ANA GABRIELA COSTA NORMANDO

Mucosite oral: Modelo ‘In Vitro’ e revisão sistemática de tratamento com inibidores naturais de mTOR

BRASÍLIA, 2019

UNIVERSIDADE DE BRASÍLIA
FACULDADE DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

ANA GABRIELA COSTA NORMANDO

Mucosite oral: Modelo ‘In Vitro’ e revisão sistemática de tratamento com inibidores naturais de mTOR

Dissertação apresentada como requisito parcial para a obtenção do título de Mestre em Ciências da Saúde pelo programa de Pós-Graduação em Ciências da Saúde da Universidade de Brasília.

Orientadora: Profª. Drª. Eliete Neves da Silva Guerra
Coorientadora: Drª. Caroline Lourenço de Lima

BRASÍLIA, 2019

AGRADECIMENTOS

Em primeiro lugar, à Deus por me permitir trilhar minha jornada com saúde e sabedoria.

Aos meus pais, Álvaro e Silvânia, por me transmitirem suas virtudes, por me apoiarem em todos os meus sonhos, por me possibilitarem dedicar exclusivamente ao mestrado e por compreenderem minha ausência nos momentos de estudo.

Aos meus irmãos, Thadeu e Ana Luísa, pela amizade mais pura, pelo companheirismo, por me incentivarem, por me ouvirem e sempre acreditarem em mim. E à minha cunhada, Ana Paula, que considero como irmã, agradeço pelas palavras de conforto e sabedoria.

Ao Christian, meu companheiro de vida, por me manter com os pés no chão, por me apoiar nos momentos de angústia, por me acompanhar nos experimentos no laboratório em fins de semana e feriados, e principalmente por acreditar em mim.

À minha orientadora, Profª Eliete Neves Silva Guerra, que me recebeu de braços abertos no laboratório, me ensinou os primeiros passos da pesquisa básica, me incentivou a buscar sempre o meu melhor, me motivou a continuar na clínica, e que me possibilitou realizar sonhos que eu nem tinha sonhado para mim.

À minha coorientadora, Drª Caroline Lourenço de Lima, por compartilhar todo seu conhecimento e paixão pela pesquisa. Por me guiar e orientar até mesmo por ligações telefônicas. Agradeço pelo exemplo de pesquisadora, pela paciência em ensinar, e pelo interesse de aprender cada vez mais.

Às colegas Amanda e Isabela, companheiras de revisão sistemática e de liga, agradeço pela amizade, companheirismo e dedicação.

Agradeço aos meus colegas do Laboratório de Histopatologia Bucal pelo convívio diário, pela amizade e por todo apoio ao longo da minha jornada. Em especial Lídia, Cláudio, Augusto, Bruna, Gabriel, Dani Assad, Josy, Silvinha, Andreinha, Letícia, Isadora, Shélida, Ana Elízia e Cinthia.

À Prof. Ana Carolina Poppe pelas palavras de incentivo e sugestões que melhoraram consideravelmente o trabalho.

Agradeço também ao FarMol por todo suporte técnico, por me possibilitar fazer qPCR e conhecer pessoas sempre tão solícitas.

Às alunas de PIBIC, Karen e Juliana, por confiarem em mim e me possibilitarem orientar pela primeira vez.

Aos físicos do HUB, em especial Samuel, Leonardo e Luís Felipe, por sempre me atenderem com prontidão e educação, por auxiliarem no planejamento e possibilitarem a irradiação das minhas placas de células.

Agradeço ao Projeto do CACON que me possibilitou entrar em contato com os pacientes oncológicos e despertar em mim a paixão pela estomatologia. Agradeço em especial aos professores Paulo Tadeu, Nilce, André e Carla, aos residentes e aos colegas extensionistas por todo o conhecimento compartilhado e por todos os momentos vividos.

À Cynthia pela amizade, pela oportunidade de atender seus pacientes oncológicos que tanto me ensinaram e por compartilhar seus conhecimentos da prática clínica.

Agradeço à Liga de Combate ao Câncer por me fazer conhecer meu paciente como um todo e poder levar um pouco de alegria aos pacientes do HUB. Agradeço em especial às professoras Paula Diniz, Christiane Vasques e Elaine Barros por conduzirem um projeto tão lindo que eu tive o prazer de fazer parte.

Agradeço por fim à CAPES que me possibilitou realizar graduação sanduíche e que me abriu as portas para a pesquisa. Ao programa de Pós-Graduação em Ciências da Saúde por dar suporte e auxiliar na minha formação, ao CNPq pela bolsa de mestrado concedida que me possibilitou dedicação exclusiva à pesquisa, e à FAPDF pelo financiamento da apresentação do meu trabalho em um congresso internacional.

RESUMO

A mucosite oral (MO) é um dos efeitos adversos mais comuns das atuais terapias antineoplásicas. Sua fisiopatologia é complexa, envolvendo interações dinâmicas de todos os tipos celulares que compõem o epitélio e o tecido conjuntivo subjacente. Atualmente existem estratégias eficazes para prevenção e tratamento da mucosite, embora não exista um protocolo padrão de tratamento da condição. Diversas terapias continuam em desenvolvimento e produtos naturais com menos efeitos adversos e mais acessíveis são potenciais estratégias terapêuticas. Diante disso, para melhor compreender a etiopatogenia da MO e possíveis alvos terapêuticos, o presente trabalho foi dividido em 2 estudos, apresentados, cada um, na forma de manuscritos. O primeiro teve como objetivo estabelecer um modelo *in vitro* de MO a partir de cultura primária de fibroblastos gengivais tratados com radiação ionizante, lipopolissacarídeo (LPS) de *Escherichia coli* e extrato total da bactéria *Porphyromonas gingivalis* (*Pg*). Foi demonstrado que 12 Gray (Gy) de radiação induziu maior expressão de *IL-1*β, *IL-6*, *TNF-*α e *NF-*κ*B* após 6h, quando comparado a células não irradiadas, e tratamento com 5μg/mL de extrato proteico de *Pg* também gerou maior expressão de todas as citocinas pró-inflamatórias após 6h. A associação dos dois estímulos resultou em expressão aumentada das citocinas quando comparada a células apenas irradiadas. O segundo estudo foi uma revisão sistemática sobre os efeitos da cúrcuma e seu principal polifenol, a curcumina, um inibidor natural de mTOR, no tratamento da MO. Foi evidenciado que tanto a cúrcuma quanto a curcumina aplicadas na forma de gel ou bochecho são capazes de reduzir o grau da mucosite, dor, intensidade do eritema e tamanho da área ulcerada. Assim, esses achados apresentaram evidência científica de que a cúrcuma e a curcumina são boas alternativas naturais no controle da MO. Em conclusão, este trabalho contribui para o desenvolvimento de um modelo experimental *in vitro* de MO com potencial para se estudar o mecanismo de ação de novas estratégias terapêuticas, como os inibidores naturais de mTOR.

Palavras-chave: Mucosite oral; Modelo *in vitro*; Citocinas pró-inflamatórias; Inibidores naturais de mTOR**.**

ABSTRACT

Oral mucositis (OM) is one of the most common side effects of current antineoplastic therapies. Its physiopathology is complex, involving dynamic interactions of all cell types that comprise the epithelium and the underlying connective tissue. Currently, there are effective strategies for prevention and treatment of mucositis, although there is no standard protocol for managing the condition. Several therapies are still in development, and natural products with fewer side effects and more affordable are potential therapeutic strategies. Therefore, to better understand the pathobiology of OM and possible therapeutic targets, the present work was divided in 2 studies, presented, each, in the form of manuscripts. The first one aimed to establish an *in vitro* OM model from primary culture of gingival fibroblasts treated with ionizing radiation, lipopolysaccharide (LPS) of *Escherichia coli* and total extract of the bacterium *Porphyromonas gingivalis (Pg)*. It was shown that 12 Gray (Gy) of radiation induced greater expression of *IL-1*β, *IL-6*, *TNF-*α e *NF-*κ*B* after 6h, when compared to non-irradiated cells, and treatment with 5μg/ml of *Porphyromonas gingivalis* protein extract also generated higher expression of all proinflammatory cytokines after 6h. Association of both stimuli resulted in increased expression of citokynes when compared to cells only irradiated. The second study was a systematic review about the effects of turmeric and its main polyphenol, curcumin, a natural mTOR inhibitor, on OM management. It was evidenced that both turmeric and curcumin applied in the form of gel or mouthwash are capable of reducing mucositis grade, pain, erythema intensity and ulcerated area size, being, therefore, good natural alternatives to treat OM. In conclusion, this work contributes to the development of an *in vitro* experimental model of oral mucositis with potential to study the mechanism of action of new therapeutic strategies, such as natural inhibitors of mTOR.

Key words: oral mucositis; *in vitro* model; proinflammatory cytokines; natural mTOR inhibitors.

LISTA DE FIGURAS

Figuras da Dissertação:

Figuras do manuscrito 1:

Figuras do manuscrito 2:

LISTA DE TABELAS

Tabelas do manuscrito 1:

Tabelas do manuscrito 2:

LISTA DE ABREVIATURAS E SIGLAS

COX2 = Ciclooxigenase 2

CT = *Chemotherapy*

DMEM = *Dulbecco's Modified Eagle Medium*

E. coli = *Escherichia coli*

EGCG = Epigalocatequina Galato

FBS = *Fetal Bovine Serum*

Gy = Gray

IL = Interleucina/ *Interleukin*

LBP = Laserterapia de Baixa Potência

LPS = Lipopolissacarídeo / *Lipopolysaccharide*

MAPK = Proteína-Quinase Ativada por Mitógeno

MMP = Metaloproteinase

MO = Mucosite Oral

mTOR = *mammalian Target of Rapamycin*

NF-κB = *Nuclear Factor Kappa B*

NO = *Nitric Oxide*

OM = *Oral Mucositis*

PBS = *Phosphate-Buffered Saline*

qPCR = *quantitative Polymerase Chain Reaction*

Pg = *Porphyromonas gingivalis*

PTK = Proteína Tirosina-Quinase

QO = Queratinócitos Orais

QRT = Quimioradioterapia

QT = Quimioterapia

ROS = Espécies Reativas de Oxigênio / *Reactive Oxygen Species*

RT = Radioterapia / *radiotherapy*

SD = *Standard deviation*

TNF-α = *Tumor Necrosis Factor Alpha*

SUMÁRIO

1. INTRODUÇÃO

A mucosite oral (MO) é uma das reações adversas mais comuns da terapia antineoplásica. Essa condição apresenta grande importância devido ao impacto negativo na qualidade de vida dos pacientes oncológicos. Sabe-se que pacientes submetidos à radioterapia (RT) de cabeça e pescoço irão quase que invariavelmente desenvolver algum grau de MO, assim como pacientes em tratamento quimioterápico. Trata-se de uma lesão extremamente dolorosa, que por vezes é responsável pela interrupção da radioterapia, piorando o prognóstico do câncer. A lesão inicia-se como uma área eritematosa em regiões de mucosa não queratinizada e, ao decorrer do tratamento, evolui para lesões erosivas ou ulceradas, cobertas por uma pseudomembrana extremamente friável (Epstein *et al.*, 2012; Villa & Sonis, 2016).

Atualmente existem diversas estratégias de prevenção e tratamento da MO. Recomenda-se iniciar com a orientação do paciente quanto ao controle da higiene oral. Algumas das estratégias mais amplamente utilizadas no tratamento da mucosite têm sido a fotobiomodulação e a crioterapia, com excelentes resultados de prevenção e redução da gravidade (Oberoi *et al*., 2014; Reis *et al*., 2016). Outras estratégias incluem bochechos e géis anti-inflamatórios, analgésicos, anestésicos ou a associação destes. Apesar da ampla gama de possibilidades terapêuticas, ainda não há um protocolo definitivo para a prevenção ou tratamento da MO. Os procedimentos variam muito de paciente para paciente, terapia antineoplásica utilizada, localização do tumor e grau de gravidade (Lalla *et al*., 2014; Peterson *et al*., 2015).

Diante deste panorama, faz-se necessário investigar a etiopatogenia da mucosite oral para, assim, se formular medicamentos que atuem de forma seletiva e eficaz. A maior parte das informações que suportam o modelo da etiopatogenia da mucosite são derivados de experimentos em animais, porém existem restrições para testes *in vivo* em algumas situações. Dessa forma, os modelos *in vitro* são uma alternativa para se investigar melhor os mecanismos envolvidos no desenvolvimento da lesão, assim como analisar possíveis alvos de tratamento (Shin *et al*., 2013; Tra *et al*.,

2013; Lambros *et al*., 2015). Entretanto, ainda existe uma lacuna na literatura no que tange a um modelo *in vitro* mais fidedigno que permita testar novos tratamentos naturais que apresentem poucos efeitos adversos, mais acessíveis e mais facilmente aplicáveis.

2. REVISÃO DE LITERATURA

2.1 TERAPIAS ANTINEOPLÁSICAS ASSOCIADAS À MUCOSITE ORAL

As neoplasias malignas, comumente conhecidas como câncer, são umas das principais causas de morte ao redor do mundo e sua incidência e mortalidade têm aumentado rapidamente (Bray, 2018). A maioria dos tipos de câncer é tratada com cirurgia, quimioterapia (QT), radioterapia (RT) ou associação de quimioradioterapia (QRT), que atualmente são as estratégias mais eficazes. Entretanto, os efeitos das terapias antineoplásicas não são limitados às células tumorais, afetando também tecidos normais, causando efeitos adversos que incluem a mucosite oral e a gastrointestinal, a hepatotoxicidade, a nefrotoxicidade, a cardiotoxicidade e a neurotoxicidade (Shapiro, 2016).

O epitélio oral devido ao seu alto poder de renovação celular é afetado pelo tratamento oncológico, tornando-se atrófico e levando à mucosite oral (MO). Essa desordem é o efeito adverso mais comum na cavidade oral causado pela medicação antineoplásica. Outras complicações orais também comuns incluem xerostomia, disgeusia e disfagia, bem como infecções fúngicas, virais e bacterianas (Migliorati *et al*., 2015; Carvalho *et al*., 2018). A primeira manifestação clínica da MO é eritema em uma ou mais regiões de mucosa oral não queratinizada, em especial a mucosa de revestimento e borda lateral de língua. Com o avançar do tratamento, as lesões podem evoluir para úlceras dolorosas acompanhadas de odinofagia, disfagia, má-nutrição e perda de peso (Chaveli-López, 2014; Cinausero *et al*., 2017).

