was the most frequently used and has the advantage of avoiding interference of genetic/phenotypical inter-subject variability influencing the outcomes, besides allowing for an adequate statistical power with a smaller number of participants in comparison with the parallel design. On the other hand the split-mouth design is prone to biased estimates of treatment efficacy if any “leakage” occurs due

to carry-across effects[82]. Both, split-mouth (seven)[59,62-64,69,71,72] and parallel (one)[58] studies were included and combined in this meta-analysis, adding important heterogeneity. Moderate and high heterogeneity was observed when both study designs were pooled and analyzed. Interestingly, either no heterogeneity (for PPD) or low (for CAL) heterogeneity was observed at 180-day follow up. The sustained significance of the clinical improvements associated with local application of curcumin at 180-day follow up further supports a beneficial effect of curcumin as a local adjuvant to non-surgical periodontal treatment.

It is important to note that only one study[72] included a placebo control, which is important for the blinding of participants, for the assessment of placebo/nocebo effects and also of possible vehicle-associated adverse effects[83]. Clinical assessment protocol may also influence the estimated treatment efficacy. In this meta-analysis, seven of nine studies reported using a method (inter/intra-examiner calibration or use of acrylic stents) to ensure reproducible measures; and all performed a partial-mouth assessment. The partial-mouth assessment of selected sites is not necessarily a drawback in the experimental design, considering that local application of curcumin formulations may be most beneficial in recurrent/recalcitrant sites or in sites with greater disease severity.

In addition, after sensitivity analysis, we detected that a specific study[62] generated this variability twice. This RCT included 40 patients and applied the highest concentration of turmeric (50mg/ml) of all reviewed studies using a slow-release vehicle (94% of turmeric was released at 72h). These peculiarities (high dose, slow-releasing vehicle) may have influenced the outcomes in this study and skewed the results of the meta-analysis. Dosing regimens and vehicles varied in the reviewed studies: six studies tested 10-50mg/ml *Curcuma Longa*/turmeric extract gel or chip, and four RCTs applied 0.05-20mg/ml curcumin (loaded in PGLA nanoparticles or in gel formulations). Moreover, some studies used turmeric extracts, in which the relative abundance of curcumin may vary depending on the geographic origin of the plant and protocol for preparation of the extract, possibly affecting its biological effect[84].

An important of this meta-analysis is the quality of the RCTs analyzed. Most of the reviewed studies was considered as having “unclear” risk of bias, and only three of them were classified as “low risk” of bias. Consequently, the estimated added effect should be considered as ‘moderate level evidence’. Thus, large-scale and better

conducted randomized clinical trials are needed to verify and conclude the evidence-based clinical efficacy of curcumin as an adjunct to the SRP in periodontal treatment.
Due to its antimicrobial and immune-inflammatory properties widely reported in preclinical studies, curcumin may be a complementary alternative to periodontal therapy, modulating the host response and the microbial biofilm, especially, in sites with recurrent/severe disease in healthy or systemically-compromised subjects[46,85].
These biological effects coupled with the limitations in bioavailability after systemic administration makes local application particularly promising. New vehicles and/or chemical modifications on the structural composition of curcumin should be investigated to maximize its biological effects and enhance its clinical efficacy.
We conclude that local administration of curcumin as an adjunct to the non-surgical periodontal therapy promotes statistically significant reduction in PPD and gain in CAL sustained up to 180-days of follow up, and significant decrease in SBI at 90-day of follow up, with no adverse effects. However, considering the magnitude of the clinical improvements, the relatively poor quality of the available studies and the high efficacy of non-surgical periodontal treatment, we think that this evidence is insufficient to recommend and justify its clinical use in systemically-healthy patients or in previously untreated sites.

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Conflict of Interest

Authors declare no conflict of interest related to this study

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Tables, figures and figures legends

Table 1. Electronic database and search strategy

Pubmed		
#1 ((((((((((((((((((((((((((periodontitis[MeSH Terms]) OR periodontal diseases[MeSH Terms]) OR chronic periodontitis[MeSH Terms]) OR periodontal pocket[MeSH Terms]) OR dental scaling[MeSH Terms]) OR root planing[MeSH Terms]) OR subgingival curettage[MeSH Terms]) OR periodontal debridement[MeSH Terms]) OR dental prophylaxis[MeSH Terms]) OR periodontitis[Title/Abstract]) OR "periodontal diseases"[Title/Abstract]) OR "chronic periodontitis"[Title/Abstract]) OR "periodontal pocket"[Title/Abstract]) OR "periodontal disease"[Title/Abstract]) OR "adult periodontitis"[Title/Abstract]) OR "dental scaling"[Title/Abstract]) OR "root planing"[Title/Abstract]) OR "subgingival curettage"[Title/Abstract]) OR "periodontal debridement"[Title/Abstract]) OR "dental prophylaxis"[Title/Abstract]) OR "periodontal recall"[Title/Abstract]) OR "periodontal therapy"[Title/Abstract]) OR SRP[Title/Abstract]) OR "periodontal treatment"[Title/Abstract]) OR "scaling root planing"[Title/Abstract]) OR "ultrasonic scaler"[Title/Abstract]) OR "nonsurgical periodontal"[Title/Abstract]))	#2 (((((((curcumin[MeSH Terms]) OR curcuma[MeSH Terms]) OR curcumin[Title/Abstract]) OR curcuma[Title/Abstract]) OR "turmeric"[Title/Abstract]) OR "curcuminoid synthase"[Title/Abstract]) OR "curcuma longa"[Title/Abstract]) OR diferuloylmethane[Title/Abstract]))	#3 (((((randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized controlled trials[mh] OR random allocation[mh] OR double-blind method[mh] OR single-blind method[mh] OR clinical trial[pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR (placebos[mh] OR placebo*[tw] OR random*[tw] OR research design[mh:noexp] OR comparative study[pt] OR evaluation studies as topic[mh] OR follow-up studies[mh] OR prospective studies[mh] OR control*[tw] OR prospective*[tw] OR volunteer*[tw]) NOT (animals[mh] NOT humans[mh]))))))
#1 AND #2 AND #3 50 results (02.06.20)		
Embase		
#1 'periodontitis'/exp OR 'periodontal diseases'/exp OR 'dental prophylaxis'/exp OR periodontitis:ab,ti OR 'periodontal pocket':ab,ti OR 'periodontal diseases':ab,ti OR 'periodontal disease':ab,ti OR 'dental scaling':ab,ti OR 'root planing':ab,ti OR 'subgingival curettage':ab,ti OR 'periodontal debridement':ab,ti OR 'dental prophylaxis':ab,ti OR 'periodontal recall':ab,ti OR 'periodontal therapy':ab,ti OR srp:ab,ti OR 'periodontal treatment':ab,ti OR 'ultrasonic scaler':ab,ti OR 'subgingival curettage'/exp	#2 'curcumin'/de OR 'curcuma'/exp OR curcumin:ab,ti OR curcuma:ab,ti OR 'turmeric':ab,ti OR 'curcuminoid synthase':ab,ti	#3 'randomized controlled trial'/exp OR 'controlled clinical trial'/exp OR 'double blind procedure'/exp OR 'single blind procedure'/exp OR 'clinical trial'/exp OR 'placebo'/exp OR 'randomized controlled trial':ab,ti OR 'controlled clinical trial':ab,ti OR 'double blind procedure':ab,ti OR 'single blind procedure':ab,ti OR 'clinical trial':ab,ti OR placebo:ab,ti
#1 AND #2 35 results (02.06.20)		
Cochrane		
#1: MeSH descriptor: [Periodontitis] explode all trees #2: MeSH descriptor: [Periodontal Diseases] explode all trees #3: MeSH descriptor: [Subgingival Curettage] explode all trees #4: MeSH descriptor: [Dental Prophylaxis] explode all trees #5: ("periodontal treatment"):ti,ab,kw OR ("ultrasonic scaler"):ti,ab,kw OR ("scaling root planing"):ti,ab,kw OR (SRP):ti,ab,kw OR ("periodontal therapy"):ti,ab,kw (Word variations have been searched) #6: #1 OR #2 OR #3 OR #4 OR #5	#7: MeSH descriptor: [Curcumin] explode all trees #8: MeSH descriptor: [Curcuma] explode all trees #9: (curcumin):ti,ab,kw OR (curcuma):ti,ab,kw OR ("Curcuma longa"):ti,ab,kw OR (turmeric):ti,ab,kw OR (curcuminoid):ti,ab,kw (Word variations have been searched) #10: #7 OR #8 OR #9	
#11: #6 AND #10 33 results (02.06.20)		
Web of Science		
#1 TOPIC: (periodontitis) *OR* TOPIC: ("periodontal diseases") *OR* TOPIC: ("periodontal disease") *OR* TOPIC: (chronic periodontitis) *OR* TOPIC: ("periodontal pocket") *OR* TOPIC: ("adult periodontitis") *OR* TOPIC: ("dental scaling") *OR* TOPIC: ("root scaling") *OR* TOPIC: ("subginginval curettage") *OR* TOPIC: ("periodontal debridement") *OR* TOPIC: ("dental prophylaxis") *OR* TOPIC: ("periodontal recall") *OR* TOPIC: ("periodontal therapy") *OR* TOPIC: ("periodontal treatment") *OR* TOPIC: ("scaling root planing") *OR* TOPIC: ("ultrasonic scaler") *OR* TOPIC: ("non surgical periodontal") Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED Timespan=All years	#2 TOPIC: (curcum*) *OR* TOPIC: (curcuma) *OR* TOPIC: ("curcuma longa") *OR* TOPIC: (turmeric) *OR* TOPIC: ("curcuminoid synthase") *OR* TOPIC: (diferuloylmethane) Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED Timespan=All years	
#1 AND #2 Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED Timespan=All years 95 results (02.06.20)		
Scopus		
#1 ((TITLE-ABS-KEY (periodontitis) OR TITLE-ABS-KEY ("periodontal diseases") OR TITLE-ABS-KEY ("chronic periodontitis") OR TITLE-ABS-KEY ("periodontal pocket") OR TITLE-ABS-KEY ("dental scaling") OR TITLE-ABS-KEY ("root scaling") OR TITLE-ABS-KEY ("subgingival curettage") OR TITLE-ABS-KEY ("periodontal debridement") OR TITLE-ABS-KEY ("dental prophylaxis") OR TITLE-ABS-KEY ("periodontal recall") OR TITLE-ABS-KEY ("periodontal therapy") OR TITLE-ABS-KEY (srp) OR TITLE-ABS-KEY ("periodontal treatment") OR TITLE-ABS-KEY ("scaling root planing") OR TITLE-ABS-KEY ("ultrasonic scaler") OR TITLE-ABS-KEY ("nonsurgical periodontal")))	#2 ((TITLE-ABS-KEY (curcumin) OR TITLE-ABS-KEY (curcuma) OR TITLE-ABS-KEY ("turmeric") OR TITLE-ABS-KEY ("curcuminoid synthase") OR TITLE-ABS-KEY ("curcuma longa") OR TITLE-ABS-KEY (diferuloylmethane)))	
#1 AND #2 145 results (02.06.20)		
LILACS/BBO		
#1 periodontitis	#2 curcumin	
#1 AND #2 3 results (02.06.20)		

Table 2. Characteristics of the studies, participants, interventions and outcomes

Study ID	Study Design	Subjects' age mean ± SD [range]	Sample size [Gender distribution]	Country/ Setting	Control Group	Test Group/AF	Clinical parameters	Follow-up	Side-effect
Anuradha et al 2015	Split-mouth	NR [25-60y]	30 patients [NR]	India/ University	SRP+OHI	SRP + 10mg/mL C. Longa extract gel (Curenext) + PerioD x 7 days + OHI/ Single application	PPD CAL GI: Loe and Silness, 1963 PI: Turesky-Gilmore-Glickman modification of Quigley-Hein, 1970	30 and 45 days	No
Behal et al 2011	Split-mouth	NR	30 patients; [NR]	India/ University	SRP+OHI	SRP + 2% turmeric gel + PerioD x 7 days+ OHI/ Single application	PPD CAL SBI: Muhlemann and Son, 1971 GI: Loe and Silness,1963 PI: Turesky-Gilmore-Glickman modification Quigley-Hein, 1970	30 and 45 days	No
Bhatia et al 2014	Split-mouth	NR [21-45y]	25 patients [15 males (60%) - 10 females (40%)]	India/ University	SRP+OHI	SRP + 1mg/ml curcumin gel + PerioD + OHI/ Multiple application (BL, 1, 3 and 6 months)	PPD CAL SBI: Muhlemann and Son, 1971 PI: Silness and Loe, 1964	30, 90 and 180 days	No
Dave et al 2019	Parallel	NR [20-59y]	20 patients [NR]	India/ University	SRP+OHI	SRP + curcuma gel + OHI / Multiple application (NR)	PPD CAL SBI PI: NR	30 and 60 days	No
Kaur et al 2018	Parallel	NR [20-65y]	15 patients [20 males (69%) – 9 females (31%)]*	India/ University	SRP+OHI	SRP +10mg/mL C. Longa extract gel (Curenext) + PerioD x 7 days + OHI/ Single application	PPD RAL PI: Silness and Loe, 1964) SBI: Muhlemann and Son, 1971	30 and 90 days	No
Nagasri et al 2015	Split-mouth	38 ±4 [35-60y]	30 patients [18 males (60%) - 12 females (40%)]	India/ University	SRP+OHI	SRP + 10mg/mL C. Longa extract gel (Curenext) +OHI/ Single application	PPD CAL GI: Loe and Silness, 1963 PI: Silness and Loe, 1964	30 days	NR
Nasra et al 2017	Parallel	NR [35-55y]	10 patients [NR]	Egypt/ University	SRP	SRP + 2% Curcumin gel Multiple application/ (once a week per 3 weeks)	PPD Bleeding Index: Checchi, 2009 PI: Loe and Silness, 1964	30 days	No
Perez-Pacheco et al 2020	Split-mouth	49±6 [37-62y]	20 patients [14 males (70%) - 6 females (30%)]	Brazil/ University	SRP + vehicle + OHI	SRP + 0.05mg/ml curcumin-loaded nanoparticles +OHI/ Single application	PPD CAL BOP GBI: Ainamo & Bay, 1975 PI: O'Leary-Drake-Naylor, 1972	30, 90 and 180 days	No
Raghava et al 2019	Split-mouth	NR [20-45y]	10 patients [5 males (50%)-5 females (50%)]	India/ University	SRP	SRP + 10mg/mL C. Longa extract gel (Curenext) + PerioD x 7 days/ Single application	PPD CAL GI: Loe and Silness, 1963 PI: Silness and Loe, 1964	30 days	NR
Singh et al 2018	Split-mouth	34 [30-50y]	40 patients; [22 males (55%) - 18 females (45%)]	India/ University	SRP+OHI	SRP + 5% turmeric chip + OHI/ Single application	PPD RAL GI: NR PI: NR	30 and 90 days	No
Sreedhar et al 2015	Split-mouth	48.73±3.5 [35-55y]	15 patients [7 males (46.67%) - 8 females (53.33%)]	India/ Institute	SRP+OHI	SRP + 10mg/ml C. Longa extract (Curenext) + OHI/ Single application	PPD CAL SBI: Muhlemann and Son, 1971 PI: Silness and Loe, 1964	30 and 90 days	NR

ID: identification; **SD:** standard deviation; **NR:** not reported; **SRP:** Scaling root and planning; **OHI:** Oral hygiene instructions; **PPD:** Probing Pocket Depth; **CAL:** Clinical Attachment Loss; **GI:** Gingival Index; **PI:** Plaque index; **SBI:** Sulcus bleeding index; **BOP:** Bleeding on probing; **GBI:** Gingival Bleeding Index; **BL:** Baseline; **AF:** application frequency; **PerioD:** Periodontal dressing; *Gender proportion after drop-outs.

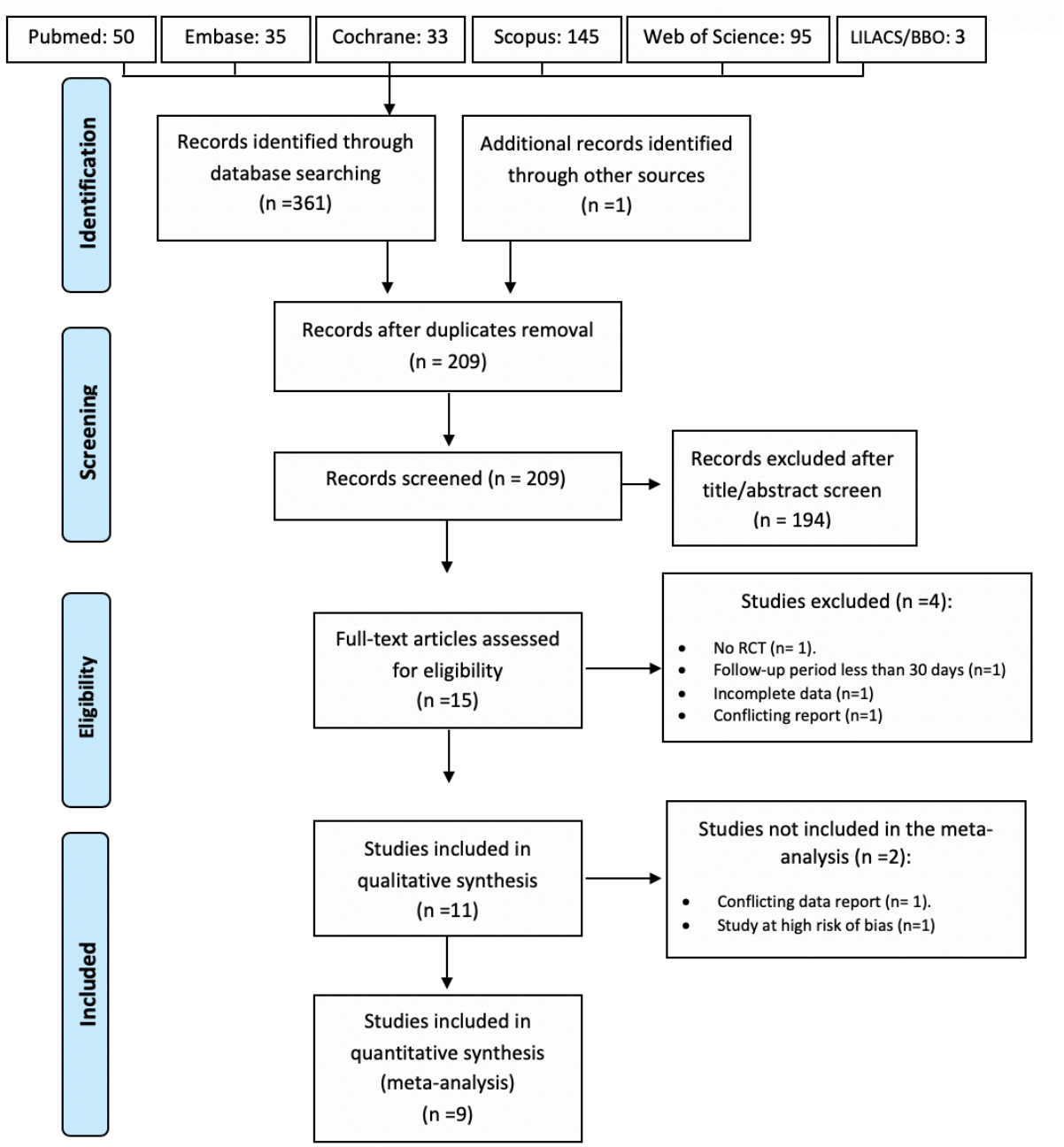

Figure 1 – Flow diagram of study identification

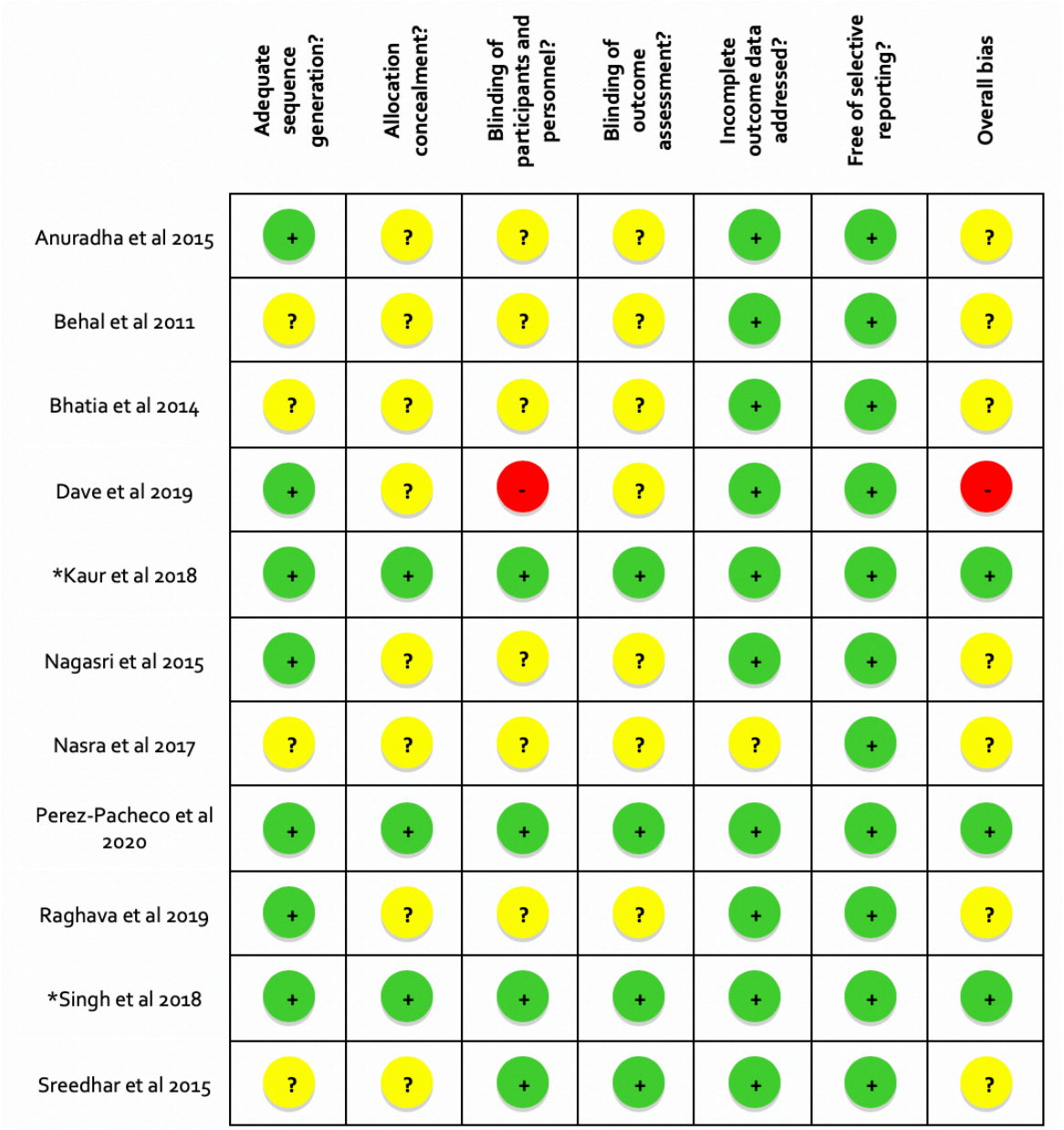

Figure 2– Summary of the risk of bias assessment according to the Cochrane Collaboration tool.