As diferentes drogas quimioterápicas têm como alvo moléculas de diversas partes do ciclo celular ou metabolismo e por isso variam em relação ao grau de mucotoxicidade. Além disso, outros fatores que também podem influenciar no surgimento de lesões em mucosa, incluem a dose e a interação entre diferentes agentes prescritos em um protocolo (Pico *et al*., 1998). Drogas quimioterápicas que usualmente são associadas a toxicidades em mucosa incluem antimetabólitos como 5-Fluorouracil e metotrexato, irinotecano, agentes alquilantes, como a ciclofosfamida e a cisplatina, e as antraciclinas e taxanos (Chaudhry *et al*., 2016; Mayo *et al*., 2017;

Curra *et al*., 2018). Além disso, infusão em *bolus* (administração intravenosa realizada em até 1 minuto) e associação de agentes quimioterápicos à radioterapia tendem a causar reações mais graves (Pico *et al*., 1998; Cinausero *et al*., 2017). A MO causada por quimioterapia tem um curso clínico razoavelmente previsível, com os primeiros sinais aparecendo 3 a 4 dias após a infusão e pico máximo entre 7 a 14 dias, resolvendo espontaneamente na semana seguinte (Lalla *et al*., 2014).

A RT de cabeça e pescoço também está associada a um risco elevado de desenvolver algum grau de mucosite, uma vez que a mucosa oral é diretamente exposta à radiação. O protocolo de tratamento geralmente varia entre 60 e 70 Gy, em doses fracionadas diárias de 2 Gy, com os primeiros sinais e sintomas, como sensação de queimação e eritema, aparecendo já ao final da primeira semana. As úlceras tipicamente surgem entre a segunda e terceira semana e tornam-se extremamente dolorosas em doses cumulativas de radiação em torno de 30-40 Gy, podendo persistir até 4 semanas após término do tratamento (Villa & Sonis, 2015). Em uma revisão sistemática, foi confirmada a alta incidência de mucosite em pacientes com câncer de cabeça e pescoço submetidos a RT, com taxas de 97% relatadas durante RT convencional, 100% durante RT com fracionamentos alterados (acelerado ou hiperfracionado) e 89% durante QRT (Trotti *et al*., 2003).

Na última década, novas terapias contra o câncer surgiram, incluindo a terapia-alvo, que inibe receptores moleculares e vias de sinalização envolvidas na progressão do câncer (Lacouture & Sibaud, 2018). Essas novas drogas têm sido associadas a um risco aumentado de lesões em mucosa oral, porém com características clínicas que diferem da mucosite causada pelas terapias convencionais (Vigarios *et al*., 2017). As lesões apresentam-se como aftas únicas ou múltiplas, bem circunscritas, arredondadas, superficiais e bastante dolorosas que surgem em mucosa não queratinizada (Peterson *et al*., 2016; Lacouture & Sibaud, 2018). Embora a fisiopatologia e as características clínicas das lesões em mucosa oral causada por terapias-alvo sejam diferentes, essas lesões compartilham dos mesmos tratamentos e ações preventivas preconizadas para as lesões de mucosite causadas pelas terapias convencionais (Peterson *et al*., 2016).

2.2 FISIOPATOLOGIA DA MUCOSITE ORAL

A MO é um processo biologicamente complexo que envolve interações dinâmicas de todos os tipos de células que formam o epitélio e a lâmina própria. Antigamente, acreditava-se que a mucosite fosse consequência da agressão direta da RT ou quimioterapia apenas sobre as células do tecido epitelial, que se proliferam rapidamente (Sonis *et al*., 1994). Entretanto, sabe-se hoje que alterações no endotélio do tecido conjuntivo precedem a lesão epitelial. Dessa forma, para melhor explicar o mecanismo de desenvolvimento da mucosite, Sonis (2004) propôs um modelo teórico que divide a evolução da lesão em cinco estágios: iniciação, sinalização, amplificação de sinal, ulceração e cicatrização (Figura 1).

A fase de iniciação ocorre rapidamente após administração da RT ou QT que induzem lesões ao DNA, levando à lesão das células do epitélio basal e do conjuntivo subjacente (lâmina própria). Apesar de haver morte celular no epitélio, a destruição das células do tecido conjuntivo subjacente é o que mais contribui para o desenvolvimento da MO (Denahm & Hauer-Jensen, 2002). Simultaneamente, espécies reativas de oxigênio (ROS) são geradas, que são importantes mediadores de eventos biológicos (Sonis, 2004).

A resposta primária ocorre em consequência de uma complexa série de eventos envolvendo lesões ao DNA e geração de ROS. Quebras das fitas de DNA levam à ativação de diversas vias de transdução que, por sua vez, ativam fatores de transcrição como o fator nuclear κB (NF-κB) (Sonis, 2002). A ativação do NF-κB pode resultar em um aumento na expressão de aproximadamente 200 genes, dos quais muitos têm potencial efeito na toxicidade da mucosa. A superexpressão desses genes leva à produção de citocinas pró-inflamatórias, incluindo o TNF-α , a IL-1β e a IL-6. Os níveis elevados dessas citocinas foram observados inclusive em amostras de sangue de ratos com mucosite induzida por quimioterapia e em saliva de pacientes com câncer de cabeça e pescoço submetidos à quimioradioterapia (Logan *et al*., 2008; Bossi *et al*., 2016). Essas citocinas, então, amplificam o sinal primário ou podem ativar o NF-κB em outras células, resultando na expressão de moléculas sinalizadoras de proteína-quinase ativada por mitógeno (MAPK),

ciclooxigenase 2 (COX2) e proteína tirosina-quinase (PTK). Essas vias de sinalização levam à ativação de metaloproteinases de matriz (MMPs) em células epiteliais e do conjuntivo subjacente, como fibroblastos, macrófagos e células endoteliais (Sonis, 2004). As MMPs, por sua vez, causam destruição da matriz de colágeno subepitelial e rompem a interface entre o epitélio e o tecido conjuntivo, possibilitando a disseminação de outros sinais destrutivos (Figura 2) (Sonis, 2007; Al-Dasooqi *et al*., 2010).

Como consequência da superexpressão gênica que ocorre devido à ativação inicial de fatores de transcrição, um grande número de proteínas se acumula e tem como alvo o tecido epitelial e o conjuntivo subjacente. Algumas delas, em especial as citocinas pró-inflamatórias, não só lesionam o tecido, como também, geram um *feedback* positivo que amplifica a lesão iniciada pela RT ou QT, aumentando e prolongando a agressão tecidual. Embora possa ocorrer eritema durante essas fases, geralmente o tecido mantém-se íntegro e os pacientes relatam poucos sintomas (Sonis, 2002; Sonis, 2007).

A fase de ulceração da MO é a mais significante, uma vez que a perda da integridade da mucosa resulta em lesões extremamente dolorosas e propensas à colonização bacteriana superficial. Componentes da parede celular dessas bactérias podem penetrar no tecido conjuntivo, onde ativam macrófagos a produzirem e liberarem citocinas pró-inflamatórias adicionais. Isso acaba promovendo a expressão de genes pro-apoptóticos que potencializam a lesão tecidual, gerando úlceras clinicamente visíveis (Al-Ansari *et al*., 2015; Stringer & Logan, 2015).

Na maioria dos casos, a MO é um fenômeno agudo que se resolve espontaneamente dias após o término do tratamento antineoplásico. Sinais provenientes da matriz extracelular (MEC) regulam a migração e a proliferação celular e sua diferenciação em tecido de cicatrização (Sonis, 2004).

Compreender a etiopatogenia da MO é fundamental para o desenvolvimento de novas medidas terapêuticas, uma vez que moléculas que conduzem cada fase representam potenciais alvos de intervenção, como o NF-κB, as ROS e as citocinas pro-inflamatórias (Sonis, 2004; Cinausero *et al*., 2017).

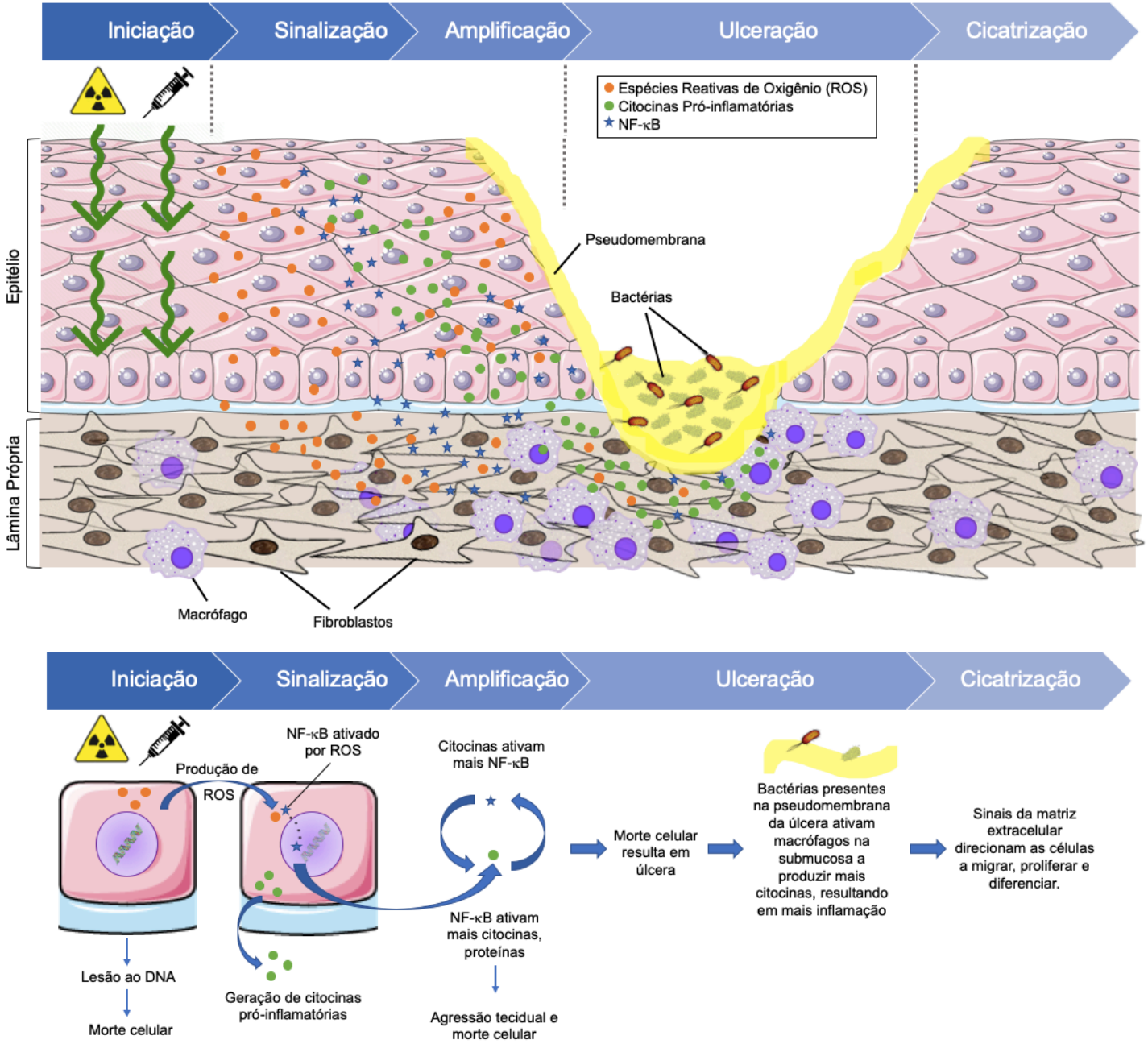

Figura 1. Estágios da fisiopatologia da mucosite oral, adaptado de Sonis, 2009.

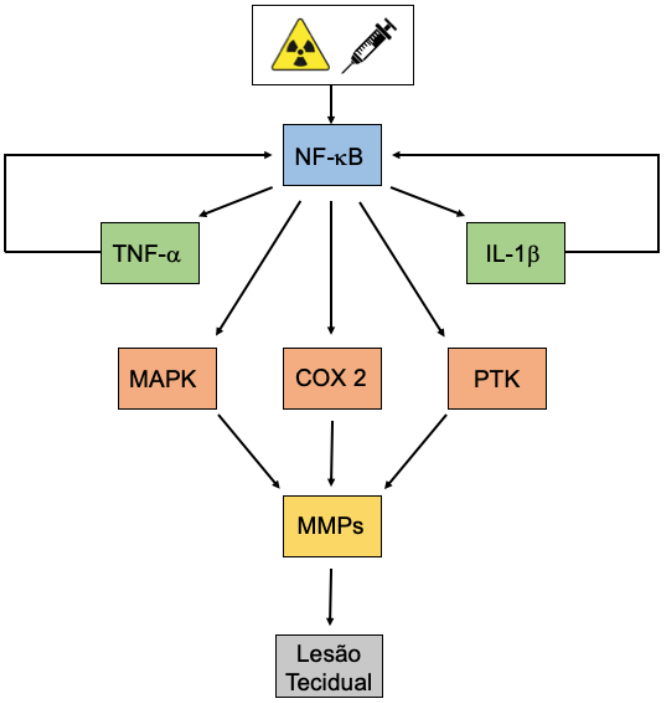

Figura 2. Amplificação de sinal no desenvolvimento da mucosite, adaptado de Sonis, 2004.

2.3 MEDIDAS TERAPÊUTICAS

Avanços no conhecimento sobre a fisiopatologia da mucosite têm resultado na identificação de inúmeros alvos promissores para tratamento da condição. Embora existam limitações na qualidade da evidência que permita estabelecer um protocolo padrão de prevenção e tratamento para mucosite, existem diretrizes que oferecem potenciais estratégias (Lalla *et al*., 2014; Peterson *et al*., 2015). Tais diretrizes não são definitivas e passam por constantes atualizações à medida que novos estudos são publicados e demonstram níveis mais altos de evidência para suportar ou refutar um determinado tratamento (Cinausero *et al*., 2017).

A estratégia preventiva mais básica é a orientação do paciente quanto às toxicidades que poderão surgir ao longo do tratamento antineoplásico. Dessa forma, o paciente fica alerta quanto ao surgimento de sintomas iniciais e possibilidade de intervenção precoce. Boa saúde oral antes e durante o tratamento do câncer parece ter um impacto positivo no curso da mucosite, uma vez que protocolos de cuidado

oral eliminam fontes de irritação e infecção da mucosa, reduzindo a microbiota patogênica e consequentemente o risco de infecção (McGuire *et al*., 2013; Peterson *et al*., 2015).

Agentes antioxidantes, anti-inflamatórios e antiapoptóticos parecem ser os mais eficazes no controle da MO, uma vez que agem diretamente sobre vias da fisiopatologia da mucosite, inibindo seu desenvolvimento. A cascata de sinalização e amplificação da resposta inflamatória da MO é gerada principalmente a partir das ROS. Essas moléculas são importantes alvo terapêuticos, uma vez que reduzindo sua produção ou eliminando-as do tecido reduz-se o desenvolvimento da MO (Kwon, 2016; Cinausero *et al*., 2017). Assim, agentes antioxidantes como amifostina, glutamina, zinco e vitamina E têm sido recomendados no controle da mucosite por suprimirem ROS ou aumentarem a produção endógena de enzimas antioxidantes (Lalla *et al*., 2014; Tsujimoto *et al*., 2015; Chaitanya *et al*., 2017; Rambod *et al*., 2018).

Uma outra estratégia para controlar o curso de evolução da MO é inibindo a inflamação e a produção de citocinas. Um dos medicamentos recomendados como agente preventivo da MO é o bochecho de cloridrato de benzidamina que possui efeito anti-inflamatório ao inibir a produção e a atividade de citocinas como o TNF-α, além de ter propriedades anestésica, analgésica e antimicrobiana (Nicolatou-Galitis *et al*., 2013). A pentoxifilina, uma outra classe de inibidores de citocinas pró-inflamatórias, demonstrou reduzir a expressão de TNF-α, IL-1β e óxido nítrico após sua administração em modelos animais. Apesar de ainda serem necessários estudos em humanos, a pentoxifilina parece ter potencial na prevenção da MO por inibir citocinas essenciais na sua patogênese (Moura *et al*., 2015; Gruber *et al*., 2017).

Uma potente moduladora da inflamação é a laserterapia de baixa potência (LBP), que tem sido amplamente utilizada como estratégia profilática e terapêutica da MO (Migliorati *et al*., 2013). Foi demonstrado que a LBP induz reparo tecidual ao influenciar diferentes fases da resolução, incluindo as fases inflamatória e proliferativa ao reduzir expressão de COX2 e estimular macrófagos e fibroblastos, e a fase de remodelação, auxiliando na deposição de colágeno (Zecha *et al*., 2016).

Basso *et al.* (2015) demonstraram em modelo *in vitro* de MO a expressão gênica e proteica reduzida de TNF-α, IL-6 e IL-8 em fibroblastos estimulados com LPS de *E. coli* após aplicação de LBP, confirmando seu efeito anti-inflamatório. Resultados semelhantes foram encontrados em um ensaio clínico randomizado que demonstrou expressão proteica reduzida de IL-1β, TNF-α e IL-10 em saliva de pacientes submetidos à QRT de cabeça e pescoço e tratados com LBP (Oton-Leite *et al*., 2015).

Tem sido cada vez mais investigado o potencial de agentes naturais na prevenção e tratamento da MO, principalmente devido ao seu mecanismo de ação que simultaneamente reduz estresse oxidativo, inflamação e infecção (Zhang *et al*., 2018). Produtos naturais incluem extratos brutos, frações enriquecidas com componentes bioativos e compostos puros que derivam de ervas (Sanders *et al*., 2016). Em particular, os inibidores naturais da via mTOR, tais como a curcumina, a epigalocatequina galato (EGCG) e o resveratrol, parecem ser potenciais agentes preventivos de mucosite oral. Estudo *in vitro* demonstrou que inibição de mTOR previne a perda de células-tronco epiteliais proliferativas durante a radioterapia e aumenta a capacidade de repovoamento, preservando a integridade da mucosa oral e protegendo-a da mucosite induzida por radiação. Esse efeito protetivo da inibição de mTOR é mediado pela expressão aumentada da enzima superóxido dismutase mitocondrial e consequente supressão de ROS (Beevers *et al*., 2009; Zhou, Luo & Huang, 2010; Iglesias-Bartolome *et al*., 2012).

A curcumina, principal polifenol extraído da cúrcuma, tem sido extensivamente estudada por suas propriedades anti-inflamatórias, antioxidantes, anticarcinogênicas e antimicrobianas. Atua principalmente pela modulação de citocinas pró-inflamatórias, proteínas apoptóticas, NF-κB e COX2 (Nagpal & Sood, 2013; Devaraj & Neelakantan, 2014). Este agente mostrou-se promissor no tratamento de várias doenças inflamatórias, incluindo a MO, sendo útil na reversão dos sinais e sintomas da condição (Rao *et al.,* 2014; Patil *et al*., 2015). Por ser uma estratégia terapêutica eficaz em outras desordens inflamatórias, faz-se necessário sumarizar as evidências presentes na literatura acerca dos efeitos da curcumina no manejo da MO.

As evidências ainda são poucas e, por isso, mais estudos experimentais ainda são necessários de forma a melhor compreender o mecanismo de ação dos inibidores naturais de mTOR sobre a fisiopatologia da MO e confirmar sua eficácia (Nagi *et al*., 2018). Nesse sentido, culturas de células utilizadas como modelos *in vitro* de MO aparecem como ferramentas úteis.

2.4 MODELOS *IN VITRO* DE MUCOSITE ORAL

A maioria das informações que suportam o modelo da fisiopatologia da MO proposto por Sonis (2004) são derivadas de experimentos em animais. Entretanto, existem diferenças significativas entre a biologia de roedores e humanos, e, portanto, modelos animais podem não replicar precisamente a condição clínica (Colley *et al*., 2013). Modelos *in vitro* têm sido propostos como complementos a experimentos em animais, especialmente em estudos que visam caracterizar propriedades farmacológicas e toxicológicas de novas substâncias. Entretanto, vale ressaltar que curvas dose-resposta geradas a partir de dados *in vitro* não são diretamente aplicáveis na prática clínica e devem, portanto, ser extensivamente investigadas (Algharably *et al*., 2019).

Monoculturas de queratinócitos orais (QO) são usualmente utilizadas para avaliar os efeitos da radiação ionizante *in vitro* (Donetti *et al*., 2009; Tobita *et al*., 2010; Colley *et al*., 2013; Shin *et al*., 2013). Colley *et al*. (2013) observaram redução na viabilidade celular 72h após irradiar QOs com dose única de 20 Gy, quando comparada aos QOs não irradiados. Utilizando a mesma dose de radiação, Shin *et al*. (2013) também observaram diminuição na viabilidade dos queratinócitos irradiados e concluíram que a epicatequina, um inibidor natural de mTOR extraída da folha do chá verde, protegeu significativamente as células da citotoxicidade induzida pela radiação. Além disso, a epicatequina protegeu as células da indução de apoptose e inibiu a geração de ROS intracelulares. Utilizando doses mais baixas de radiação sobre monocamada de queratinócitos, foi demonstrado que 2 Gy causa redução na expressão de desmogleína 3, mas não afeta a espessura epitelial

(Donetti *et al*., 2009), enquanto que doses crescentes entre 1 e 8 Gy de radiação geram redução na viabilidade celular e aumento na expressão de IL-1α e IL-8 (Tobita *et al*., 2010).

Modelos *in vitro* associando os diferentes tipos celulares que compõem a mucosa oral também têm sido uma estratégia de estudar os efeitos da radiação ionizante, uma vez que representam mais fielmente a condição clínica, apesar de serem mais complexos e onerosos (Rakhorst *et al*., 2006; Lambros *et al*., 2011; Colley *et al*., 2013; Tra *et al*., 2013). Foi observado em modelos tridimensionais compostos por queratinócitos e fibroblastos que irradiação com 12 Gy causou alterações drásticas na morfologia celular, resultando em maior expressão de quebras de fita de DNA, além de indução de apoptose e superexpressão de diversas citocinas inflamatórias, incluindo *IL-1*β, *IL-8* e *NF-*κ*B* (Rakhorst *et al*., 2006; Lambros *et al*., 2011). Com doses ainda maiores de radiação, entre 16,5 e 20 Gy, modelos de mucosite compostos por fibroblastos e queratinócitos também apresentaram viabilidade reduzida ao longo do tempo, maior lesão ao DNA, alteração na expressão de desmogleína 3, indução de apoptose e maior expressão de IL-1β e IL-1α, quando comparados a modelos não irradiados (Tra *et al*., 2013; Colley *et al*., 2013).

Poucos estudos avaliaram os efeitos da radiação sobre fibroblastos *in vitro*. Colley e colaboradores (2013) observaram significante perda da viabilidade celular em monocamada de fibroblastos, após irradiação com 20 Gy, além de expressão elevada de IL6 quando comparada à monocultura de queratinócitos e às células controle não irradiadas ($p<0.01$). Similarmente, Vuyyuri *et al*. (2008) utilizaram fibroblastos humanos para investigar o efeito protetor do aminoácido essencial metionina na prevenção de mucosite radio-induzida. Foi observado que o tratamento com metionina 1 hora antes de 10 Gy de radiação gerou aumento significativo na sobrevivência celular de fibroblastos quando comparados às linhagens de carcinoma espinocelular. Sabe-se que, após exposição à radiação ionizante, a apoptose dos fibroblastos precede a agressão epitelial (Sonis *et al*., 2000). Dessa forma, faz-se necessária investigação dos efeitos da radiação sobre esse tipo celular.

3. PROBLEMAS E HIPÓTESES

Existem na literatura diversas tentativas de modelos *in vitro* de MO, mas nenhum associando radiação ionizante a componentes bacterianos em cultura de fibroblastos orais humanos, como proposto no modelo da fisiopatologia de Sonis (2004). Assim, faz-se necessário um modelo mais fidedigno e facilmente reproduzível, para ser futuramente utilizado na investigação de novas medidas terapêuticas, tais como o potencial uso da cúrcuma e da curcumina que apresentam propriedades biológicas promissoras na prevenção e no tratamento da MO. Diante desse panorama, formularam-se as seguintes perguntas:

Pergunta 1: Como desenvolver um modelo de mucosite oral em laboratório para uso futuro em estudos *in vitro*?

Hipótese 1: O estímulo inflamatório com LPS de *E. coli*, Extrato proteico de *Pg* ou radiação ionizante aumentam a expressão gênica de mediadores químicos inflamatórios em linhagem de fibroblastos gengivais humanos.

Hipótese 2: O uso da radioterapia associado ao estímulo bacteriano irá induzir maior expressão de mediadores químicos inflamatórios em células de mucosa oral.

Pergunta 2: Quais os efeitos da cúrcuma e da curcumina (inibidores naturais de mTOR) na prevenção e tratamento da MO?

Hipótese 1: Os inibidores naturais de mTOR atuam como anti-inflamatórios, reduzindo a gravidade da mucosite oral.

Hipótese 2: Os inibidores naturais de mTOR são efetivos agentes preventivos pois suprimem a produção de ROS.

4. ARTIGOS

4.1 MANUSCRITO 1

O artigo a seguir foi escrito de acordo com as normas da revista *Oral Diseases*, ISSN 1601-0825 (versão online), classificada como periódico B1 na Qualis-Capes Medicina II, onde será posteriormente submetido para publicação. A escolha da revista foi influenciada pelo seu escopo, onde são publicadas investigações laboratoriais originais na área de Patologia oral.

In vitro model of oral mucositis induced by ionizing radiation and bacterial challenge

Ana Gabriela Costa Normando[a], D.D.S.
Samuel Ramalho Avelino[b], M.P., M.Sc.
Karina Gonzales Silvério[c], D.D.S., M.Sc., Ph.D.
Caroline Lourenço de Lima[a], D.D.S., M.Sc., Ph.D.
Eliete Neves Silva Guerra[a]*, D.D.S., M.Sc., Ph.D.

Affiliations
[a] Laboratory of Oral Histopathology, Health Sciences Faculty, University of Brasilia, Brasilia, Brazil, Health Sciences Faculty, University of Brasilia, Brasilia, Brazil.
[b] Radiotherapy department of Unit of High Complexity in Oncology of the University Hospital of Brasilia, Brasilia, Brazil.
[c] Department of Prosthodontics and Periodontics, Division of Periodontics, Piracicaba Dental School, University of Campinas, Piracicaba, São Paulo, Brazil.
*Corresponding author

ABSTRACT

Background and Objectives: Investigation of the physiopathology of oral mucositis (OM) may provide a better understanding of its mechanisms and the interactions with new targeted therapies. Several *in vitro* OM models have been developed to assess the effects of ionizing radiation, but none have associated radiation to bacteria nor assessed its effect on human oral fibroblasts. Therefore, this study aimed to establish an *in vitro* model of OM from human gingival fibroblasts induced by ionizing radiation and bacterial challenge.

Methods: Primary cultures of gingival fibroblasts were established from healthy patients using explant method. Cells were stimulated with *Escherichia coli* lipopolysaccharides (LPS) (0, 1 and 10 μg/mL), *Porphyromonas gingivalis (Pg)* protein extract (0, 1, 2 and 5 μg/mL) or ionizing radiation (0 and 12 Gy). Gene expression of nuclear factor kappa B (*NF-κB*) and pro-inflammatory cytokines *IL-6*, *IL-1β* and tumor necrosis factor alpha (*TNF-α*) were assessed 4, 6 and 24h after cell stimulation by RT-qPCR. Data were subjected to Kruskall-Wallis test followed by Dunn's post test ($P < 0.05$).

Results: Fibroblasts treated with *E. coli* LPS had increased expression of *IL-1β* and *TNF-α* in a dose-dependent manner at all times, with highest expression of *NF-κB*, *IL-6* and *IL-1β* 24h after stimuli. *Pg*-treated fibroblasts had increased expression of all genes in a dose-dependent manner. The greatest expression happened 6h after challenging the cells with 5μg/mL *Pg* protein extract with statistical significance for *IL-6* ($p<0.05$). Irradiated cells showed extremely low expression of all cytokines, with its peak happening 6h after 12 Gy. Association of radiation to *Pg* extract led to gene expression more pronounced than in cells stimulated with radiation only. Dose-dependent increase in expression of *IL-6* ($p<0.05$) and *IL-1β* could be also observed.

Conclusion: *Pg* better stimulates pro-inflammatory cytokines expression and association with radiation has provided additional gene expression. Although further experiments are still needed, the proposed model could be useful in future investigation of mechanism of new target therapies.

Key Words: oral mucositis; *in vitro* model; fibroblasts; *Escherichia coli; Porphyromonas gingivalis;* pro-inflammatory cytokines.

INTRODUCTION

Oral mucositis (OM) is among the most prevalent side effects of both radiotherapy (RT) and chemotherapy (CT), affecting almost a half million patients per year in the US (Sonis *et al*., 2015). OM is a very relevant condition since it negatively impacts the cancer patient's quality of life in terms of difficulty feeding, weight loss and treatment break due to oral pain. Also, it can be associated with local and systemic infection, leading to additional use of healthcare resources and increased hospital expenses (Villa & Sonis, 2015).

Historically, it was suggested that the mucosal injury was solely the result of RT or CT damaging effects on rapid dividing epithelial cells (Sonis, 2004). However, recent research has shown that the physiopathology of OM involves a complex multi-phase biological events within the epithelium and the connective tissue, with damaged fibroblasts and infiltrating leukocytes contributing to apoptosis, atrophy and ulceration (Sonis, 2007). An *in vitro* study observed quick death of cultured fibroblasts after treatment with chemotherapeutic agents, resulting in apoptosis, what supports the hypothesis that these cells play an important role in the pathogenesis of OM (Chrzanowski *et al*., 2001). Also, morphologic evidence from microscopy confirms damage to connective tissue before clinical signs of erythema or ulceration (Sonis, 2007).