*Authors provided additional information

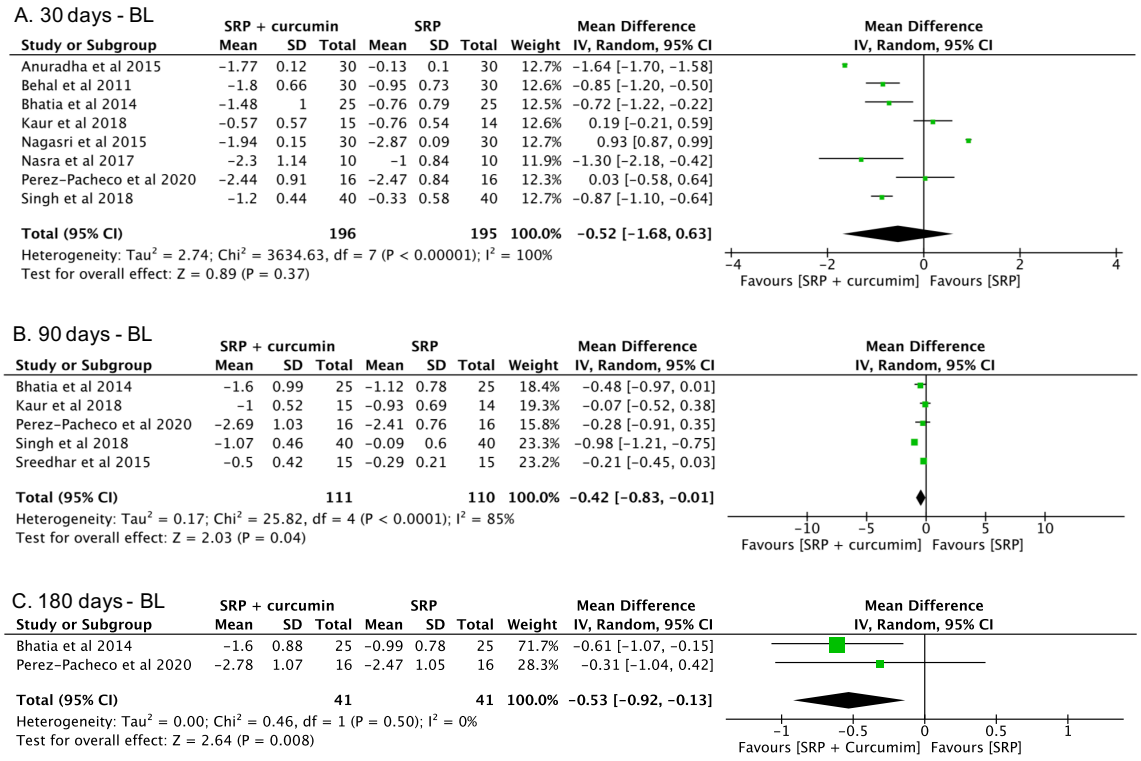

Figure 3. Forest plot of meta-analysis assessing probing pocket depth reduction from selected studies in the different periods (A, B, C).
SRP: Scaling and root planing; BL: Baseline; CI: Confidence Interval

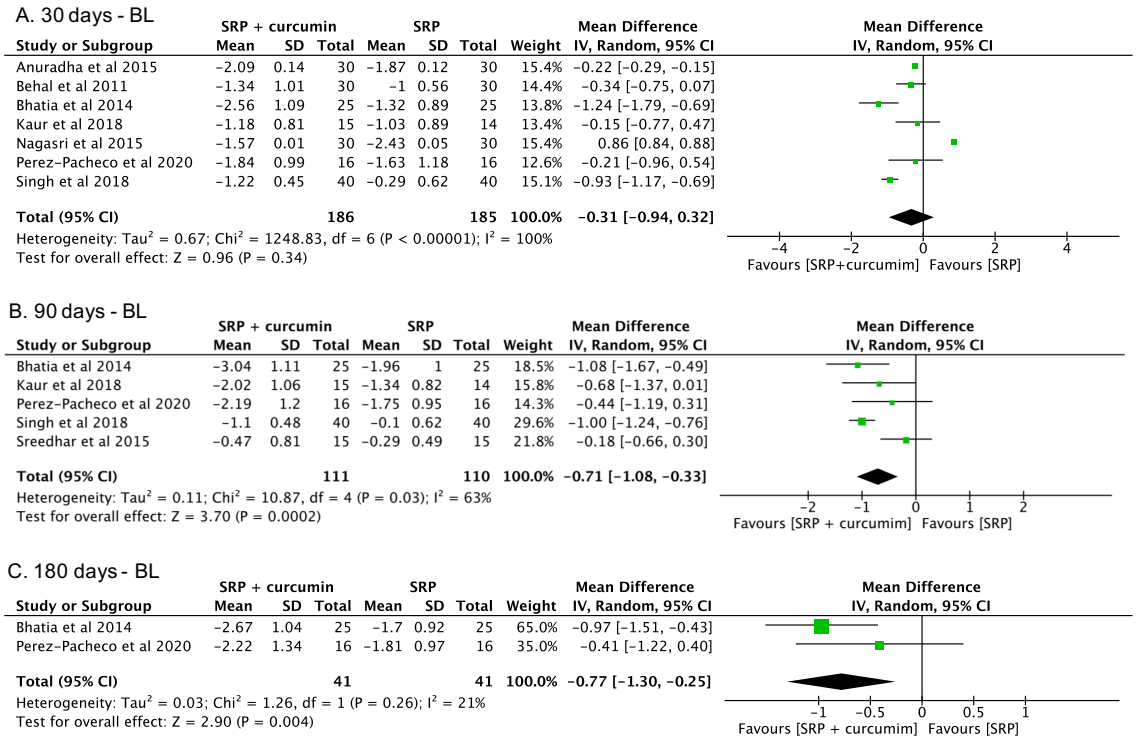

Figure 4. Forest plot of meta-analysis assessing clinical attachment level gain from selected studies in the different periods (A, B, C).
SRP: Scaling and root planing; BL: Baseline; CI: Confidence Interval

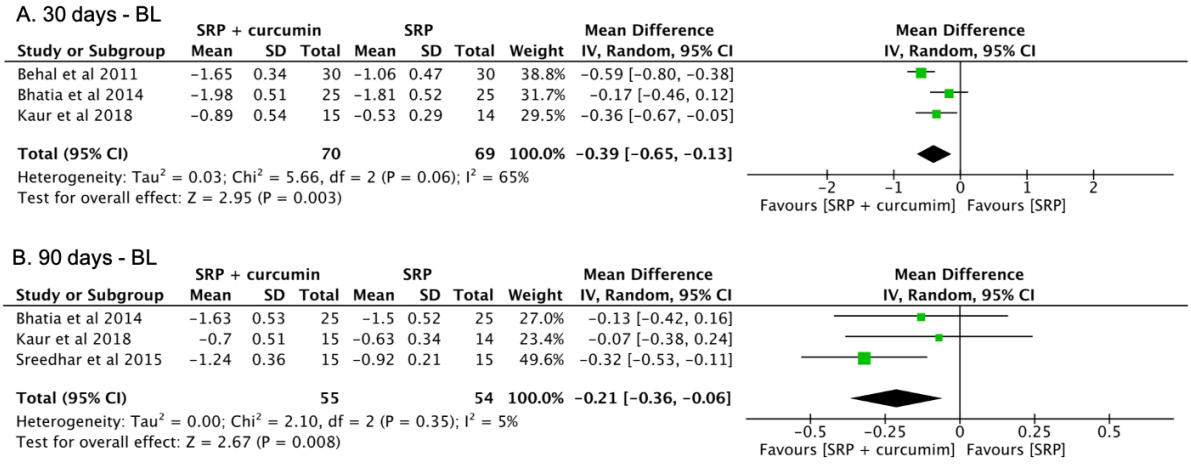

Figure 5. Forest plot of meta-analysis assessing sulcus bleeding index from selected studies in the different periods (A, B).
SRP: Scaling and root planing; BL: Baseline; CI: Confidence Interval

Appendix 1

Table A1.1. Selection criteria and periodontitis definition

Study ID	Previous PT	Type/Extension	Severity	FM/PM	Description
Anuradha et al 2015	NR	Chronic/ Localized or generalized	NR	PM - 2 sites	PDD of 5-7 mm in two non-adjacent sites in different quadrants. Clinical and rx features were considered.
Behal et al 2011	NR	Chronic/ Localized or generalized	NR	PM - 2 sites	PDD 5-7mm in two non-adjacent sites in different quadrants
Bhatia et al 2014	No PT in previous 6 months	Chronic/ NR	NR	PM - 2 sites	PPD of >5mm in two sites in contralateral quadrants
Dave et al 2019	No PT in previous 6 months	Chronic/ NR	mild	NR	NR
Kaur et al 2019*	No PT in previous 6 months	Chronic/ NR	Mod-sev	PM- 4 teeth	PPD ≥ 5mm on at least 4 sites in one or both arches
Nagasri et al 2015	No PT in previous 6 months	Chronic/ NR	NR	PM- 2 sites	PPD of >5 mm and radiographic evidence of bone loss
Nasra et al 2017	NR	Chronic/ Localized	Mod-sev	NR	PPD >5 mm in addition to marks of BOP
Perez-Pacheco et al 2020	No PT in previous 6 months	Periodontitis/ Generalized	Stage III	PM- 4 sites	PPD ≥ 5mm on at least 2 non-adjacent sites in 2 different quadrants, with BOP and Rx bone loss
Raghava et al 2019	No PT in previous 6 months	Chronic/ NR	Severe	PM-2 sites	PPD of ≥5 mm
Singh et al 2018	No PT in previous 6 months	Chronic/ Localized	mild-to-moderate	PM- 3 sites	3 test sites with PPD of 5– 8 mm
Sreedhar et al 2015	No PT in previous 6 months	Chronic/ NR	NR	PM- 4 sites	"at least one tooth with PPD ≥5 mm in each quadrant"

NR: not reported; PT: periodontal treatment; Mod: moderate; Sev: severe or advanced; PM: partial-mouth (selected sites); FM: full-mouth (all sites); PPD: probing pocking depth; BOP: bleeding on probing; Rx: radiographic
*Author provided information about the number of teeth assessed by group

Appendix 1

Table A1.2. Clinical outcome assessment and other outcomes.