OM development starts with RT or CT directly injuring DNA, causing death of basal epithelial cells and generation of reactive oxygen species (ROS). ROS initiate a series of biological events that culminate in the activation of many transcription factors, especially NF-κB, which regulates the expression of several molecules related with the pathogenesis of mucositis, such as TNF-α, IL-6 and IL-1β pro-inflammatory cytokines. These molecules can generate a positive feedback, leading to additional NF-κB activation, and amplifying its response. The consequence of signal amplification is the ulcerative phase of OM, which is the most clinically and symptomatically significant stage. In this phase, bacterial colonization of ulcers surface contributes to exacerbate the mucositis process once several microbial components, such as lipopolysaccharides (LPS), can penetrate the mucosa and stimulates additional pro-inflammatory cytokines

release (Sonis, 2002, 2009). It has been recently demonstrated that oral mucosal microbiota dysbiosis occurs during radiation therapy with variations in the abundances of *Prevotella, Fusobacterium, Treponema* and *Porphyromonas* thoughout the course of radiotherapy, with its peaks coinciding with severe mucositis (Hou *et al*., 2018).

Understanding the biological complexity behind mucosal injury caused by cytotoxic cancer therapy reflects the importance of advances in molecular and cell biology in this subject. The development of *in vitro* models may provide a better understanding of individual mechanisms and their interactions with new targeted therapies (Sonis, 2004). Different *in vitro* OM models have been developed to assess the effects of ionizing radiation, but none have associated radiation to bacterial challenge (Rakhorst *et al*., 2006; Lambros *et al*., 2011; Colley *et al*., 2013; Tra *et al*., 2013). Indeed, it has been recently suggested that further investigation should be performed to better characterize the potential contribution of the oral microbiome in the pathobiology of mucositis (Cinausero *et al*., 2017). Also, radiation-induced effects have been extensively studied on oral keratinocytes, but little is known about its effect on fibroblasts (Donetti *et al*., 2009; Tobita *et al*., 2010; Shin *et al*., 2013). The connective tissue beneath the epithelium is mainly composed by fibroblasts that act as physical and biochemical base, and when these cells are damaged by radiation, the epithelium collapses, revealing the importance of maintaining its integrity (Sonis, 2007).

Thus, the present study aimed to develop an *in vitro* model of oral mucositis from primary culture of human gingival fibroblasts induced by ionizing radiation and bacterial challenge. Nitric oxide synthesis and gene expression of *NF-κB*, as well as *TNF-α*, *IL-1β* and *IL-6* pro-inflammatory cytokines, was assessed in order to quantify the efficacy of the inflammatory stimulus.

MATERIALS AND METHODS

Cell culture

Gingival fibroblasts were isolated from gingival mucosa of five young healthy volunteer donors (aged 20 to 23 years) who underwent third molar extraction surgery. The fragments collection was undertaken with the understanding and written consent of each subject. The study has been conducted in full accordance with ethical principles (Declaration of Helsinki). Prior approval had been obtained from the Human Research Ethics Committee of the Health Sciences Faculty of the University of Brasilia (# 78679717.6.0000.0030).

After obtained, the gingival tissues were immersed in cold Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, St. Louis, MO) supplemented with 20% fetal bovine serum (FBS) (Gibco®, Invitrogen, Carlsbad, *CA*) and 10% penicillin/streptomycin (Sigma-Aldrich), and immediately transported to the Laboratory of Oral Histopathology. Cultures were stablished by using the explant technique (Kedjarune *et al*., 2001; Hendijani, 2017). Briefly, the gingival tissues were washed twice with Phosphate-Buffered Saline (PBS) 1x, minced into small fragments, also known as *explants*, and then placed on a 6-well plate. The fragments were stabilized with a glass coverslip and covered by 2 mL of DMEM high-glucose with 20% FBS and antibiotics. Cells were incubated at 37ºC in a humidified incubator at 5% CO_2 and culture medium was replaced every 2-3 days. When 80-90% confluency was reached, cells were detached with 0.25% trypsin (Sigma-Aldrich) and subcultured to a 100 mm dishes with DMEM plus 10% FBS and antibiotics. Cells on second passage were stored at -80ºC in freezing solution containing FBS and 10% dimethyl sulfoxide (DMSO) until future use. Gingival fibroblasts cultures on passage five were used in all experiments of this study.

Bacterial inflammatory stimulus

Liposaccharides (LPS) from *Escherichia coli* (*E. coli* O111:B4; Sigma-Aldrich) or protein extract of *Porphyromonas gingivalis* (*Pg*) were used to mimic the release of bacterial cells wall products into mucosal lesions. The LPS (*E. coli*) was solubilized in PBS and stored at -20ºC. The *Pg* protein extracts were prepared as previously described (Albiero *et al*., 2017), diluted in DMEM and stored at -80°C.

Gingival fibroblasts were seeded in DMEM with 10% FBS. After 24h, the medium was replaced by fresh serum-free DMEM, and the cells were treated with 1 and 10 μg/mL of LPS (*E. coli*) or with 1, 2 and 5 μg/mL of *Pg* protein extract for 1 hour. Cells without treatment, incubated only with medium, were used as control group.

Ionizing irradiation protocol

After one hour of treating or not the cells with bacterial stimulus, the plates were transported to the Unit of High Complexity in Oncology of the University Hospital of Brasilia to be irradiated. The gingival fibroblasts were exposed to a single dose of 2 or 12 Gy. Control plates were maintained in a non-irradiated environment. Irradiated and control fibroblast cultures were immediately returned to the incubator at 37ºC and 5% CO_2 for subsequent Nitric Oxide and Gene Expression assays. Irradiation was performed using the PRIMUS™ Linear Accelerator (*Siemens Medical Solutions*, Concord, CA) with a maximum rated power of 6 MeV photon beam. The source-culture plate distance was 98 cm and the dose rate was 200 MU/min.

Nitric Oxide (NO) synthesis

To indirect determine nitric oxide production, spectrophotometric measurement of its stable decomposition products (nitrite and nitrate) was determined by the Griess reaction (Grisham *et al*., 1996; Bryan & Grisham, 2007). Cells (2 x 10^5 cells/well) were seeded in 12-well plates and treated as protocols described. After 24, 48 and 72h post-treatments, a 100μL media aliquot of each well was collected and mixed with 100 μl of Griess Reagent (Sigma-Aldrich) (1:1 of 1% N-(1-Naphthyl)ethylenediamine dihydrochloride (NEED) and 0,1% Sulfanilamide 1% in 5% H_3PO_4) in a 96-well plate. The NO production was determined by comparing with a standard curve of sodium nitrite (0,18 - 200,0 μmol/L). Optical density was measured at a wavelength of 540 nm in a spectrophotometer (Multimode Plate Reader EnSpire®, *PerkinElmer*, Waltham, MA).

RT-qPCR gene expression assay

Gingival fibroblasts were seeded (2.5 x 10^5 cells/well) in 6-well plates, and 4, 6 and 24h after the treatments, total RNA was extracted with TRI Reagent® (Sigma-Aldrich) according to the manufacturer's protocol. Samples quality and concentration were determined by spectrophotometry using the NanoVue Plus (GE Healthcare Life Sciences, UK, EU). Genomic DNA contamination was removed by treating RNA samples with DNAse I (Sigma-Aldrich). The cDNA was synthesized from 400 ng total RNA by using High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA) in 20 μL reactions containing MultiScribe™ Reverse Transcriptase, 10X RT Buffer, 25X dNTP mix, and 10X RT Random Primers. The samples were stored at -20º C until use.

Quantitative polymerase chain reaction (qPCR) was performed by using Power Up™ SYBR® Green Master Mix (Applied Biosystems) in a StepOnePlus™ Real-Time PCR System (Applied Biosystems). Reactions were prepared in duplicate or triplicate in 96-well plates (MicroAmp™ Optical, *Applied Biosystems*) to 10 μL final volume containing 0.5 μL of cDNA (10 ng), 5 μL of 2X PowerUp™SYBR® Green Master Mix, 0.2 μL (100nM) of each forward and reverse primer, and 4.1 μL o nuclease free water. Data analysis was carried out on StepOne Software v2.1 (Applied Biosystems). Primers sequences (Table 1) for gene analysis (*IL-1β*, *IL-6*, *TNF-α*, and *NF-κB*) were compared to sequences available in the non-redundant NCBI (National Center for Biotechnology Information) database, and provided by IDT® (*Integrated DNA Technologies*, Coralville, IA) or Invitrogen (Carlsbad, CA). Beta-actin was used as a housekeeping gene, and relative quantification of gene expression was calculated by comparative cycle threshold (Ct) method by using the formula $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct_{treatment} - \Delta Ct_{calibrator/control}$ (Schmittgen & Livak, 2008).

Table 1. Primer sequences selected for gene expression analysis.

Target gene	Primer Sequences	Size (bp*)
IL-1β	**F** 5' ATGATGGCTTATTACAGTGGCAA 3' **R** 5' GTCGGAGATTCGTAGCTGGA 3'	132
IL-6	**F** 5' CCTGAACCTTCCAAAGATGGC 3' **R** 5' TTCACCAGGCAAGTCTCCTCA 3'	75
NF-κB	**F** 5' AACAGAGAGGATTTCGTTTCCG 3' **R** 5' TTTGACCTGAGGGTAAGACTTCT 3'	104
TNF-α	**F** 5' GGAGAAGGGTGACCGACTCA 3' **R** 5' CTGCCCAGACTCGGCAA 3'	71
ACTB	**F** 5' TCACCCACACTGTGCCCATCAATG 3' **R** 5' CAGCGGAAACCGTCATTGCCAATG 3'	295

* Bp: base pairs.

Statistical analysis

Data were obtained from at least one experiment in triplicate or from three independent experiments, using cultures from different donors. NO synthesis and gene expression data were presented as mean (range) and mean ± standard deviation (SD), respectively. All data were analyzed by the non-parametric Kruskall-Wallis test followed by Dunn's post-test, by using the GraphPad Prism® version 8 for Windows (GraphPad Software, La Jolla, CA). $P < 0.05$ was considered statistically significant.

RESULTS

In order to establish the concentration of *E. coli* LPS, *Pg* protein extract and radiation dose that would better stimulate inflammatory reaction, the synthesis of nitric oxide, an important inflammatory mediator, was first investigated (Kendall *et al*., 2000; Daghigh *et al*., 2002). The results of the Nitric Oxide (NO) synthesis by human gingival fibroblasts 24, 48 and 72h post-treatment with different concentrations of *E. coli* LPS or *Pg* protein extract associated or not with ionizing radiation are shown in Tables 2 and 3.

Regardless of the concentration of *E. coli* LPS, the production of NO was more pronounced at the radiation dose of 12 Gy, with a greater expression after 72h of stimulus, although not statistically significant. Surprisingly, the highest absorbance values were found in control samples 72h after irradiation with 12 Gy, followed by control samples not irradiated in 48h. On the other hand, in the cells treated with *Pg* protein extract there was a greater release of NO at the radiation dose of 2 Gy in all samples, independent of time after inflammatory exposure or *Pg* concentration, again not statistically significant.

Table 2. Nitric Oxide (NO) synthesis by human gingival fibroblasts 24, 48 and 72h post-treatment with different concentrations of *E. coli* LPS associated or not with ionizing radiation.

		Radiation Doses		
Time	**LPS Concentration (μg/ml)**	**0 Gy**	**2 Gy**	**12 Gy**
24h	0 (control)	51 (44-56)	55 (46-76)	52 (41-63)
	1	52 (47-63)	47 (42-58)	55 (48-60)
	10	56 (49-68)	53 (47-62)	61 (47-72)
48h	0 (control)	65 (49-87)	49 (44-54)	62 (50-100)
	1	43 (40-47)	40 (38-42)	48 (45-56)
	10	44 (40-51)	41 (40-43)	52 (39-71)
72h	0 (control)	51 (42-66)	48 (39-57)	67 (59-76)
	1	45 (43-47)	46 (40-52)	60 (47-73)
	10	49 (40-51)	48 (40-71)	60 (58-61)

*Values are represented in nm as mean x 10^3 (range) of one experiment in triplicate.

Table 3. Nitric Oxide (NO) synthesis by human gingival fibroblasts 24, 48 and 72h post-treatment with different concentrations of *Pg* protein extract associated or not with ionizing radiation.

		Radiation Doses		
Time	***Pg* Concentration (μg/ml)**	**0 Gy**	**2 Gy**	**12 Gy**
24h	0 (control)	64 (46-79)	52 (43-66)	58 (44-76)
	1	57 (42-84)	65 (46-103)	41 (40-42)
	2	42 (41-43)	91 (74-113)	50 (40-66)
	5	42 (41-43)	81 (67-92)	46 (43-50)
48h	0 (control)	56 (44-70)	89 (72-108)	43 (42-44)
	1	44 (41-49)	87 (82-95)	43 (40-47)
	2	46 (43-49)	60 (44-90)	49 (45-55)
	5	56 (42-83)	73 (56-82)	61 (42-76)
72h	0 (control)	76 (42-97)	101 (76-120)	70 (43-97)
	1	44 (42-45)	73 (54-95)	56 (44-67)
	2	44 (43-44)	70 (48-90)	44 (42-45)
	5	43 (43-43)	106 (92-131)	80 (68-95)

*Values are represented in nm as mean x 10^3 (range) of one experiment in triplicate.

Because the results from NO synthesis assay were inconsistent, not very enlightening and with no statistical significance, irradiation with the dose of 12 Gy was selected for the gene expression assay based on the scientific literature. Also, this dose of radiation coincides with the development of the first clinical signs of oral mucositis in patients and, therefore, more similar to reality.

In order to analyze the effectiveness of each stimulus in up-regulate inflammatory gene expression, cells were firstly treated with *E. coli* LPS, *Pg* protein extract or 12 Gy irradiation in separate. Gingival fibroblasts treated with *E. coli* LPS have shown an increased expression trend for all assessed genes while compared to untreated controls. However, time and concentration that induced the highest expression were little consistent among genes (Figure 1). The relative expression of *IL-1β* and *TNF-α* tended to enhance in a dose-dependent manner in all times, while *NF-κB* and *IL-6*

expression had this pattern only 4h after stimuli. Treatment with LPS induced highest relative *NF-κB*, *IL-6* and *IL-1β* gene expression 24h after stimuli, whereas the degree of *TNF-α* expression was smaller after 24h-treatment.