Study ID	Sites/ tooth	No. examiners	Calibration	Probing type	Probing quality	Analysis	Other outcomes
Anuradha et al 2015	1	NR	NR	Manual (UNC- 15)	Stent	Site-based	No
Behal et al 2011	1	NR	NR	Manual (William)	NR	Site-based	Microbiologic
Bhatia et al 2014	1	NR	NR	Manual	Stent	Site-based	Microbiologic
Dave et al 2019	NR	NR	NR	Manual (UNC- 15)	NR	NR	No
Kaur et al 2018*	4	1	NR	Manual (UNC- 15)	Stent	Tooth-based	Immunologic
Nagasri et al 2015	1	NR	NR	Manual (Williams)	Stent	Site-based	Microbiologic
Nasra et al 2017	6	NR	NR	Manual	NR	NR	No
Perez-Pacheco et al 2020	1	1	Yes	Manual (UNC- 15)	NR	Site-based	Immunologic Microbiologic
Raghava et al 2019	1	NR	NR	Manual (UNC-15)	NR	Site-based	No
Singh et al 2018	1	NR	NR	Manual (UNC-15)	Stent	Site-based	No
Sreedhar et al 2015	1	1	NR	Manual (UNC-15)	Stent	Site-based	Microbiologic

NR: not reported;
*Author provided information about the number of sites/tooth considered for analysis

Appendix 1

Table A1.3. Features of the tested products.

Study ID	Descriptive name	Brand name/s	Company/Provider	Concentration/Composition
Anuradha et al 2015	Curcuma oral gel	Curenext	Abbott Health Care	10mg/ml *C. longa* / *C. longa* extract
Behal et al 2011	Turmeric gel	No brand	The Poona College of Pharmacy, Pune, Maharashtra, India.	2% turmeric (20mg/ml turmeric) / "The composition of the gel was turmeric extract, 2%; pluronic polymer, 20%; and water q.s. N-benzoyl-L-arginine–p-nitroanilide (BAPNA; Sigma, St. Louis, Missouri, USA)".
Bhatia et al 2014	Curcuma oral gel	No brand	NR	1mg/ml curcumin / "The ingredients of curcumin gel were Curcumin (Natural Remedies Pvt Limited), Pluronic F-127 (Sigma-Aldrich USA) and Double Distilled water".
Dave et al 2019	Curcuma gel	NR	NR	NR
Kaur et al 2018	Curcuma oral gel	Curenext	Abbott Healthcare Pvt. Ltd., Haryana	10mg/ml *C. longa* / *C. longa* extract
Nagasri et al 2015	Curcuma oral gel	Curenext	Abbott Healthcare Pvt. Ltd., 4. Corporate Park, Sion Trombay Road, Chembur, Mumbai 400 071, India.	10mg/ml *C. longa* / *C. longa* extract
Nasra et al 2017	Curcumin gel	No brand	NR	2% curcumin (20mg/ml curcumin) / "Curcumin Cur (Hebei Food Additive Co., Ltd, China) (purity 95%), polyethylene glycol (PEG) 400, potassium di-hydrogen phosphate and sodium lauryl sulfate (SLS) (El-Nasr Pharmaceutical Co., Egypt) and tri-ethanol amine (TEA) (Nice Chemicals, Pvt Ltd, Kochi, India), Pluronic 127 (Kolliphore 407) (Kind Gift from BASF, Ludwigshafen, Germany), PEG 7-glyceryl-cocoate (Galaxy, Navi, Mumbai, India), Carbopol P 934 (Lubrizol, Belgium.) "
Perez-Pacheco et al 2020	Curcumin gel	No brand	Department of Chemistry, University of Sao Paulo at Ribeirao Preto	0.05 mg/ml curcumin-loaded nanoparticles / Curcumin (Sigma-Aldrich Co. cat# C1386, Lot# 081M1611V) + 1:1 ratio of polylactic and polyglycolic acids (Sigma-Aldrich Co)
Raghava et al 2019	Curcuma oral gel	Curenext	Abbott Healthcare Pvt. Ltd., Haryana	10mg/ml *C. longa* / *C. longa* extract
Singh et al 2018	Curcumin chip	No bfrand	NR	5% turmeric (50mg/ml turmeric) / "A biodegradable 1000mg hydroxy propyl cellulose matrix with 5% turmeric by weight"
Sreedhar et al 2015	Curcuma oral gel	Curenext	Abbott Healthcare Pvt. Ltd. Mumbai, India.	10mg/ml *C. longa* / *C. longa* extract

NR: not reported; *C. longa: Curcuma longa*

4 DISCUSSÃO

A repetida administração local de curcumin veiculado por nanopartículas como adjuvante à remoção do estímulo indutor de doença favoreceu significativamente o reparo do osso alveolar e a produção de matriz extracelular em periodontite experimental induzida em ratos. Porém, uma única aplicação local de curcumin no mesmo veículo e concentração não adicionou melhoras significativas nos parâmetros clínicos, microbiológicos e immunológicos em pacientes sistémicamente saudáveis submetidos a RAR. Estes resultados do estudo clínico discordam da literatura avaliada pela revisão sistemática e metanálise, a qual indicou benefícios clínicos significativos, com redução significativa da profundidade de sondagem e um ganho do nível de inserção clínica aos três e seis meses, e redução da inflamação gengival no primeiro e terceiro meses. É importante ressaltar que não houve efeitos adversos associados à aplicação local do curcumin nos estudos pré-clínico e clínico, bem como não há relatos de efeitos adversos nos ensaios clínicos incluídos na revisão sistemática, apoiando a segurança deste composto. A segurança no uso clínico é um aspecto importante, pois possibilita a investigação de veículos, posologias e dosagens/concentrações alternativas.

No estudo in vivo, o favorecimento do reparo dos tecidos periodontais foi associado à redução do infiltrado inflamatório, que é fato comumente observado em estudos pré-clínicos de desenho experimental “preventivo”, em que a aplicação de curcumin se dá na presença continuada do estímulo indutir de doença. Considerando o modelo experimental utilizado em que o curcumin foi aplicado após a remoção do estímulo indutor de doença, os resultados sugerem um efeito biológico do curcumin sobre células estromais não inflamatórias, como fibroblastos e osteoblastos. Considerando que o processo de resolução da inflamação participa ativamente do reparo tecidual, não podemos descartar um efeito do curcumin também sobre o fenótipo de macrófagos (por exemplo) e sobre a expressão de mediadores de reparo produzidos por tipos celulares imunes participantes do processo de resolução/reparo, como resolvinas e lipoxinas. Há carência de informações sobre os efeitos do curcumin no processo de reparo, assim estudos subsequentes deverão explorar os mecanismos biológicos envolvidos, incluindo as possíveis influências sobre diferenciação, migração e fenótipo celular, relevantes no processo de reparo. A investigação de diferentes concentrações do curcumin, bem como de veículos alternativos que

proporcionem liberação controlada e sustentada também podem contribuir para maximização do resultado biológico de promoção do reparo.

A tradução dos resultados promissores obtidos em estudos pré-clínicos para aplicações clínicas é uma constante do método científico, assim não é característica peculiar dos estudos com curcumin ou quaisquer outros fitoterápicos. Desta forma, a discrepância entre a magnitude do benefício observado no estudo pré-clínico e os resultados do estudo clínico podem estar relacionadas à limitações intrínsecas do modelo experimental animal, porém existem outros aspectos a serem considerados: influências do *background* genético variável de susceptibilidade à doença periodontal em humanos em contraste à maior "homogeneidade" das características genéticas de animais de uma mesma linhagem; a influência de aspectos comportamentais relacionados aos hábitos de higiene bucal em humanos; a concentração de curcumin efetivamente alcançada nos tecidos periodontais considerando as dimensões anatômicas e volume dos sítios de interesse em animais e em humanos; a diferença na frequência de aplicação local do curcumin; e a notável efetividade do tratamento não cirúrgico na amostra de voluntários incluídos no estudo clínico, a qual dificulta a detecção de efeitos benéficos aditivos.

A abordagem experimental utilizada no estudo clínico (uma única aplicação) tem como vantagem principal a conveniência/aplicabilidade clínica, porém muito provavelmente limitou os efeitos biológicos que poderiam ser favorecidos por aplicações repetidas, como sugerem os resultados do estudo pré-clínico. De fato, estudos clínicos em que múltiplas aplicações de curcumin foram realizadas relatam melhoras clínicas significativas[77-79]. Embora os resultados destes estudos sejam mais favoráveis do que no ensaio clínico controlado randomizado que faz parte deste trabalho, é preciso considerar os controles positivos e negativos (ou a ausência destes controles) utilizados nos diferentes estudos. Assim, existem estudos comparando uma única aplicação de curcumin veiculado em gel associada ao tratamento não-cirúrgico com outros agentes antimicrobianos locais como clorexidina[74] e ordinazol[80], mas que não incluem um grupo controle negativo (tratamento não cirúrgico apenas, com e/ou sem aplicação do veículo/placebo).

Apesar dos resultados do ensaio clínico não indicarem diferenças estatisticamente significativas nos níveis protéicos das citocinas inflamatórias no fluido subgengival segundo a aplicação local do curcumin, observou-se uma maior redução de citocinas inflamatórias (IL-6 e TNF-α) nos sítios experimentais, tratados com aplicação tópica

de curcumin. De forma similar, a análise microbiológica apresentou uma diminuição não estatisticamente significativa nas contagens de *Porphyromonas gingivalis* aos 15 dias, e aumento significativo na contagem de espécies do complexo púrpura (*Veillonella parvula*) aos 7 dias; enquanto os sítios-controles que receberam o veículo, o aumento nas contagens desses microrganismos só atingiu significância estatística aos 15 dias. Além disso, apenas o grupo controle apresentou uma significativa recolonização do *Aggregatibacter actinomycetemcomitans*, entre 3 e 15 dias. Considerados coletivamente, estes resultados do ensaio clínico podem ser interpretados como sugestivos da atividade anti-inflamatória/antimicrobiana do curcumin aplicado localmente, e indicadores de um potencial promissor como adjuvante do tratamento periodontal. A ausência de diferenças estatisticamente significativas pode estar relacionada ao tamanho da amostra e, especialmente, à influência das características da amostra e do desenho experimental sobre a magnitude dos efeitos biológicos do curcumin, além de outros aspectos comentados anteriormente. Assim, especulamos que a magnitude do efeito do curcumin aplicado localmente pode ser maior (e atingir a significância estatística) em situações clínicas associadas à menor eficácia do tratamento periodontal não cirúrgico, como: pacientes com comprometimento sistêmico ou sítios periodontais recalcitrantes e associados à condições anatômicas (bolsas profundas, regiões de furca) que podem reduzir a eficácia do tratamento periodontal mecânico não cirúrgico ou cirúrgico.

Com base nos resultados dos estudos que compõe este trabalho, e considerando as limitações dos mesmos, sugerimos o curcumin tem potencial como adjuvante no tratamento de periodontite.