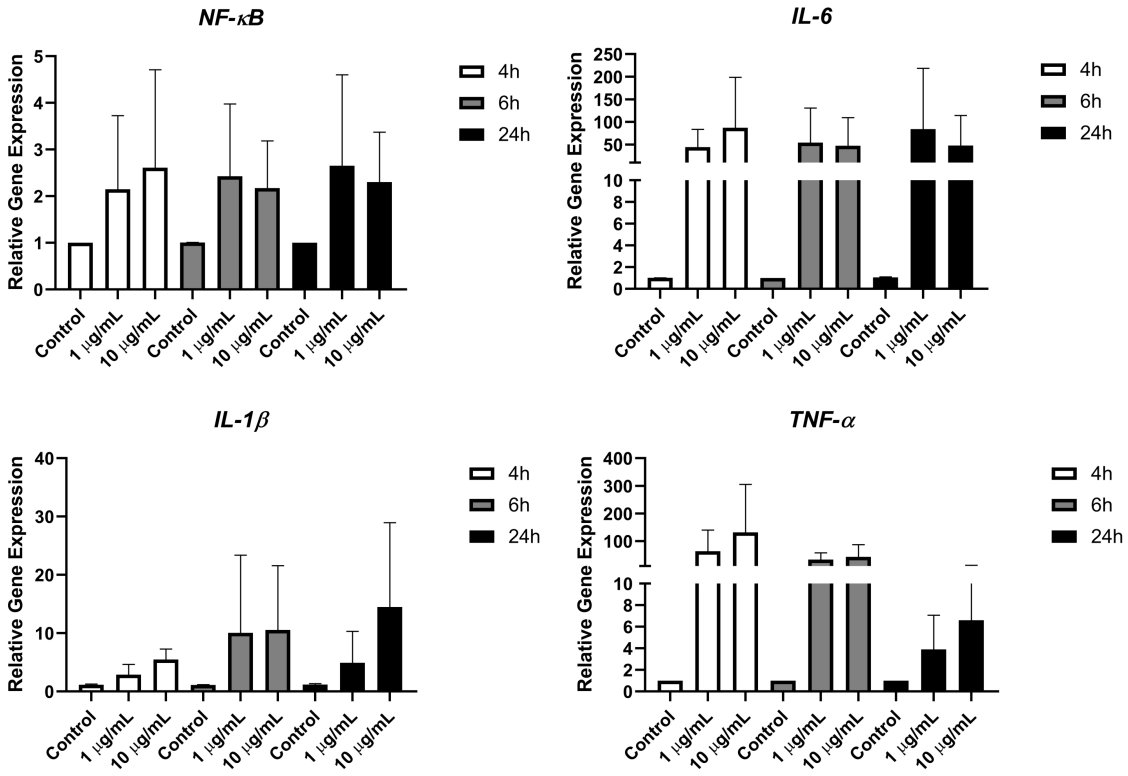

Figure 1. Relative gene expression of *NF-κB*, *IL-6*, *IL-1β* and *TNF-α*, 4, 6 and 24h after treatment with 0 (control), 1 and 10 μg/mL *E. coli* LPS. Results are shown as mean ± SD of three independent experiments, using cultures from different donors.

Pg-treated fibroblasts exhibited an increase in relative expression of all genes in a dose-dependent manner, except for *TNF-α* in 24h, where low expression was observed for all concentrations tested (Figure 2). Further, the greatest expression of all genes happened 6h after challenging the cells with 5μg/mL *Pg* protein extract, with statistically significant difference for *IL-6* ($p<0.05$). Importantly, 5 μg/mL of *Pg* protein extract

induced statistically significant *IL-6* expression in all assessed times, when compared to respective control groups ($p<0.05$).

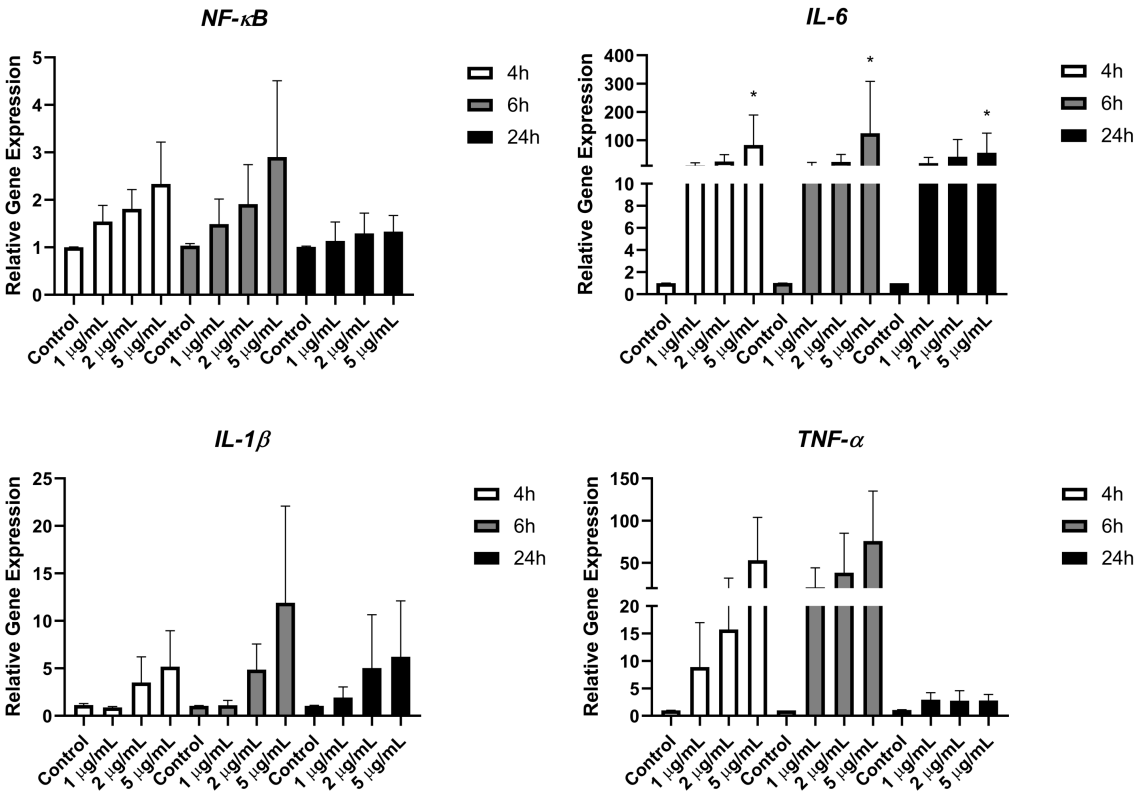

Figure 2. Relative gene expression of *NF-κB*, *IL-6*, *IL-1β* and *TNF-α*, 4, 6 and 24h after treatment with 0, 1, 2 and 5 μg/mL *Pg* protein extract. Results are shown as mean ± SD of three independent experiments, using cultures from different donors. *p< 0.05 when compared to respective controls (Kruskall-Wallis test followed by Dunn's post-test).

Finally, cells receiving only irradiation showed an extremely low expression for all inflammatory genes with little variation between the control group (untreated) and the irradiated group. Interestingly, the highest relative expression of all genes could be observed 6h after 12 Gy of irradiation, although not statistically significant (Figure 3). Undetermined expression of *TNF-α* 4h after irradiating the cells led to the impossibility of analyzing the expression of this cytokine in this period.

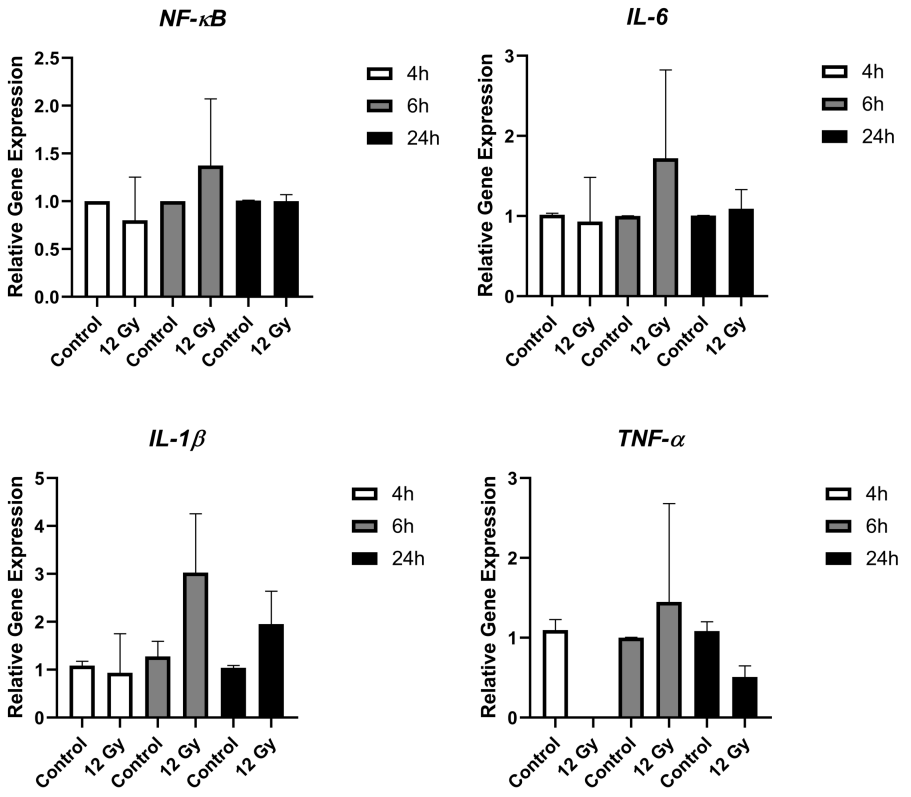

Figure 3. Relative gene expression of *NF-κB*, *IL-6*, *IL-1β* and *TNF-α*, 4, 6 and 24h after 12 Gy of ionizing radiation. Results are shown as mean ± SD of three independent experiments, using cultures from different donors.

Afterward, the gene expression was assessed by associating one of the bacterial challenges with 12 Gy of radiation. Because the highest expression of all pro-inflammatory genes was observed 6h after 5 μg/mL *Pg* stimulation and 12 Gy irradiation, these doses and time were selected to test the combinate stimuli effects. The hypothesis was that applying two combined inflammatory stimuli could produce additional increase in gene expression. Association of 12 Gy to increasing concentrations of *Pg,* led to enhanced expression of interleukins in a dose-dependent manner, demonstrating that this association increases interleukin expression when compared to cells only irradiated. Statistically significant IL-6 expression was observed after stimuli with 5 μg/mL *Pg* associated to 12 Gy, when compared to non-stimulated cells ($p < 0.05$) (Figure 4).

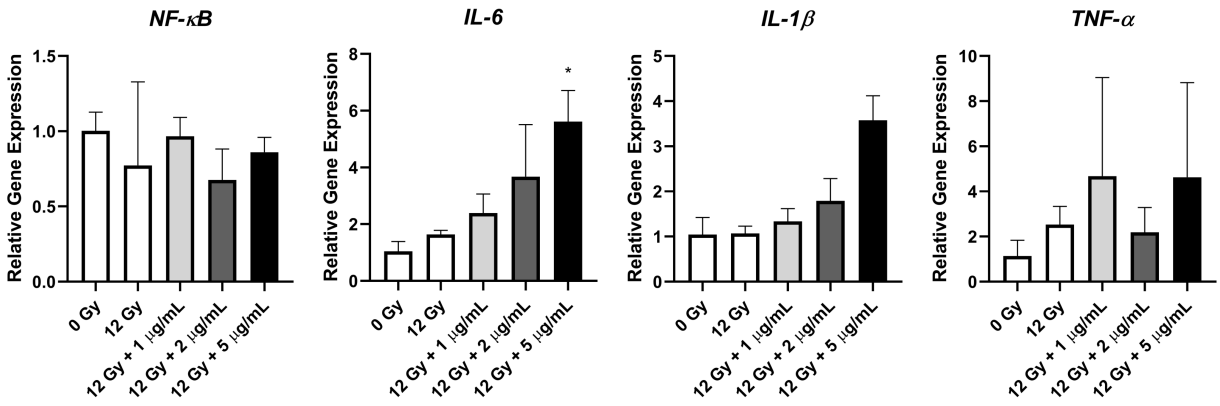

Figure 4. Relative gene expression of *NF-κB*, *IL-6*, *IL-1β* and *TNF-α*, 6h after treatment with 5 μg/mL *Pg* protein extract associated to 12 Gy ionizing radiation. Results are shown as mean ± SD of one experiment in triplicate. * p< 0.05 when compared to control (Kruskall-Wallis test followed by Dunn's post-test).

DISCUSSION

The increasing knowledge about the physiopathology of the OM has provided opportunities for the development of new approaches based on the underlying molecular pathways involved in its development. Although several possibilities of treatments have emerged, no standard protocol have been established so far. Further, the potential role of the oral microbiome in the pathobiology of mucositis associated with targeted agents should be better characterized (Peterson *et al*., 2015; Cinausero *et al*., 2017).

In highlight of this scenario, the development of a new experimental model of OM, which can be easily reproduced, may be useful to better understand the biological processes behind mucositis, and could aid the development of new therapies that target molecules involved in its pathobiology. Morphological observations indicate that changes in the endothelium and fibroblasts precede epithelial injury (Sonis *et al*., 2000; Sonis, 2004). For this reason, the present study aimed to establish an *in vitro* model from primary culture of human gingival fibroblasts, which seemed more reliable than using immortalized cells. The

immortalization method may introduce variations in some important metabolic functions and generate genotypic, karyotypic and phenotypic modifications during prolonged culture time (Gomez-Lechon *et al*., 2008). To the best of our knowledge, this is the first attempt of an *in vitro* model of OM that associates ionizing radiation to bacterial challenge in a primary culture of human oral fibroblasts to simulate the pathogenesis previously proposed by Sonis (2004).

In vitro OM models are mostly based on keratinocyte monocultures, although fibroblasts monolayers and three-dimensional (3D) models with keratinocytes and fibroblasts co-cultures have also been designed (Rakhorst *et al*., 2006; Vuyyuri *et al*., 2008; Lambros *et al*., 2011; Colley *et al*., 2013; Tra *et al*., 2013). Despite the 3D models more accurately represent the OM microenvironment *in situ*, they are complex and very expensive, often unviable, depending on the laboratory conditions. Therefore, a model from gingival tissue fragments, or explants, seems to be a simple and reliable alternative for analyzing the expression of inflammatory mediators that may be useful to assess the effectiveness of new treatments (Hendijani, 2017).

Cytokines are small proteins produced by a variety of cells throughout the body and primarily involved in host responses to inflammation or infection (Dinarello, 2000; Logan *et al*., 2007). In the development of OM, inflammatory cytokines are produced in response to the activation of *NF-κB*, which has been implicated in the control of a broad range of biological events, including apoptosis (Sonis, 2002). Increased levels of IL-1β, IL-6 and TNF-α positively associated to radiation dose and OM severity have been detected in serum and salivary samples of head and neck cancer patients submitted to chemoradiotherapy (Citrin *et al*., 2012; Bossi *et al*., 2016; Normando *et al.,* 2017). It has also been demonstrated the overexpression of NF-κB in biopsy samples of oral mucosa of patients during administration of cytotoxic chemotherapy (Logan *et al.,* 2007). However, expression of this important transcription factor as well as cytokines have not been previously assessed in gingival fibroblasts until then.