5 CONCLUSÃO

Os dados obtidos indicam que:

O tratamento com múltiplas aplicações tópicas de nanopartículas carregadas com 0.05mg/ml de curcumin promove o reparo dos tecidos periodontais em modelo experimental de periodontite;

A aplicação única e local de nanopartículas carregadas com 0.05mg/ml de curcumin associada ao tratamento periodontal não cirúrgico em pacientes saudáveis sistemicamente não promoveu benefício clínico significativo estatisticamente;

A análise de estudos clínicos avaliando a aplicação local de curcumin indica benefícios estatisticamente significativos na redução da PS e ganho de inserção clínica, o qual é sustentado até 180 dias de acompanhamento; bem como diminuição significativa da inflamação gengival aos 90 dias de acompanhamento, sem efeitos adversos.

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APÊNDICE A - MATERIAIS E MÉTODOS

1 PUBLICAÇÃO 1

1.1 Preparo do Curcumin Nanoparticulado

O curcumin nanoparticulado, bem como as nanopartículas 'vazias' (veículo) foram preparados nos laboratórios dos Profs. Drs. Antonio Claudio Tedesco (Departamento de Química da Universidade de São Paulo em Ribeirão Preto) e Fernando Lucas Primo (Departamento de Bioprocessos e Biotecnologia - Faculdade de Ciências Farmacêuticas de Araraquara-Univ. Estadual Paulista). As partículas foram preparadas usando uma combinação de ácido poli-láctico (PLA) e ácido poliglicólico (PGA) na proporção de 1:1 e carreadas com 0.05 mg/mL de curcumin (61081M1611V, Sigma-Aldrich Co, São Paulo.Brazil) (Figura 2). Os polímeros foram inicialmente dissolvidos em di-clorometano e então adicionados a uma solução aquosa contendo 1% de álcool polivinílico e curcumin. A mistura foi submetida à agitação vigorosa (10.000 – 15.000 rpm) para obter a emulsificação água em óleo (w/o). O solvente orgânico foi removido da solução por agitação a temperatura ambiente e evaporação sobre pressão reduzida. As partículas foram centrifugadas (4–10°C; 1100–4600 g) em intervalos de 10 a 20 minutos. A preparação foi lavada três vezes em água destilada, suspensa em 1 mL e PBS, estocada a 4°C e usada em até 3 meses.

1.2 Modelo In Vivo de Reparo Periodontal

O protocolo experimental foi aprovado pelo Comitê de Ética em Uso Animal (CEUA) da Faculdade de Odontologia de Araraquara - UNESP (processo nº 37/2017) e realizado de acordo com as diretrizes do Conselho Nacional de Controle da Experimentação Animal do Colégio Brasileiro de Experimentação Animal (CONCEA). Este estudo foi relatado seguindo as diretrizes da ARRIVE (Pesquisa com Animais: Relato de Experiências In Vivo).

Um total de 24 ratos Wistar adultos (Rattus norvegicus albinus, Wistar), pesando entre 200 e 250g, foram utilizados neste estudo. Os animais foram mantidos em gaiolas de polipropileno (4 animais / gaiola) com temperatura (21 ± 1C) e umidade (65-70%) controlada e um ciclo claro-escuro de 12 horas. Os ratos receberam alimentação granulada (Labina / Purina) e água ad libitum. O tamanho da amostra foi

baseado em estudos anteriores, nos quais o desfecho primário foi a alteração do volume ósseo no modelo experimental de periodontite em ratos**.** Os animais foram distribuídos aleatoriamente nos três grupos experimentais a seguir (n = 8 animais / grupo): (i) nanopartículas carregadas com 0,05mg / ml de curcumin (N-Curc), (ii) nanopartículas vazias (veículo) e (iii) soro fisiológico (Ctrl). Para induzir a periodontite, os animais foram anestesiados por administração intramuscular de ketamina (Dopalen, Sespo Ind. E Com. Ltd., São Paulo, Brasil) e xilazina (Anasedan, Sespo Ind. E Com. Ltd., São Paulo, Brasil) a 0,08 ml / 100g e 0,04ml / 100g de peso corporal, respectivamente. A instalação das ligaduras (n ° 24; Coats Corrente, São Paulo, SP, Brasil) foi feita ao redor dos primeiros molares inferiores, bilateralmente. Após 15 dias, os animais foram anestesiados por inalação (Fluovac, Harvard Apparatus Holliston, Massachusetts, EUA) usando isoflurano, as ligaduras foram removidas, as superfícies dos dentes limpas e os sítios de ligadura dos dois primeiros molares inferiores imediatamente tratados de acordo com os grupos experimentais. Um volume de 30 µl de nanopartículas carregadas com curcumin a 0,05 mg/ml ou nanopartículas vazias ou soro fisiológico foi aplicado localmente no sulco gengival ao redor dos primeiros molares com o auxílio de uma seringa descartável estéril de 1 ml e agulha de 13 x 0,38 mm (INJEX, Ourinhos, SP, Brasil).

Os tratamentos experimentais foram administrados no dia 0 (dia da remoção das ligaduras), 3, 5, 7, 9 e 11, em um total de seis aplicações. Os animais foram eutanasiados por aprofundamento de anestesia 72 horas após o último tratamento.

As hemimandíbulas foram removidas, fixadas em paraformaldeído a 4% por 24 horas a 4° C, posteriormente lavadas em agua corrente por 24 horas e armazenadas em etanol a 70% para digitalização por tomografia computadorizada (µCT). Após escaneamento, doze hemi-mandíbulas (n = 4 / condição experimental) foram descalcificadas em EDTA 10%, pH 7,2 durante 6-8 semanas. As amostras foram reduzidas obtendo o primeiro e o segundo molar com seu processo alveolar e tecidos moles para inclusão em parafina para posterior análise histológica.

1.3 Avaliação da Perda Óssea por Microtomografia Computadorizada (µCT)

As amostras foram escaneadas com cortes de 18 µm de espessura, usando configurações pré-otimizadas (50 kV, 500 mA, filtro de alumínio de 0,5 mm, passo de

rotação de 0.5mm, e frame averaging de 3) em um microtomógrafo SkyScan 1176 (Bruker, Kontich, Bélgica). As imagens foram submetidas à reconstrução tridimensional com o software SkyScan NRecon® versão 1.6.6.0 (Bruker, Kontich, Bélgica) e rotacionadas em orientação tridimensional padronizada usando o software Data Viewer versão 1.4.4 de 64 bits (Bruker, Kontich, Bélgica). Posteriormente, uma região de interesse (ROI) foi determinada usando imagens bidimensionais no plano sagital com o software CTAnalyser (CTAn) versão 1.12.4.0 (Bruker, Kontich, Bélgica), para analisar a região interproximal entre o primeiro e o segundo molar.

Uma ROI interproximal poligonal foi posicionada em todas as imagens tridimensionais reconstruídas de acordo com os seguintes marcos anatômicos: junção cemento-esmalte (CEJ) da raiz distal do primeiro molar (limite mesial-superior) e CEJ da raiz mesial do segundo molar (limite distal-superior) ao ápice dessas raízes (limites inferiores mesial e distal do ROI). A extensão da ROI no plano vestíbulo-lingual (frontal) foi de 100 cortes (1.800 µm), começando o primeiro corte no qual foi possível ver o aspecto bucal da raiz distal a partir do primeiro molar.

Foi estabelecido uma escala de cinza (thresholds) padronizado (255-65) para diferenciar tecidos não mineralizados e mineralizados, a fim de quantificar a fração de volume ósseo (BF / TV), espessura e separação de trabéculas na ROI, utilizando o software CTAn.

1.4 Análise Estereométrica

Cortes seriados de 4 µm foram obtidos no plano sagital e corados com Hematoxilina e Eosina (H / E). Fotomicrografias de quatro cortes (duas da região vestibular e duas da região lingual) de espécimes de quatro animais diferentes em cada grupo experimental foram capturadas com magnificação de 200x usando um microscópio óptico (Diastar-Cambridge Instruments) acoplado a uma câmera digital (Leica Application Suite 3.8, Wetzlar, Alemanha). Um intervalo de 240 µm foi dado no plano buco-lingual entre esses cortes.

A análise estereométrica determinou a proporção de células (células inflamatórias, fibroblastos, etc.) e matriz extracelular usando uma grade de 240 mm^2 com 80 pontos distribuídos simétricamente em imagens padronizadas de 200X de magnificação. Essa grade foi posicionada nas imagens considerando os seguintes

pontos de referência: base do epitélio juncional entre o primeiro e o segundo molar (limite superior) e a superfície radicular do primeiro molar (limite lateral).

A análise histomorfométrica de osteoblastos e osteócitos foi realizada em imagens na magnificação de 200X. A área de interesse foi definida como a crista alveolar entre o primeiro e o segundo molares, desde o aspecto mais coronal da crista óssea até os 200 µm apicalmente. Os osteoblastos foram determinados como células cuboidais ao longo da superfície óssea alveolar e foram contados e normalizados pelo perímetro da superfície óssea (mm). Osteócitos, definidos como células aprisionadas na matriz óssea mineralizada, foram contados no processo ósseo alveolar e normalizados pela área óssea (mm^2).

Todas as análises foram realizadas em um total de 16 cortes por grupo, usando o software ImageJ (v. 1.51 s, Institutos Nacionais de Saúde, EUA - http://imagej.nih.gov/ij). Cada análise foi conduzida por dois examinadores independentemente, calibrados (IC: 0,96) e cegos para a codificação dos grupos experimentais.

1.5 Imunoistoquímica

A Osteopontin (OPN) foi selecionada como marcador da formação / maturação óssea, por ser considerado um potente inibidor da mineralização, associado a doenças inflamatórias crônicas, como a artrite reumatoide, devido à sua regulação positiva observada nas células inflamatórias. O fator de transcrição relacionado ao runt 2 (Runx-2) foi selecionado devido ao seu papel crítico na diferenciação osteoblástica e na proliferação de progenitores osteoblásticos, processos essenciais para o desenvolvimento e regeneração óssea.

As análises imunoistoquímicas da expressão Runx-2 (Abcam # 76956) e OPN (Abcam # 8448) foram realizadas pelo método da biotina-estreptavidina-HRP-DAB (kit Envision FLEX, Dako, # K800021-2) de acordo com as instruções do fabricante. Foram analisadas 3 cortes semi-seriados obtidos no plano sagital (espessura de 4 µm, abrangendo uma distância de 320 µm) de quatro amostras de diferentes animais em cada grupo experimental. Resumidamente, os cortes foram desparafinados e reidratados em concentrações decrescentes de etanol. Após o bloqueio da atividade endógena da peroxidase, a recuperação do antígeno foi realizada em tampão de pH

alto, a ligação inespecífica foi bloqueada com BSA a 2% em PBS a 1% (OPN) ou com um tampão de bloqueio sem soro (Runx2-) (proteínas sem soro, pronto para usar, Dako, # X0909). As seções foram incubadas com os anticorpos primários (diluição 1: 100 para OPN e Runx2) durante a noite (16 horas) a 4^{o}C. A detecção de anticorpos primários e a visualização foram realizadas usando anticorpos secundários conjugados à peroxidase e a reação desenvolvida com substrato DAB.

As seções foram contrastadas com hematoxilina de Carrazi e montadas com Permount. Fotomicrografias (aumento de 200X) foram tiradas na mesma área de interesse da análise morfométrica com uma câmera digital (Leica Application Suite 3.8, Wetzlar, Alemanha). A porcentagem de coloração positiva na área de interesse foi avaliada por um examinador treinado, cego às condições experimentais, usando o software ImageJ e o perfil IHC do plug-in (v. 1,51 s, Institutos Nacionais de Saúde, EUA - http: //imagej.nih .gov / ij).

1.6 Análise de Dados

Os dados obtidos foram analisados usando o GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, EUA). As análises estatísticas visaram comparar os resultados de acordo com as diferentes condições experimentais. Após analisar a distribuição normal pelo teste de Shapiro-Wilk, os dados foram analisados pelo teste paramétrico ANOVA One-Way para amostras independentes ou pelo teste não paramétrico de Kruskal-Wallis e pelo teste post hoc de Tukey ou Dunn, respectivamente.

2 PUBLICAÇÃO 2

2.1 Preparo do Curcumin Nanoparticulado

O curcumin nanoparticulado, bem como as nanopartículas 'vazias' (veículo) foram preparados nos laboratórios dos Profs. Drs. Antonio Claudio Tedesco (Departamento de Química da Universidade de São Paulo em Ribeirão Preto) e Fernando Lucas Primo (Departamento de Bioprocessos e Biotecnologia - Faculdade de Ciências Farmacêuticas de Araraquara-Univ. Estadual Paulista). As partículas foram preparadas usando uma combinação de ácido poli-láctico (PLA) e ácido

poliglicólico (PGA) na proporção de 1:1 e carreadas com 0.05 mg/mL de curcumin (61081M1611V, Sigma-Aldrich Co, São Paulo.Brazil). Os polímeros foram inicialmente dissolvidos em di-clorometano e então adicionados a uma solução aquosa contendo 1% de álcool polivinílico e curcumin. A mistura foi submetida à agitação vigorosa (10.000 – 15.000 rpm) para obter a emulsificação água em óleo (w/o). O solvente orgânico foi removido da solução por agitação a temperatura ambiente e evaporação sobre pressão reduzida. As partículas foram centrifugadas (4– 10°C; 1100–4600 g) em intervalos de 10 a 20 minutos. A preparação foi lavada três vezes em água destilada, suspensa em 1 mL e PBS, estocada a 4°C e usada em até 3 meses.

2.2 Voluntários

Para este estudo clínico randomizado, duplo-cego, tipo boca-dividida, foram selecionados 20 pacientes dentre os que procuraram atendimento na Faculdade de Odontologia de Araraquara-UNESP. Todos os pacientes atenderam os critérios de inclusão quanto a presença de periodontite, estágio III grau A, em pelo menos 2 sítios em 2 quadrantes diferentes, com PS ≥ 5 mm e doença periodontal ativa, verificada pela presença de sangramento à sondagem. A perda óssea alveolar foi avaliada por radiografias periapicais. Todos tiveram participação voluntária, concedida mediante assinatura de um termo de consentimento livre e esclarecido. Importante destacar que todos os pacientes voluntários receberam tratamento periodontal básico (Raspagem e Alisamento Radicular- RAR) independente do grupo experimental. Uma vez que o estudo avalia o potencial do curcumin nanoparticulado como adjuvante ao tratamento periodontal básico, não houve qualquer tipo de prejuízo ou “negativa de tratamento” a nenhum dos voluntários.

Os critérios para exclusão de pacientes foram: presença de doenças sistêmicas que contra-indiquem o tratamento periodontal, que requeiram a profilaxia antibiótica ou uso de medicações que interfiram na evolução da doença periodontal e/ou no resultado do tratamento; mulheres em período gestacional ou lactantes; fumantes ou ex- fumantes que cessaram o hábito há menos de 5 anos; ter feito uso de antimicrobianos nos últimos seis meses; ter feito uso de medicamentos anti-inflamatórios esteróides ou não esteróides de forma contínua por período superior a 7 dias nos últimos 3 meses; ter recebido tratamento periodontal no último ano; ter menos do que 15 dentes presentes.

2.3 Cálculo Amostral

O parâmetro clínico utilizado para a realização do cálculo amostra (desfecho de interesse principal) foi a profundidade à sondagem. Considerando a capacidade de detectar como significativa uma diferença de 1mm nas médias deste parâmetro nos sítios selecionados (PPD 5-8mm) entre os grupos, e uma variabilidade associada à determinação deste parâmetro de 1.0 mm e utilizando um poder (β) de 80% (para discriminação dos falsos negativos) e nível de significância (α) de 95% (para discriminação dos falso-positivos), serão necessários 16 pacientes. Considerando a possibilidade de desistências, ou necessidade de exclusão de algum paciente, serão selecionados 20 pacientes para a realização desse estudo.

2.4 Protocolo de Tratamento

a. Fase pré-tratamento:

Avaliação Clínica: A avaliação clínica foi realizada por um único examinador responsável, que foi previamente submetido à calibração. A calibração intra-examinador foi feita para os parâmetros de profundidade de sondagem (PS) e recessão gengival (RG), com um intervalo de 1 semana entre as avaliações.

Foi realizado exame clínico-anamnésico e os dados obtidos foram registrados em uma ficha clínica padronizada. A avaliação periodontal incluiu o registro do índice de placa bacteriana visível (IP), índice de sangramento marginal (IG), sangramento à sondagem (SS), profundidade de sondagem (PS), nível de inserção clínica (NIC), recessão gengival (RG). A mensuração dos índices PS, SS, NIC e RG foi feita com uma sonda periodontal Carolina do Norte (PUNC # 15, Hu- Friedy) em seis sítios de cada dente. Porém, a análise comparativa entres os grupos será feita baseada na avaliação dos sítios selecionados.

Os retornos dos pacientes para reavaliação de todos os parâmetros clínicos periodontais ocorreram após 30, 90 e 180 dias do tratamento.

Instrução de Higiene Oral e remoção de fatores retentivos: Inicialmente, os pacientes passaram por uma fase de preparo para remoção de fatores retentivos de placa como cálculos supragengivais, restaurações sem polimento ou com sobrecontorno. Ainda nesta fase, todos os pacientes receberam orientações de

higiene oral (OHO) padronizadas, recomendando-se a utilização da técnica de Bass modificada, o uso de escovas macias e de fio dental.

b. Fase do Tratamento:

Seleção dos sítios-teste: A partir dos dados do exame clínico inicial foram selecionados 4 sítios: 2 sítios em 2 quadrantes diferentes apresentando PS: 5-8 mm e sangramento à sondagem. Estes sítios estavam localizados em faces dentais não-contíguas; não foram selecionados sítios de dentes com próteses mal adaptadas, tratados com endodontia ou com lesões de cáries.

Quando houve mais de dois sítios por quadrante, a seleção se priorizou segundo a melhor acessibilidade dos sítios, nesta ordem: face mésio-vestibular, mésio-lingual, disto- vestibular e disto-lingual.

Coleta de biofilme subgengival e fluido crevicular gingival: Amostras de biofilme subgengival e de fluido crevicular gengival foram coletadas no baseline e após 3, 7 e 15 dias, em 4 sítios por paciente: 2 sítios tratados com RAR e aplicação do curcumin nanoparticulado e 2 sítios tratados com RAR e aplicação do veículo.

Após a remoção cuidadosa do biofilme supragengival, foi feito o isolamento relativo dos dentes de interesse, os quais foram secos com um jato de ar da seringa tríplice para a coleta do fluido crevicular gengival. Uma tira de papel absorvente (Periopaper, Oraflow, New York, EUA) foi inserida no sulco gengival a 2mm de profundidade e deixadas nesta posição durante 30 segundos. Em seguida, foi feita a mensuração do volume coletado por meio de equipamento específico, Periotron (Oraflow, New York, EUA). Em seguida, a tira foi colocada em microtubo de centrífuga de 1,5 ml contendo 250 µL de tampão fosfato salino (PBS). Esses tubos foram previamente identificados com o código do indivíduo. Todas as amostras coletadas foram imediatamente armazenadas a -80^{o}C até serem analisadas por meio do teste de ELISA, para a presença das citocinas (IL1α, IL6, TNFα e IL-10) seguindo as recomendações do fabricante dos ensaios (PeproTech, Rocky Hill, NJ, USA).

Logo após a coleta do fluido crevicular, amostras de biofilme subgengival dos mesmos dentes foram obtidas com o uso de curetas Mini-Gracey estéreis, as quais foram posicionadas na porção mais apical dos sítios e retiradas em um único golpe

de raspagem no sentido ápico- coronal. Essas amostras foram colocadas em microtubos de centrífuga de 1,5 mL contendo 200 µL de solução tampão TE (10mM Tris-HCL (Invitrogen Life Technologies, Carlsbad, CA, EUA), 1mM EDTA (Labsynth Produtos para Laboratórios Ltda, Diadema, Sp, Brasil), pH 7,6) e foi acrescentado 100uL de NaOH a 0,5M para que o DNA bacteriano permaneça viável por um longo período de tempo. Esses tubos foram previamente identificados com o código do indivíduo, data e sítio da coleta. Todas as amostras coletadas foram imediatamente armazenadas a -20ºC até serem analisadas por meio da técnica do checkerboard DNA-DNA hybridization.

Raspagem e alisamento radicular: A instrumentação mecânica (RAR) foi feita sob anestesia local, com instrumentos manuais (curetas tipo Gracey - Hu-Friedy) e ultra-sônicos em uma única sessão. Todos os tratamentos foram realizados por um mesmo operador cego aos sítios selecionados.

Randomização dos sítios-teste: Para a randomização dos sítios, foi gerada uma sequência de números através de um gerador randomizado de sequência de número 1-20. Envelopes sequencialmente numerados, lacrados e não transparentes foram usados para ocultar a alocação do tratamento e foram abertos apenas imediatamente antes no momento do tratamento. Estabeleceu-se que números pares, quando gerados, indicariam a aplicação do tratamento A no quadrante de menor numeração, (considerando-se 1° quadrante: hemi-arcada superior direita, 2° quadrante: hemi-arcada superior esquerda, 3° hemi-arcada inferior esquerda, 4° hemi-arcada inferior direita). O tratamento B, portanto, deveria ser aplicado no outro quadrante selecionado. O contrário também foi considerado como verdadeiro, quando o número gerado era ímpar, o tratamento B deveria ser aplicado no quadrante de menor numeração, devendo o tratamento A ser aplicado no outro quadrante.

Consideramos como tratamento A: Curcumin nanoparticulado, e tratamento B: veículo contendo as nanopartículas.

Aplicação do curcumin ou veículo: A aplicação foi feita por um operador distinto daquele que realizou o tratamento, a randomização dos sítios e avaliação dos mesmos. Os pacientes, também, foram cegos ao tratamento.

Os sítios selecionados receberam uma das modalidades de tratamento: aplicação de uma emulsão contendo nanopartículas carregadas com curcumin ou administração do mesmo volume do veículo utilizado na diluição do composto natural (nanopartículas neutras/"vazias"). Curcumin nanopartículado ou o veículo (volume padronizado de 100 µL) foram aplicados no interior do sítio selecionado com auxílio de uma seringa descartável utilizada na aplicação intra-dérmica de insulina (BD Biosciences). Esta aplicação foi feita de forma não invasiva, isto é, o conteúdo da seringa foi depositado no interior do sulco gengival sem a punção / introdução no interior dos tecidos gengivais, apenas preenchendo o espaço existente entre os tecidos moles e a raiz do dente.

No dia do tratamento foi fornecido a todos os pacientes um kit de higiene bucal contendo 2 escovas dentais Twister (Colgate, São Paulo, Brasil), 12 tubetes de dentifrício Colgate Total 12 (Colgate, São Paulo, Brasil) de 30g e um fio dental de 500 metros, para que pudessem ser utilizados durante os próximos 6 meses do estudo.