Initially, to confirm that radiation and bacterial stimuli were being effective, the NO production by the fibroblasts was measured. This method was preferred for its simplicity and low-cost to detect inflammation injury (Bryan & Grisham, 2007).

Accumulating evidence suggests that NO may play a key role in mediating tissue damage in inflammatory conditions and that human fibroblasts produce NO while stimulated by cytokines (Daghigh *et al*., 2002). Also, it has been demonstrated that the stimulation of human gingival fibroblasts cultures with *Porphyromonas gingivalis* LPS is capable of increasing NO production (Kendall *et al*., 2000).

In view of this, it was hypothesized that the association of radiation with different bacterial stimuli would exacerbate NO production. However, the results were somewhat heterogeneous and little pronounced, with the maximum values oscillating among the different stimulus conditions. Basso *et al*. (2016) also found considerably heterogeneous results of NO synthesis after stimulating gingival fibroblasts with different concentrations of TNF-α, IL-1β, IL-6, or IL-8. Therefore, the results of NO production in fibroblasts were interpreted with caution and the selection of the best radiation dose equal to 12 Gy was based on the scientific literature, that supports that low doses of radiation, around 2 Gy, do not cause considerable morphological changes nor impact the release of inflammatory cytokines (Rakhorst *et al*., 2006; Tobita *et al*., 2010; Lambros *et al*., 2011).

Since the results were heterogeneous in the NO assay, it seemed more coherent to analyze the inflammatory relative gene expression after stimulating the cells with one of the three stimuli in separate to observe the gene expression profile among them. It was observed that the greatest expression of *NF-κB* and *IL-6* was detected on fibroblasts challenged with *Pg* protein extract, whereas cells challenged with *E. coli* LPS expressed higher levels of *IL-1β* and *TNF-α*. Gamma radiation at a dose of 12 Gy tended to increase the expression of all mediators 6h post-irradiation, although extremely lower level than the other inflammatory stimuli. Interestingly, the peak expression of all the mediators occurred 6h after irradiation and *Pg* challenge, raising the hypothesis that this is probably the best time for the analysis of these genes.

Finally, additional increase in the gene expression of *NF-κB* and inflammatory cytokines was expected after exposing the cells to *Pg* extract and radiation stimuli in association. Indeed, an enhance in expression of the interleukins could be observed in a dose-dependent manner after irradiating cells stimulated by *Pg* extract when

compared to cells only irradiated. On the other hand, this pattern of expression did not occur to *NF-κB* neither to *TNF-α.*

Similarly, Tobita *et al*. (2010) observed that IL-1α and IL-8 secretion tended to increase in a dose-dependent manner after irradiating a tissue-engineered three-dimensional human oral mucosa with 0, 1, 3 and 8 Gy. Further, pronounced release of IL-1α and IL-8 increased after exposing the cells to 8 Gy, with statistical significance ($p<0.01$). Oral mucosal models composed by keratinocytes and fibroblasts demonstrated wide-ranging alterations in cell morphology after irradiation with 12 Gy. It induces great expression of DNA strand breaks, apoptosis and upregulation of NF-κB and some inflammatory cytokines, including IL-1β, IL-8 and, what corroborates our results (Rakhorst *et al*., 2006; Lambros *et al*., 2011). With even higher doses of radiation, between 16.5 and 20 Gy, three-dimensional models also showed greater DNA damage, more apoptosis and increased secretion of IL-1β and IL-1α while compared to non-irradiated models (Colley *et al*., 2013; Tra *et al*., 2013). Colley *et al*. (2013) observed greater IL-6 production in fibroblasts monolayer after 20 Gy of irradiation when compared to irradiated keratinocytes monoculture and non-irradiated control cells ($p<0.01$).

Cell viability has not been evaluated in this study, however, similar research has observed that irradiation of oral fibroblasts and keratinocytes with 20 Gy was capable of reducing cell viability over time, with statistically significance 72h after irradiation ($p<0.001$) (Colley *et al*., 2013). Lower doses of radiation, such as 8 Gy, have also been shown to cause a statistically significant drop in cell viability of oral keratinocytes ($p<0.05$) (Tobita *et al*., 2010). Vuyyuri *et al*. (2008) observed that the surviving fraction of fibroblasts dropped as the irradiation dose increased from 0, 2, 4, 6 to 8 Gy.

Although the stimuli have been effective in up-regulate inflammatory mediators gene expression in gingival fibroblasts, some potential limitations of the present study should be pointed out. For example, the sample size per experiment, not having tested the gene expression with lower doses of radiation, the storage form of *Pg* protein extract, which maybe had impaired its stability, contributing for partial loss of its inflammatory stimulus capacity, and not having tested fibroblasts viability after

bacterial and radiation challenge. Therefore, further investigations must be conducted in order to complement the presented results.

CONCLUSION

Primary culture of human gingival fibroblasts seems to be a simple, inexpensive and reliable method to simulate oral mucositis since the effects of radiation and chemotherapy affect these cells prior to epithelial injury. Dose of 12 Gy generates greater expression of all inflammatory mediators when compared to the non-irradiated control. *E. coli* LPS better stimulated the expression of *IL-1*β and TNF-α, while *Pg* protein extract induced greater expression of *NF-*κ*B* and *IL-6*. *Pg*-stimulated cells had a maximal peak for all inflammatory mediators with the same concentration (5μg/mL) and time (6h). Association of radiation to *Pg* extract led to increased interleukin expression in a dose-dependent manner when compared to non-irradiated cells. Therefore 5μg/mL of *Pg* associated to 12 Gy seems the most suitable combination for OM *in vitro* model.

ACKNOWLEDGEMENTS

The authors thank the volunteers who participated in the study, Dra. Sandra Márcia Mazutti from the Natural Products Laboratory for assisting in the experiments of NO synthesis and the medical physicists Luis Felipe Oliveira and Leonardo Bicudo for assisting in the irradiation of the cells. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare no potential conflicts of interest.

REFERENCES

1. Albiero, M. L., Stipp, R. N., Saito, *et al*. (2017). Viability and osteogenic differentiation of human periodontal ligament progenitor cells are maintained after incubation with Porphyromonas gingivalis protein extract. *Journal of periodontology*, *88*(11), e188-e199.
2. Bossi, P., Bergamini, C., Miceli, R., *et al* (2016). Salivary cytokine levels and oral mucositis in head and neck cancer patients treated with chemotherapy and radiation therapy. *International Journal of Radiation Oncology* Biology* Physics*, *96*(5), 959-966.
3. Bryan, N. S., & Grisham, M. B. (2007). Methods to detect nitric oxide and its metabolites in biological samples. *Free Radical Biology and Medicine*, *43*(5), 645-657.
4. Chrzanowski, K., Bielawska, A., Bielawski, K., *et al* (2001). Cytotoxicity and effect on collagen biosynthesis of proline analogue of melphalan as a prolidase-convertible prodrug in cultured human skin fibroblasts. *Il Farmaco*, *56*(9), 701-706.
5. Cinausero, M., Aprile, G., Ermacora, P., *et al*. (2017). New frontiers in the pathobiology and treatment of cancer regimen-related mucosal injury. *Frontiers in pharmacology*, *8*, 354.
6. Citrin, D. E., Hitchcock, Y. J., Chung, E. J., *et al* (2012). Determination of cytokine protein levels in oral secretions in patients undergoing radiotherapy for head and neck malignancies. *Radiation Oncology*, *7*(1), 64.
7. Colley, H. E., Eves, P. C., Pinnock, A., *et al* (2013). Tissue-engineered oral mucosa to study radiotherapy-induced oral mucositis. *International journal of radiation biology*, *89*(11), 907-914.
8. Daghigh, F., Borghaei, R. C., Thornton, R. D., *et al* (2002). Human gingival fibroblasts produce nitric oxide in response to proinflammatory cytokines. *Journal of periodontology*, *73*(4), 392-400.
9. Dinarello CA (2000). Proinflammatory cytokines. *Chest*, 118(2): 503–8.

10. Donetti, E., Bedoni, M., Capone, P., *et al* (2009). An in vitro model of human oral explants to study early effects of radiation mucositis. *European journal of oral sciences*, *117*(2), 169-174.
11. Gomez-Lechon, M. J., Donato, M. T., Lahoz, A., & Castell, J. V. (2008). Cell lines: a tool for in vitro drug metabolism studies. *Current drug metabolism*, *9*(1), 1-11.
12. Grisham, M. B., Johnson, G. G., & Lancaster Jr, J. R. (1996). Quantitation of nitrate and nitrite in extracellular fluids. In *Methods in enzymology* (Vol. 268, pp. 237-246). Academic Press.
13. Hendijani, F. (2017). Explant culture: An advantageous method for isolation of mesenchymal stem cells from human tissues. *Cell proliferation*, *50*(2), e12334.
14. Hou, J., Zheng, H., Li, P., *et al* (2018). Distinct shifts in the oral microbiota are associated with the progression and aggravation of mucositis during radiotherapy. *Radiotherapy and Oncology*, *129*(1), 44-51.
15. Kedjarune, U., Pongprerachok, S., Arpornmaeklong, P., *et al* (2001). Culturing primary human gingival epithelial cells: comparison of two isolation techniques. *Journal of cranio-maxillo-facial surgery*, *29*(4), 224-231.
16. Kendall, H. K., Haase, H. R., Li, H., *et al* (2000). Nitric oxide synthase type-II is synthesized by human gingival tissue and cultured human gingival fibroblasts. *Journal of periodontal research*, *35*(4), 194-200.
17. Lambros, M. P., Parsa, C., Mulamalla, H., *et al* (2011). Identifying cell and molecular stress after radiation in a three-dimensional (3-D) model of oral mucositis. *Biochemical and biophysical research communications*, *405*(1), 102-106.
18. Logan, R. M., Stringer, A. M., Bowen, J. M., *et al* (2007). The role of pro-inflammatory cytokines in cancer treatment-induced alimentary tract mucositis: pathobiology, animal models and cytotoxic drugs. *Cancer treatment reviews*, *33*(5), 448-460.
19. Logan, R. M., Gibson, R. J., Sonis, S. T., & Keefe, D. M. (2007). Nuclear factor-κB (NF-κB) and cyclooxygenase-2 (COX-2) expression in the oral mucosa following cancer chemotherapy. *Oral oncology*, *43*(4), 395-401.

20. Normando, A. G. C., Rocha, C. L., de Toledo, I. P., *et al* (2017). Biomarkers in the assessment of oral mucositis in head and neck cancer patients: a systematic review and meta-analysis. *Supportive Care in Cancer*, *25*(9), 2969-2988.
21. Peterson, D. E., Boers-Doets, C. B., Bensadoun, R. J., & Herrstedt, J. (2015). Management of oral and gastrointestinal mucosal injury: ESMO Clinical Practice Guidelines for diagnosis, treatment, and follow-up. *Annals of oncology*, *26*(suppl_5), v139-v151.
22. Rakhorst, H. A., Tra, W. M., Posthumus-Van Sluijs, *et al* (2006). Quantitative analysis of radiation-induced DNA break repair in a cultured oral mucosal model. *Tissue engineering*, *12*(12), 3395-3403.
23. Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C T method. *Nature protocols*, *3*(6), 1101.
24. Shin, Y. S., Shin, H. A., Kang, *et al* (2013). Effect of epicatechin against radiation-induced oral mucositis: in vitro and in vivo study. *PLoS One*, *8*(7), e69151.
25. Sonis, S. T., Peterson, R. L., Edwards, L. J., *et al* (2000). Defining mechanisms of action of interleukin-11 on the progression of radiation-induced oral mucositis in hamsters. *Oral Oncology*, *36*(4), 373-381.
26. Sonis, S. T. (2002). The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with anti- neoplastic therapy. *Crit. Rev. Oral Biol. Med.* 13, 380–389.
27. Sonis, S. T. (2004). The pathobiology of mucositis. *Nat. Rev. Cancer* 4, 277–284. doi: 10.1038/nrc1318
28. Sonis, S. T. (2007). Pathobiology of oral mucositis: novel insights and opportunities. *J. Support Oncol*. 9 (Suppl 4), 3–11.
29. Sonis, S. T. (2009). Mucositis: the impact, biology and therapeutic opportunities of oral mucositis. *Oral oncology*, *45*(12), 1015-1020.
30. Sonis, S., Elting, L., Keefe, D., *et al*. (2015). Unanticipated frequency and consequences of regimen-related diarrhea in patients being treated with radiation or chemoradiation regimens for cancers of the head and neck or lung. *Support. Care Cancer* 23, 433–439.

31. Tobita, T., Izumi, K., & Feinberg, S. E. (2010). Development of an in vitro model for radiation-induced effects on oral keratinocytes. *International journal of oral and maxillofacial surgery*, *39*(4), 364-370.
32. Tra, W. M. W., Tuk, B., van Neck, J. W., *et al* (2013). Tissue-engineered mucosa is a suitable model to quantify the acute biological effects of ionizing radiation. *International journal of oral and maxillofacial surgery*, *42*(8), 939-948.
33. Villa, A., and Sonis, S. T. (2015). Mucositis: pathobiology and management. *Curr. Opin. Oncol.* 27, 159–164.
34. Vuyyuri, S. B., Hamstra, D. A., Khanna, D., *et al* (2008). Evaluation of D-methionine as a novel oral radiation protector for prevention of mucositis. *Clinical Cancer Research*, *14*(7), 2161-2170.

5.2 MANUSCRITO 2

A revisão sistemática a seguir foi submetida para publicação na revista *Phytotherapy Research*, ISSN 1099-1573 (versão online), classificada como periódico B1 na Qualis-Capes Medicina II. O registro do envio está sob número PTR-18-1291 e encontra-se sob "minor revision". A escolha da revista foi influenciada pelo seu escopo, onde se encontram diversas publicações na área de interesse, além de apresentar prévias publicações de revisões sistemáticas.

Effects of turmeric and curcumin on oral mucositis: a systematic review

Ana Gabriela Costa Normando[a], D.D.S.
Amanda Gomes de Menêses[b], R.N., M.Sc.
Isabela Porto de Toledo[a, c], S.L.P., M.Sc.
Gabriel Álvares Borges[a], D.D.S., M.Sc.
Caroline Lourenço de Lima[a], D.D.S., M.Sc., Ph.D.
Paula Elaine Diniz dos Reis[b], R.N., Ph.D.
Eliete Neves Silva Guerra[a]*, D.D.S., M.Sc., Ph.D.

Affiliations
[a] Laboratory of Oral Histopathology, Health Sciences Faculty, University of Brasilia, Brasilia, Brazil, Health Sciences Faculty, University of Brasilia, Brasilia, Brazil.
[b] Interdisciplinary Laboratory of Applied Research on Clinical Practice in Oncology, Health Sciences Faculty, University of Brasilia, Brasilia, Brazil.
[c] Brazilian Centre for Evidence-Based Research, Department of Dentistry, Federal University of Santa Catarina, Florianópolis, Brazil.