2.5 Determinação da Expressão Gênica a Nível Protéico (ELISA)

Alíquotas de cada amostra de fluido crevicular gengival foram examinadas, por meio do teste ELISA, para a presença das citocinas (IL1α, IL6, IL10, TNFα) seguindo as recomendações do fabricante dos ensaios (PeproTech, Rocky Hill, NJ, USA). A normalização dos resultados de concentração das citocinas foi feita pela quantidade (volume) de fluido gengival coletado, determinada imediatamente após a coleta em equipamento específico (Periotron 8000, Oraflow Inc. Plainview, NY).

2.6 Análise Microbiológica - Checkerboard DNA-DNA Hybridization

A identificação dos microorganismos presentes no biofilme subgengival dos sítios selecionados foi realizada através da técnica do Checkerboard hibridização DNA-DNA descrita por Socransky et al., 1994. Foram usadas sondas de DNA específicas para 40 espécies, selecionadas devido à sua associação com diferentes tipos de doença ou saúde periodontal. Ao final do experimento, a leitura dos filmes

radiográficos foi realizada por um único examinador treinado. Estas análises foram realizadas em laboratório da colaboradora neste projeto (Profa. Dra. Magda Feres, Universidade de Guarulhos).

2.7 Acompanhamento dos Pacientes Voluntários e Critérios para Cessar o Estudo

Com base em nossos estudos anteriores in vitro e em modelos animais não antecipamos qualquer tipo de reação adversa ao curcumin nanoestruturado ou ao veículo de nanopartículas. Evidências de estudos clínicos também demonstram a segurança e ausência de efeitos adversos associados à administração sistêmica do curcumin. No entanto, como medida de cautela e para a máxima segurança dos voluntários participantes, ao aceitarem participar e serem efetivamente incluídos na amostra de estudo, os pacientes receberão orientações de entrar em contato em caso de qualquer dúvida ou de qualquer um dos sintomas abaixo: (i) sensação de ardência ou queimação no local; (ii) desconforto gástrico; (iii) alteração intestinal; (iv) qualquer tipo de mal-estar (tontura, dores de cabeça, alterações de pressão).

Esta comunicação poderá ser feita por meio telefônico (telefone da clínica C, Depto. Diagnostico e Cirurgia da FOAr/UNESP ou por meio de linha telefônica celular específica divulgada aos pacientes participantes) ou pessoalmente.

Na ausência de relato de efeitos adversos pelo paciente, caso sejam detectadas pelo operador reações teciduais locais não-usuais nos retornos dos pacientes (como agravamento de sinais inflamatórios ou infecciosos, reação tipo corpo estranho, abscessos), estas serão registradas e documentadas.A ocorrência de qualquer tipo de efeito adverso (relatados pelos pacientes ou detectados pelos operadores) em 10% ou mais dos pacientes indicará a encerramento do estudo.

2.8 Variáveis do Estudo

As diferenças na PS média seis meses após o tratamento periodontal foram determinadas como o desfecho primário. Os desfechos secundários foram NIC, RG, SS, concentração de citocinas e dados microbiológicos.

2.9 Análise de Dados

O nível de significância foi estabelecido em 5%. Os dados foram tabulados e avaliados quanto à distribuição normal usando o teste de normalidade D'Agostino-Pearson. Testes paramétricos ou não paramétricos foram utilizados com base na distribuição dos dados.

As diferenças médias de PS, NIC e RG foram analisadas com um modelo linear de efeitos mistos contendo efeitos fixos para grupo, mês e grupo*mês. As diferenças de SS foram testadas independentemente para cada mês pelo teste do Qui-quadrado da razão de verossimilhança.

As comparações de IP e IG foram avaliadas por ANOVA de uma via para medidas repetidas, teste post hoc de Tukey. Diferenças na concentração de citocinas e contagem bacteriana foram analisadas pelo teste de Wilcoxon, e diferenças no mesmo grupo pelo teste de Friedman e Dunn.

3 PUBLICAÇÃO 3

3.1 Pergunta Focada

Em pacientes saudáveis com periodontite, o uso local/tópico do curcumin como adjuvante favorece a resposta clínica à raspagem e alisamento radicular?

3.2 Protocolo e Registro

O protocolo do estudo foi registrado no banco de dados do International Prospective Register of Systematic Reviews (PROSPERO/ número de registro: CRD42020158512) e foram seguidas as recomendações dos principais itens para relatar revisões sistemáticas e meta-análises (PRISMA).

3.3 Fontes de Informação e Estratégia de Pesquisa

O vocabulário controlado (termos MeSH) e as palavras-chave na estratégia de pesquisa foram definidas em base nas seguintes categorias do PICO:

• População (P): Indivíduos sistemicamente saudáveis com periodontite

• Intervenção (I): Raspagem e alisamento radicular (RAR) mais aplicação local de curcumin (em qualquer veículo, concentração e regime de administração)

• Comparações (C): RAR mais aplicação local de um placebo ou controle positivo (somente RAR)

• Resultados (O): Desfecho primário: redução da PS. Desfechos secundários: ganho na inserção clínica e sangramento à sondagem.

• Desenho do estudo (S): ECRs com pelo menos 30 dias de acompanhamento.

A estratégia de busca foi aplicada nas seguintes bases de dados eletrônicas, sendo a última atualização o dia 6 de fevereiro de 2020: MEDLINE via PubMed, Scopus, Web of Science, banco de dados da Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS), Biblioteca Brasileira de Odontologia e Biblioteca Cochrane. A estratégia de busca desenvolvida para o PubMed foi modificada para os outros bancos de dados para identificar estudos elegíveis. Além disso, a pesquisa manual foi feita a partir da lista de referência dos estudos primários e em periódicos relacionados à Periodontia, incluindo Periodontology 2000, Journal of Clinical Periodontology, Journal of Dental Research e Journal of Periodontology. Não houve restrições de idioma ou data de publicação.

Foram pesquisados resumos da reunião anual da Associação Internacional de Pesquisa Odontológica (IADR) entre 1990 e 6 de fevereiro de 2020. Além disso, a literatura cinza foi explorada usando o banco de dados System for Information on Grey Literature na Europa. As dissertações e teses foram pesquisadas usando os bancos de dados ProQuest Disserts and Theses Full Text e o banco de dados de 'Periodicos' da Fundação CAPES no Brasil.

Para identificar os ensaios em andamento, foram pesquisados os seguintes registros de ensaios clínicos: Ensaios controlados atuais (www.controlled-trials.com), Plataforma Internacional de Registro de Ensaios Clínicos (http: // apps.who.int/trialsearch/), ClinicalTrials.gov (www.clinicaltrials.gov), Rebec (www.rebec.gov.br) e EU Clinical Trials Register (https: // www.clinicaltrialsregister.eu).

3.4 Critério de Seleção

3.4.1 Critério de inclusão

Neste estudo, incluímos ensaios clínicos randomizados (ECRs) que avaliam pacientes saudáveis com mais de 18 anos diagnosticados com periodontite que receberam RAR com ou sem placebo e/ou RAR associado à administração local de curcumin, em um delineamento experimental de boca toda ou sítios específicos, tratados em sessões únicas ou repetidas. ECRs com desenho paralelo ou boca dividida, com seguimento de pelo menos 30 dias e registros dos parâmetros clínicos foram selecionados.

3.4.2 Critério de exclusão

Ensaios clínicos, incluindo (i) pacientes com doenças sistêmicas; (ii) pacientes fumantes; (iii) valores de PS<4mm no baseline; (iv) usando administração sistêmica de curcumin; e (v) menos de 30 dias de acompanhamento.

3.5 Seleção de Estudos e Processo de Coleta de Dados

Após a recuperação dos estudos de acordo com a estratégia de busca, um software gerenciador de referência (Endnote, Thomson Reuters) foi usado para remover referências duplicadas antes da seleção do estudo. Os estudos foram selecionados em duas fases. A primeira fase foi a análise do título e resumo dos artigos recuperados, de acordo com os critérios de inclusão/exclusão. Dois revisores (C.P.P. e K.D.C.) selecionaram os estudos independentemente. Se a análise do título e do resumo impediu a decisão de inclusão ou exclusão do trabalho, a versão em texto completo foi analisada. Em caso de desacordo entre os revisores, um terceiro revisor (L.M.W.) foi consultado para resolver o conflito. A segunda fase foi a análise do texto completo dos trabalhos selecionados. Cada artigo recebeu um ID do estudo (autor + ano de publicação). Uma tabela com informações relevantes sobre o desenho do estudo, participantes, intervenções e resultados foi construída de forma independente pelos dois revisores.

3.6 Risco de Viés no Estudo Individual

A análise qualitativa dos estudos incluídos foram realizadas por dois revisores independentes (CPP e KDC), seguindo as recomendações descritas no Cochrane

Handbook for Systematic of Interventions 5.1.0 (http://handbook.cochrane.org). Se a análise dos dois revisores discordaram, um terceiro revisor (LMW) as analisou para resolver o conflito. Existem seis domínios na avaliação da qualidade: geração da sequência aleatória, ocultação de alocação, cegamento de participantes e profissionais, cegamento de avaliadores de desfecho, desfechos incompletos e relato de desfecho seletivo.

Para cada domínio, os revisores registraram "+" (baixo risco de viés), "-" (alto risco de viés) ou "?" (risco de viés incerto - falta de informação ou incerteza sobre o potencial de viés). Uma avaliação de “baixo” risco indicou que o estudo apresentava risco “baixo” de viés. Se um domínio foi classificado como risco de viés “incerto” ou “alto”, o estudo foi considerado como risco “incerto” ou “alto” de viés, respectivamente. Se houve alguma discordância entre os revisores na avaliação desses domínios, isso foi resolvido por meio de discussão ou consultando um terceiro revisor (L.M.W.).

3.7 Medidas Resumidas e Síntese dos Resultados

Apenas estudos classificados como risco "baixo" ou "incerto" de viés nos domínios-chaves foram incluídos na metanálise. As metanálises das mudanças na profundidade à sondagem, nível de inserção clínica e índice de sangramento (Sulcus Bleeding Index) entre o baseline e os períodos de acompanhamento de 30 dias, 90 dias e 180 dias foram estimadas com o auxilio de um software estatístico (Review Manager software, versão 5.3, The Nordic Cochrane Center, The Cochrane Collaboration, Copenhague, Dinamarca). Quando as diferenças entre os valores do baseline e dos períodos de acompanhamento não foram relatadas, elas foram calculadas usando os valores publicados. A variância da diferença foi calculada usando a fórmula: Var (XY) = Var (X) + Var (Y) -2Cov (X, Y) onde Var (XY) é a variância da diferença, Var (X) é a variância da média no baseline, Var (Y) é a variância da média do período de acompanhamento, e Cov (X, Y) = rho x (sqrt (VarX) x sqrt (VarY)). O coeficiente de correlação (rho) foi assumido como 0,5 para todos os estudos.

Para as comparações, os dados de cada estudo foram agrupados, expressos e analisados usando diferenças médias ponderadas e intervalos de confiança de 95% (IC). As estimativas específicas do estudo foram agrupadas usando o modelo de efeito fixo ou aleatório. A heterogeneidade estatística entre os ensaios foi avaliada com I^2 ($I^2 \leq 25\%$: baixo; $I^2 = 26\text{-}50\%$: moderado; $I^2 \geq 75\%$: alta heterogeneidade).

Forest plots foram criados para ilustrar os efeitos da estimativa global na metanálise.

ANEXO A - CARTA DE APROVAÇÃO ESTUDO CLÍNICO

UNESP - FACULDADE DE ODONTOLOGIA - CAMPUS ARARAQUARA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Potencial terapêutico do curcumin nanoparticulado no tratamento da doença periodontal e periimplantar. Estudo clínico randomizado.

Pesquisador: CARLOS ROSSA JUNIOR

Área Temática:

Versão: 2

CAAE: 61307416.9.0000.5416

Instituição Proponente: Faculdade de Odontologia de Araraquara - UNESP

Patrocinador Principal: MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO

DADOS DO PARECER

Número do Parecer: 1.968.124

Apresentação do Projeto:

O projeto intitulado " Potencial terapêutico do curcumin nanoparticulado no tratamento da doença periodontal e periimplantar. Estudo clínico randomizado." é um estudo clínico randomizado, duplo-cego, tipo boca-dividida, no qual serão selecionados 20 de pacientes com diagnóstico diagnóstico de periodontite crônica e 40 pacientes com Periimplantite, que que procurarem atendimento na Faculdade de Odontologia de Araraquara-UNESP. Com os resultados desse estudo será possível determinar o potencial benefício do uso tópico do curcumin nanoestruturado no tratamento de condições inflamatórias comuns da cavidade bucal. Conhecimento importante no desenvolvimento e/ou aperfeiçoamento de tecnologias que amparem sua utilização clínica de rotina.

Objetivo da Pesquisa:

Avaliar o efeito na resposta clínica da aplicação local do nanocurcumin associado ao tratamento convencional da periodontite e periimplantite.

Avaliação dos Riscos e Benefícios:

Riscos: Com base em nossos estudos anteriores in vitro e em modelos animais não antecipamos qualquer tipo de reação adversa ao curcumin nanoestruturado ou ao veículo de nanopartículas. Evidências de estudos clínicos também demonstram a segurança e ausência de efeitos adversos

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associados à administração sistêmica do curcumin. Todos os pacientes voluntários receberão o tratamentopadrão atualmente indicado segundo a filosofia da disciplina de Periodontia da FOAr/UNESP. Uma vez que o estudo avalia o potencial do curcumin nanoestruturado como adjuvante do tratamento convencional, não há qualquer tipo de prejuízo ou 'negativa de tratamento' a nenhum dos voluntários.Esta aplicação local do nanocurcumin será feita de forma não invasiva, isto é, o conteúdo da seringa será depositado no interior do sulco gengival sem a punção / introdução no interior dos tecidos gengivais, mas apenas preenchendo o espaço existente entre os tecidos moles e a raiz do dente.
Benefícios: Todos os voluntários receberão tratamento padrão de acordo com a filosofia de tratamento da Faculdade de Odontologia de Araraquara-UNESP.

Comentários e Considerações sobre a Pesquisa:
No parecer anterior o pesquisador foi questionado quanto a: Descrever o modo de aplicação das nanopartículas, se existe na literatura referências sobre a aplicação de nanopartículas vazias nas condições de uso semelhante a pesquisa a ser realizada com uso de retalho? Pois caso não haja referências, como será feita a aplicação de nanopartículas vazias? O método será diferente do tratamento preconizado atualmente pela disciplina que consta em curetagem sem aplicação de substâncias? Pois se for diferente do método preconizado, o grupo de nanopartículas vazias não se tornará
um grupo controle, correto?
O pesquisador respondeu a todas os questionamentos por meio de um documento no qual ficou esclarecido o seguinte: "Em resposta à solicitação de 'descrição do modo de aplicação', as nanopartículas serão aplicadas de forma padronizada (independentemente de serem carregadas com 50 mg/mL de cucurmin ou não) sobre a área afetada, de forma tópica, utilizando seringa descartável utilizada para aplicação intra-dérmica de insulina, sempre em volume padronizado (200 μL), como descrito no projeto.
Em relação à 'existência de dados na literatura sobre a aplicação de nanopartículas vazias' (e 'no caso de não existência, como seria feita a aplicação de nanopartículas vazias?'), não ficou muito claro para mim a natureza da pergunta, mas como há menção à 'retalho' na questão formulada, esta resposta supõe que o assessor esteja se referindo especificamente ao tratamento da periimplantite no presente projeto. O avanço na eficácia terapêutica (em qualquer área) depende de desenvolvimento de novos métodos/técnicas/abordagens e/ou (como é o caso do experimento no projeto proposto) do desenvolvimento de adjuntos terapêuticos aplicados em associação à métodos/técnicas já empregados. Obviamente, métodos ou modificações 'novas', por definição,

não foram testadas antes; assim não há 'referências' da sua utilização naquela aplicação específica. Efetivamente, não há na literatura consenso sobre o melhor método/abordagem terapêutica da periimplantite (PMID 26285807, PMID 18724855), o qual inclui abordagens cirúrgicas (PMID 23062132) e não-cirúrgicas (PMID 18724858). Frequentemente, os ensaios clínicos buscam uma maior eficácia do tratamento com o uso de estratégias ou procedimentos adjuntos ao desbridamento mecânico, como a descontaminação com irradiação por laser (PMID 23926583), uso de antibioticoterapia sistêmica ou local (PMID 18724858), incluindo microesferas contendo antibióticos (PMID 22568744, PMID 18454662). Estes estudos utilizam como controle o veículo (por ex., gel, microesferas, irradiação sem o agente fotossensibilizador) utilizado no carreamento da substância ativa em teste. Consideramos que adicionar um grupo sem a aplicação de nanopartículas (vazias ou contendo curcumin) implicaria em aumento proibitivo no custo do experimento (especificamente, do tempo necessário para seleção e inclusão de casos e número de amostras a serem avaliadas) e não resultaria em melhor qualidade interna do estudo, uma vez que o objetivo central do projeto é avaliar o efeito do curcumin como adjunto no tratamento da periimplantite. No caso da suposta eliminação do grupo controle-veículo, experimentalmente não haveria como atribuir quaisquer eventuais diferenças entre os grupos à substância-teste (no caso, o curcumin). Assim, pensamos que o controle-veículo é absolutamente essencial neste projeto para a confiabilidade dos dados, sua posterior divulgação e eventual aplicação/implementação clínica.

Considerando a ausência de consenso na literatura sobre a abordagem terapêutica da periimplantite, o procedimento mais frequentemente utilizado na FOAr/UNESP para o tratamento de periimplantite é o desbridamento/curetagem cirúrgica associado ao uso do jato de bicarbonato de sódio como descontaminante para remoção de biofilme da superfície do implante, sendo este procedimento suportado na literatura (PMID 19126114). Caso a preocupação do assessor seja com o eventual prejuízo para a qualidade do tratamento dos pacientes devido à aplicação das nanopartículas 'vazias' (controle-veículo), há que se considerar que a evidência que suporta o uso do jato de bicarbonato (por exemplo) não é conclusiva e que seu emprego trans-cirúrgico (assim como o uso de géis de clorexidina, microesferas contendo minociclina e outras terapias adjuntas preconizadas em diferentes centros e para as quais existe literatura de suporte, ainda que não conclusiva) trazem potenciais riscos aos pacientes, incluindo enfisema (injeção de ar nos tecidos), alteração do pH (devido ao bicarbonato de sódio) e citotoxicidade/necrose (devido à elevada concentração de antimicrobiano ou antibióticos em contato direto com os tecidos conjuntivos). No caso específico do uso do (preconizado) jato de bicarbonato de sódio, existe a preocupação com a

biossegurança da equipe profissional e de outros pacientes em virtude do aerosol formado. De fato, há que se considerar que qualquer terapia inclui riscos (inclusive de efeito nocebo), no entanto a escolha terapêutica sempre privilegia a maximização do benefício ao paciente acompanhada da minimização do risco (por exemplo, cirurgias de bypass coronário, quimio e/ou radioterapia para o câncer, cirurgia paraendodôntica, instalação de implantes dentais, etc). Nanopartículas de PLGA já foram empregadas clinicamente em pacientes com doença inflamatória intestinal (PMID 24084650), e este polímero biodegradável é o mais extensamente estudado pelas suas propriedades favoráveis, tanto farmacológicas quanto de segurança e ausência de efeitos colaterais (PMID 25565440), sendo inclusive aprovado pelo Food and Drug Administration (FDA) nos Estados Unidos e pela European Medicine Agency (EMA) na Comunidade Européia para uso como carreador de drogas e substâncias bioativas devido às suas propriedades farmacológicas, baixa toxicidade e biocompatibilidade (PMID 24568455).
Espero ter esclarecido a natureza essencial do grupo controle-veículo (nanopartículas vazias, neste caso) para que o estudo possa gerar informações confiáveis sobre os efeitos do curcumin no tratamento da periimplantite, que é o objetivo principal do projeto. No entanto, considerando a menção do assessor "Pois se for diferente do método preconizado, o grupo de nanopartículas vazias não se tornará um grupo controle, correto?, penso ser importante ressaltar que não embora exista base racional para antecipar qualquer efeito biológico das nanopartículas vazias (controle-veículo), apenas com a realização do grupo controle-veículo (nanopartículas vazias) será possível inferir o papel específico do curcumin neste tratamento."

Considerações sobre os Termos de apresentação obrigatória:
Os Termos de apresentação obrigatória foram adequadamente apresentados, após o primeiro parecer foi solicitado adequar o (n) da pesquisa para 60 na Folha de Rosto, o cronograma e no No TCLE, adequar o nome do Pesquisador Responsável como contato, aluna não pode ser contato. Todos os 3 itens solicitados foram adequados.

Conclusões ou Pendências e Lista de Inadequações:
Não existem pendências.

Considerações Finais a critério do CEP:
Protocolo APROVADO em reunião de 16 de Março de 2017.
O pesquisador deverá encaminhar relatórios parciais a cada 01 (um) ano até o prazo final da

ANEXO B - CARTA DE APROVAÇÃO ESTUDO IN VIVO

unesp UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Araraquara

FACULDADE DE ODONTOLOGIA

CERTIFICADO

Certificamos que a proposta intitulada ***"INFLUÊNCIA DA APLICAÇÃO LOCAL DO CURCUMIN NANOPARTICULADO SOBRE OS TECIDOS PERIODONTAIS"***, registrada com o nº 37/2017, sob a responsabilidade do(a) **Dr(a). Morgana Rodrigues Guimarães Stabili** – que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela **COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA FACULDADE DE ODONTOLOGIA DE ARARAQUARA** em reunião de 23/02/2018.

Finalidade	( ) Ensino (**X**) Pesquisa Científica
Vigência da autorização	Janeiro/2019
Espécie/linhagem/raça	Rato – Wistar
Nº de animais	84
Peso/Idade	250-300g – 90 dias
Sexo	Macho
Origem	Biotério Central da UNESP – Câmpus de Botucatu

Profa. Dra. CARINA APARECIDA FABRÍCIO DE ANDRADE
Coordenadora da CEUA

Não autorizo a publicação deste trabalho pelo prazo de até 2 anos após a data de defesa

(Direitos de publicação reservado ao autor)

Araraquara, 01 de Abril de 2020.

Cindy Grace Pérez Pacheco