* Corresponding author:
Prof. Dr. Eliete Neves Silva Guerra
SQN 205, bloco H, apto 201, Asa Norte, Brasília, DF, Brazil ZIP Code: 70843-080
E-mail: elieteneves@unb.br / Phone number: + 55 61 996684988

ABSTRACT

Objective: To evaluate the effects of turmeric and curcumin in the management of oral mucositis in cancer patients undergoing chemo and/or radiotherapy.

Methods: The systematic review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The search was performed in the following database: Cochrane Library, LILACS, LIVIVO, PubMed, Scopus, and Web of Science. A gray literature search was undertaken using Google Scholar, Open Grey and ProQuest. The methodology of included studies was evaluated by the Meta-Analysis of Statistics Assessment and Review Instrument (MAStARI).

Results: After a two-step selection process, four randomized and one non-randomized clinical trials were included in the analysis. Two studies were categorized as low and three as moderate risk of bias. Turmeric/curcumin was applied topically as a gel or as a mouthwash. Patients treated with turmeric/curcumin experienced reduced grade of mucositis, pain, erythema intensity and ulcerative area.

Conclusion: Current evidence suggests that topical application of turmeric or curcumin is effective in controlling signs and symptoms of oral mucositis. Thus, further investigation is required to confirm the promising effect of turmeric and curcumin in oral inflammatory lesions.

Key Words: mucositis; turmeric; curcumin; chemotherapy; radiotherapy; cancer; systematic review.

INTRODUCTION

Oral mucositis (OM) is one of the most common and debilitating side effects of antineoplastic therapy. It is estimated that 40 to 70% of patients undergoing conventional chemotherapy (CT) and almost all patients on head and neck radiotherapy (RT) will develop some degree of mucositis (Scully *et al*., 2003; Ruiz-Esquide *et al*., 2011). OM presents as erythema and burning sensation, and may evolve to substantially painful ulcerative lesions, impairing the patient's ability to eat and speak. Combined RT and CT increase the severity of OM, which may eventually entail a reduction in the CT dose or a RT interruption, thereby negatively affecting prognosis and quality of life (Trotti *et al*., 2003; Scully *et al*., 2004, Manzi *et al*., 2016). Furthermore, OM has a considerable economic impact, since it increases costs related to signs and symptoms management, nutritional support, secondary infection treatment and hospitalizations (Elting *et al*., 2007).

Good oral hygiene and dental status may reduce the risk, course, and severity of OM (Villa & Sonis, 2015). However, treatment interventions must be considered once OM lesions are already settled down. Treatment is focused on pain relief, bacterial load reduction and healing promotion, in order to minimize the duration and severity of the condition. The Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO) Clinical Practice Guidelines for OM recommend oral cryotherapy, low-level laser therapy, recombinant human keratinocyte growth factor-1(KGF-1/palifermin) and benzydamine mouthwash to prevent OM and suggest the use of 2% morphine or 0.5% doxepin mouthwashes as pain control (Lalla *et al.,* 2014). In spite of current recommendations and suggestions for OM management, there is still a gap in the scientific evidence regarding standard treatment. Therefore, finding an efficacious alternative with minimal side effects is essential (Patil *et al*., 2015).

In light of this, phytotherapy is deemed relevant as a treatment possibility for OM. In this sense, the therapeutic properties of turmeric (*Curcuma longa)*, a rhizomatous herb often used as food spice and traditionally applied as treatment for several illnesses in oriental medicine, have been extensively studied in the past years. The antioxidant, analgesic, anti-inflammatory, antiseptic, antimicrobial and anticarcinogenic effects of

turmeric and curcumin, a polyphenol extracted from its rhizome, have been described (Nagpal & Sood, 2013; Devaraj & Neelakantan, 2014). Further, a recent review reports the effectiveness of curcumin on pathological pain associated with several chronic conditions (Sun *et al.,* 2018). Considered the effects of turmeric and curcumin on the oral cavity, results were positive when it was applied as relief for tooth pain, cavity sealant, subgingival irrigator and treatment for aphthous and potentially malignant lesions (Nagpal & Sood, 2013; Grover *et al.,* 2015). Curcumin also inhibited tumor growth and cell viability and induced cytotoxicity, cell cycle arrest and apoptosis in *in vitro* and *in vivo* head and neck carcinoma models, as observed by our research group in a previous systematic review (Borges *et al.,* 2017). When tested in cutaneous radiation toxicity, curcumin was effective in reducing the severity of radiation dermatitis and moist desquamation in cancer patients (Ryan *et al.,* 2013; Pallaty *et al.,* 2014; Vaughn, Branum & Sivamani, 2016).

To date, many clinical studies have been performed to support the use of turmeric and curcumin on CT and RT-induced oral mucositis. Thus, the purpose of this systematic review was to evaluate the effects of turmeric and curcumin in the management of oral mucositis induced by chemo and/or radiotherapy.

METHODS

Protocol and Registration

This systematic review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Checklist (Moher *et al.,* 2010). The protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO) database under registration number CRD42018083318 (Normando *et al.,* 2018).

Study design

This is a systematic review of clinical trials that assessed the therapeutic effects of turmeric and curcumin on chemo and/or radiotherapy-induced OM in cancer patients. Although other study designs demonstrating the effects of turmeric/curcumin on OM are

available in the literature, clinical trials were selected because of their higher level of scientific evidence.

Eligibility Criteria

Inclusion criteria. The inclusion criteria for this systematic review were based on the PICOS (Population, Intervention, Comparison, Outcome and Study Design) approach. We considered (S) clinical trials, randomized or not, that evaluated the effects of (I) turmeric or curcumin compared to (C) placebo or other interventions in the (O) prevention or treatment of OM in (P) cancer patients undergoing radiotherapy and/or chemotherapy. It was considered prevention when patients started using turmeric/curcumin prior to the onset of mucositis lesions, and treatment when patients already presented mucositis at the beginning of the intervention.

Exclusion criteria. Studies were excluded for the following reasons: (1) reviews, letters, personal opinions, book chapters, and conference abstracts; (2) observational studies, *in vitro* and *in vivo* animal studies; (3) use of turmeric/curcumin to treat other oral inflammatory diseases; (4) use of turmeric/curcumin on intestinal mucositis; (5) use of turmeric/curcumin associated with other compounds; (6) data not individualized for OM; (7) language restriction; (8) full paper copy not available.

Information sources and search strategy

Individualized search strategy was developed for each of the following electronic databases: Cochrane Library, LILACS, LIVIVO, PubMed, Scopus, and Web of Science (Appendix 1). Furthermore, a gray literature search was performed through Google Scholar, Open Grey, and ProQuest Dissertations & Theses Global. The search on databases was performed on November 1st, 2017, with no time restriction. In addition, the reference lists of selected articles were hand screened for potentially relevant studies that could have been missed during the electronic database searches. Duplicated references were removed by reference manager software (EndNote®, Thomson Reuters). An updated search with the same word combinations for each database above mentioned was performed on June 1st, 2018.

Study selection

The articles were selected in two phases: screening of titles and abstracts and full text reading. In phase 1, two authors (AGCN and AGM) independently reviewed titles and abstracts of all references identified in the electronic databases and selected articles that seemed to meet the inclusion criteria. Phase 1 of study selection was performed on Rayyan, a web and mobile app developed for initial screening phase of systematic reviews (Ouzzani *et al*., 2016). In phase 2, the same two authors (AGCN and AGM) independently analyzed the full text of all articles selected in phase 1 and excluded studies that did not meet the inclusion criteria (Appendix 2). A third author (IPT) was consulted if disagreements between the two initial evaluators were not solved by consensus. Reference lists of all included articles were hand screened and the articles selected were read by AGCN and AGM.

Data collection process

One reviewer (AGCN) collected the key information from each selected study and a second reviewer (AGM) crosschecked the collected information to confirm its accuracy. Again, any disagreements were resolved by discussion and mutual agreement among the three reviewers. For each of the included studies, the following information was recorded: study characteristics (author, year, country, study design and sources of funding), population characteristics (sample, cancer site and cancer treatment) and intervention characteristics (intervention, control, administration, assessment criteria and main conclusions).

Risk of bias within studies

The risk of bias of selected studies was assessed by Meta-Analysis of Statistics Assessment and Review Instrument (MAStARI), a standardized critical appraisal instrument for risk of bias (Joanna Brigs, 2014). Risk of bias was categorized as High when the study reached up to 49% score “yes”, Moderate when the study reached 50% to 69% score “yes”, and Low when the study reached more than 70% score “yes”. AGCN and AGM scored all the 10 items as “yes”, “no”, “unclear” or “not applicable” and assessed independently the quality of each included study (Appendix 3).

Disagreements were resolved by a third reviewer (IPT).

Summary measures

The primary outcome for this systematic review was the efficacy of turmeric/curcumin in the prevention or treatment of OM. Secondary outcomes were reduction on scores of erythema, ulceration, pain intensity, and improvement in healing and ability to drink and eat. Any type of outcome measurement was considered in this review (categorical and continuous variables).

Synthesis of results

A meta-analysis was planned to be performed on Review Manager® 5.3 software (RevMan 5.3, The Nordic Cochrane Centre, Copenhagen, Denmark) to summarize the extracted data from included trials if the studies had sufficient data and if the data collected was homogeneous enough.

Risk of bias across studies

Clinical heterogeneity (by comparing variability among the participant's characteristics and outcomes assessed) and methodological heterogeneity (by comparing the variability in study design and risk of bias) were considered.

RESULTS

Study Selection

In phase 1 of the study selection, 257 citations were identified across the six electronic databases. After duplicated articles were removed, 151 citations remained. Evaluation of titles and abstracts was completed and 134 articles were excluded, remaining 17 articles. The gray literature search yielded 163 references, of which four were selected for full-text analysis. One additional study was identified from the reference lists of the identified studies and included in the analysis. On search update, 50 new references were found but none fulfilled all inclusion criteria.

A full text review was conducted on the 22 articles retrieved from phase 1 of study selection. This process led to the exclusion of 17 studies (Arantes *et al*., 2017; Belcaro *et al*., 2014; dos Santos *et al*., 2018; Elad *et al*., 2013; Francis & Williams, 2014; Ghazi *et al*., 2015; Gu *et al*., 2014; Khattry *et al*., 2012; Lueer *et al*., 2010; Lüer *et al*., 2010; Lüer *et al*., 2011; Lüer *et al*., 2012; Lüer *et al*., 2012; Lüer *et al*., 2014; Nagarale & Rathod, 2016; Rezvani, 2003; Rezvani & Ross, 2004). Finally, five articles were selected for descriptive analysis (Charantimath, 2016; Mansourian *et al.,* 2015; Patil *et al.,* 2015; Rao *et al.,* 2014; Saldanha & Almeida, 2014). A flow chart detailing the process of identification, screening, and inclusion of studies is presented in Figure 1.

Study Characteristics

Among the included studies, there were four randomized clinical trials (RCT) and one non-randomized clinical trial (NRCT). The studies were conducted in India, except for one that was conducted in Iran (Mansourian *et al.,* 2015). The five included studies were published between 2014 and 2016 and all of them were published in English.

The samples from the five selected studies totalized 217 individuals: 109 in case and 108 in control group. Sample size ranged from 10 (Patil *et al.,* 2015) to 40 (Rao *et al.,* 2014) patients affected by OM. All included patients were diagnosed with cancer in the head and neck region, and the most commonly studied sites were tongue, pharynx (naso, oro, and hypo), oral cavity and larynx. Different cancer treatment strategies were adopted, being chemoradiotherapy (CRT) and radical radiotherapy mostly applied, followed by chemotherapy alone. A summary of the descriptive characteristics for the included studies is presented in Table 1.

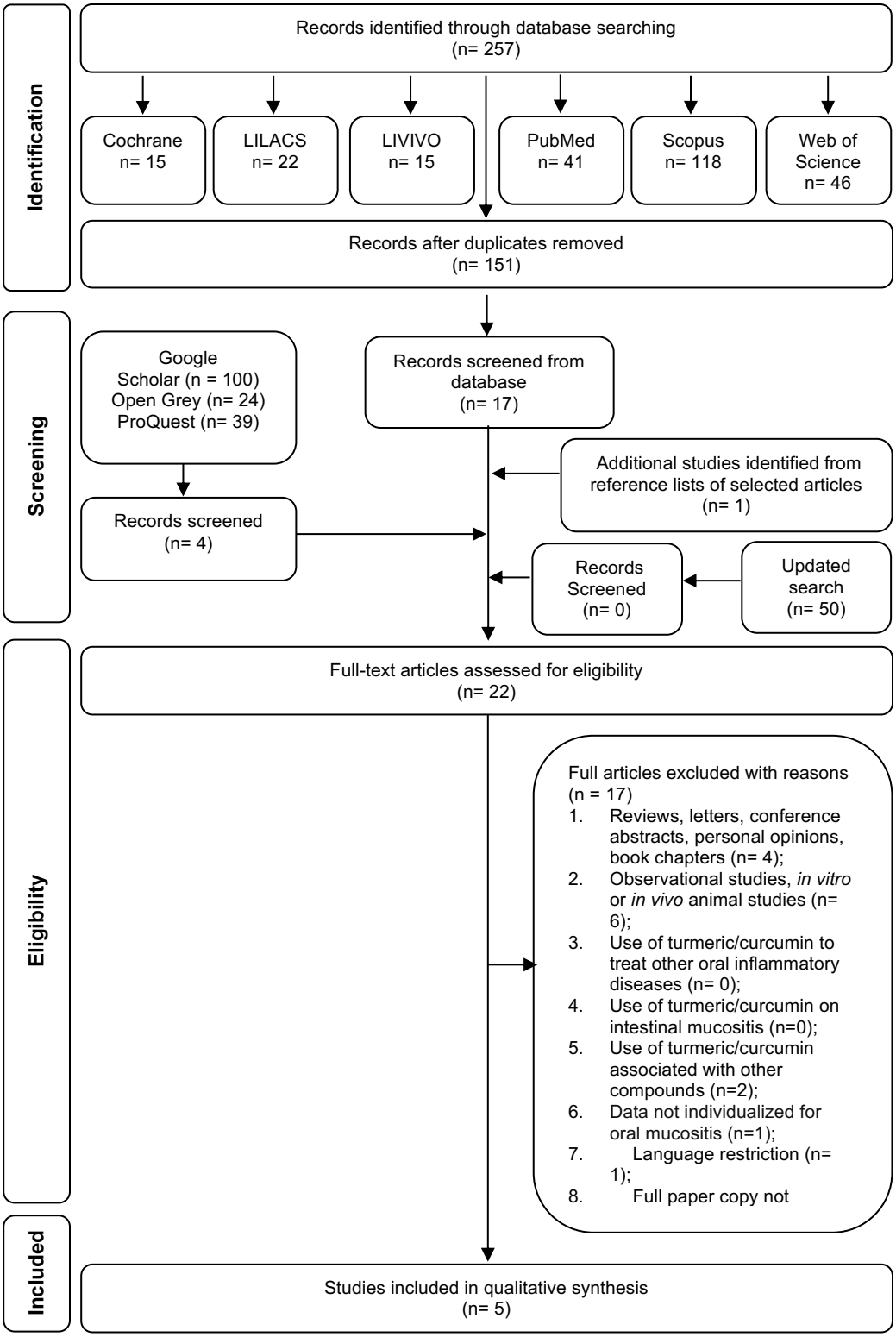

Figure 1. Flow diagram of literature search and selection criteria adapted from PRISMA (Moher *et al.,* 2010).

Table 1 - Summary of descriptive characteristics of included studies (n=5)

Study characteristics			Population characteristics			Intervention characteristics				
Author, Year and Country	**Study design**	**Funding**	**Sample**	**Cancer Site**	**Cancer Treatment**	**Intervention**	**Control**	**Administration**	**Assessment Criteria**	**Main conclusions**
Charanti-math, 2016 India	RCT	NR	20 case 20 control	Oral cavity	CRT	Curcuma oral gel - CureNext (10 mg of *Curcuma longa* extract)	Chlorhexi-dine gluconate 1.0%	Apply the gel 3 times a day on each ulcer after meals, for 2 weeks	NRS, OMAS and WHO	Curcumin is an effective and safer alternative to chlorhexidine. There was statistically significant difference between groups. Curcumin was found to be better than chlorhexidine in terms of faster wound healing and better patient compliance. No oral or systemic complications were found.
Mansourian *et al*., 2015 Iran	RCT	No financial support	19 case 18 control	Head and neck	RT (50-65 Gy)	*Curcuma longa 0.5%* topical gel	Yellowish ineffective inert placebo gel	Apply the gel 3 times a day, for 21 days	WHO and VAS	The intensity of mucositis was milder in the intervention group than in the control group. There was no grade 3 mucositis in the intervention group, while in the control group 7 patients developed grade 3 mucositis. No side effects were demonstrated. The gel could reduce the signs of oral mucositis and burning mouth sensation, but it could not be considered as a preventing agent.
Patil *et al*., 2015 India	RCT	No financial support	10 case 10 control	Oral cavity Pharynx Larynx	CRT (65-70 Gy + Cisplatin, Carbopla-tin or Taxol)	Curcumin mouthwash 0.004%	Chlorhexidi-ne mouthwash 0.2%	I - 1:5 dilution C - 1:1dilution Both use for 1 minute, 3 times a day, for 20 days	NRS, OMAS and WHO	The severity of oral mucositis increased according to the number of CT cycles, and this change was noted more in the control than in the study group. There was statistically significant difference between study and control groups in terms of NRS ($p<0.001$), erythema ($p=0.05$), ulceration ($p<0.001$) and WHO

										(p=0.003).
Rao *et al.*, 2014 India	RCT	No financial support	40 case 40 control	Head and neck	RT (70 Gy) CRT (70 Gy + Carbopla-tin)	Turmeric mouthwash (400 mg of turmeric in 80 mL of water)	Povidone-iodine mouthwash	I - Swish 10 mL of the solution for 2 minutes, 6 times a day C - Swish 10 mL, twice a day, for 6 weeks.	RTOG guidelines	The onset of mucositis was delayed in the patients using turmeric and there was a statistically significant difference between the groups throughout the study. Only 14 of 39 patients in the turmeric group developed intolerable mucositis, while in the povidone-iodine group 34 out of 40 patients developed intolerable mucositis. (p<0.0001).
Saldanha & Almeida, 2014 India	NRCT	NR	20 case 20 control	Head and neck Others	RT CRT CT	Turmeric mouthwash (1.5 g of turmeric powder in 50 mL of water)	Saline mouthwash	Swish 50 mL of the solution, 3 times a day for 5 days	Self-prepared tool	Turmeric was found to be effective in reducing the OM grade, with a reduction in mean score from 25.35 on day 1 to 18.85 on day 5. Saline mouthwash was also effective in reducing the OM grade: mean pre-test score was 25.05 and post-test score was 20.15. Only on days 4 and 5 there was a significant difference between turmeric and normal saline mouthwash (p=0.001).

* C: control; CRT: chemoradiotherapy; CT: chemotherapy; I: intervention; MPJ: Modified Patient Judged Oral Mucositis Assessment Scale; ND: not determined; NRCT: Non-randomized clinical trial; NR: Not Reported; NRS: Numerical Rating Scale; OMAS: Oral Mucositis Assessment Scale; RCT: Randomized Clinical Trial; RT: radiotherapy; RTOG: Radiation Therapy Oncology Group; VAS: Visual Analysis Scale; WHO: World Health Organization Oral Mucositis Assessment Scale

Risk of bias within studies

Risk of bias assessment of the five included studies is summarized in Figure 2. Two studies were graded as low risk of bias (Mansourian *et al*., 2015; Rao *et al*., 2014) and the other were considered as moderate risk of bias (Charantimath *et al*., 2016; Patil *et al*., 2015; Saldanha & Almeida, 2014). Some criteria were considered uncertain/unclear when they were not clearly reported in the original study, with incomplete or missing information. The items related to randomization, allocation and blinding were scored only for RCT, while the NRCT had either negative or unclear answers. In all studies, the control and treatment groups were considered comparable at entry since all patients had the same diagnosis and similar epidemiological characteristics. The groups were also considered identically treated, because in the individual studies the patients in both intervention and control groups were submitted to the same antineoplastic therapy. Most studies used appropriate statistical analysis and measured the outcomes in a reliable and reproducible way for all groups. On the other hand, most studies did not describe the outcomes of people who withdrew from the trial nor described the reasons for follow-up losses.

Figure 2. (A) Risk of bias summary: review authors' judgements about each risk of bias item for each included study (+ = yes; - = no; ? = unclear). (B) Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

Synthesis of results

Two distinct topical formulations containing turmeric/curcumin were evaluated: three studies assessed it in a mouthwash (Patil *et al*., 2015; Rao *et al.,* 2014; Saldanha & Almeida, 2014) and two studies appraised it on a gel (Charantimath, 2016; Mansourian *et al.,* 2015). Among the included trials, three used turmeric/curcumin as a treatment strategy for OM (Charantimath, 2016; Patil *et al*., 2015; Saldanha & Almeida, 2014) while the other two evaluated their potential as a preventive agent (Mansourian *et al.,* 2015; Rao *et al*., 2014).

Patil *et al*. (2015) assessed curcumin mouthwash to treat OM in 10 patients receiving CRT to the head and neck region. Statistically significant difference was found in favor of intervention group against control group (chlorhexidine) in terms of pain ($p < 0.001$), erythema intensity ($p = 0.05$), ulceration area ($p < 0.001$) and degree of severity of mucositis ($p = 0.003$). Moreover, no adverse events were noted in the intervention group. Saldanha & Almeida *et al*. (2014) also assessed the effectiveness of turmeric mouthwash to treat OM. Signs and symptoms of OM were scored on a scale of 1 to 45, in which 1 to 15 represent mild OM, 16 to 30 moderate OM and 31 to 45 severe OM. The mean post-interventional score on day 5 in the experimental group was 18.85 which was significantly lower than the mean pre-interventional score of 25.35 ($p<0.001$), showing that turmeric mouthwash was effective in reducing the OM grade. The study by Rao *et al*. (2014) investigated the preventive effects of turmeric mouthwash on head and neck cancer patients. The authors observed that turmeric mouthwash delayed the onset of OM ($p<0.001$ to $p<0.0001$) and also reduced the number of patients with intolerable mucositis ($p<0.0001$). Additionally, when compared to povidone-iodine rinse group, there was less treatment breaks and weight loss ($p<0.001$) in the turmeric group.

The efficacy of curcuma gel on OM treatment, compared to chlorhexidine gel, was evaluated in the study by Charantimath (2016). The curcuma group experienced a pronounced reduction on the Numerical Rating Scale (NRS) related to pain ($p= 0.0001$) and a significant change in WHO score both in the first ($p=0.0025$) and second follow-up ($p=0.0001$). A statistically significant change in erythema grade ($p= 0.0048$) and in size of ulcer ($p= 0.0001$) was also observed in the curcuma group when compared to control. Mansourian *et al*. (2015) also assessed the effects of curcuma in a gel formulation for OM prevention and they observed no grade 3 mucositis in the intervention group, as compared to 7 (38,9%) in the control group. The frequency of different grades of mucositis in the two groups was significantly different ($p<0.001$) and the mean size of oral lesions, oral erythema and burning mouth sensation in the intervention group was significantly lower than in control ($p<0.001$).

Figure 3 represents the mean values of mucositis grade scores before and after 5-10 days of treatment with turmeric/curcumin or control intervention in studies that used a graded scale of 0 to 4 (WHO or self-prepared tool), in which 0 means no mucositis and 4 means very severe mucositis. Although not statistically significant, a

tendency to a reduced OM severity in the turmeric/curcumin groups could be observed. Number of studies that reported secondary outcomes related to the use of turmeric/curcumin is represented in figure 4, being reduced erythema and ulcer size the most reported outcomes.

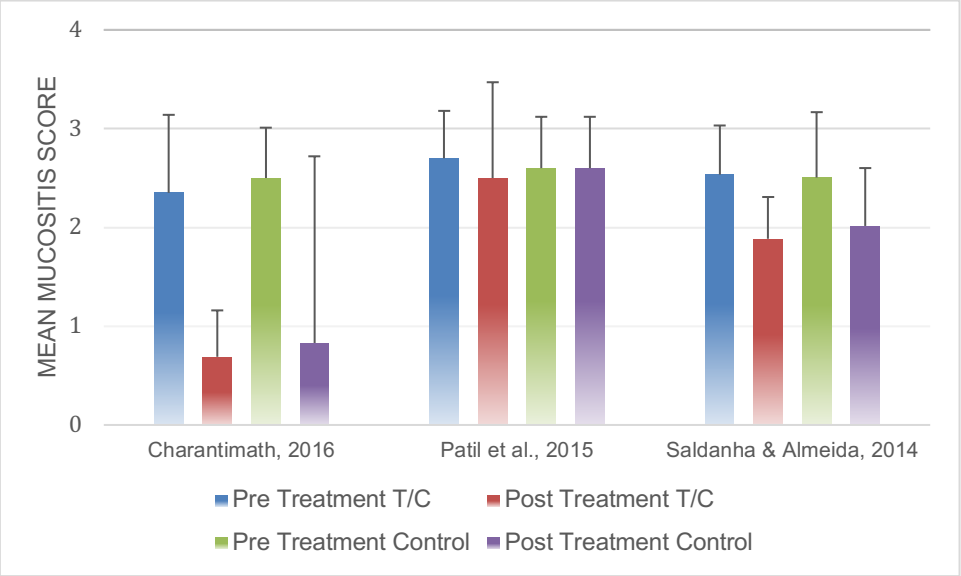

Figure 3. Graphical representation of mean mucositis score and standard deviation in the intervention with turmeric/curcumin (T/C) and control groups before and after treatment (mucositis score ranges from 0 - no mucositis to 4 - severe mucositis).

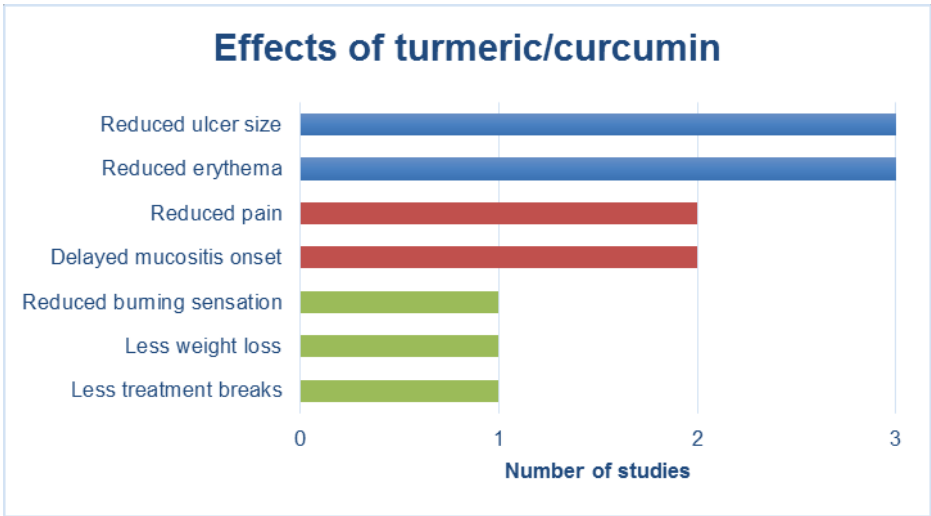

Figure 4. Secondary outcomes observed in the included studies.

Risk of bias across studies

Although all included trials assessed topical application of turmeric/curcumin on OM, a meta-analysis was not feasible since heterogeneity among the included studies was considerable, especially regarding formulations and concentrations. Therefore, only the descriptive assessment of the included data was provided.

DISCUSSION

Summary of evidence

Cancer patients suffer various side effects induced by antineoplastic therapy, and OM is among the most debilitating adverse effects of chemo and radiotherapy (Sonis, 2009). Although many palliative measures and therapeutic agents have been investigated, no effective prevention or treatment standard protocol has been completely successful to handle mucositis (dos Santos Filho *et al.,* 2018). Hence, the search for alternative products continues, and natural phytochemicals with anti-inflammatory and antioxidant properties, such as turmeric and its main polyphenol, curcumin, have been considered as a good alternative source (Nagpal & Sood, 2013).

According to the pathobiology model of mucositis proposed by Sonis (2007), OM can be divided into five stages: initiation, signaling, amplification, ulceration, and healing. Briefly, radiation and chemotherapy directly injure DNA, causing strand breaks that result in death of basal epithelial cells and generation of reactive oxygen species (ROS). These ROS initiate a series of biological events that culminates in the activation of several transcription factors, such as nuclear factor kB (NF-κB). NF-κB governs the expression of approximately 200 genes, of which some are associated with the production of molecules that play an important role in the pathogenesis of mucositis, including cytokines, cytokine modulators, stress responders and cell adhesion molecules (Sonis, 2002). Accordingly, targeting and inhibiting NF-κB could be an efficient strategy to decrease pro-inflammatory cell responses and consequently reduce mucositis lesions.

A well-known NF-κB inhibitor is the turmeric's main constituent, curcumin, which is a powerful anti-inflammatory agent that strongly inhibits cytoplasmic NF-κB activation and subsequent synthesis of cytokines such as Tumor Necrosis Factor-α

(TNF-α), Interleukin-6 and -8, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Monocyte Chemoattractant Protein-1 (MCP-1), and Vascular Endothelial Growth Factor (VEGF) (Lüer, Troller & Aebi, 2012). In addition, curcumin has been reported to have strong antibacterial effects by inhibiting bacterial growth, epithelial cell adherence, and cellular invasion in a mucositis model of epithelial cells *in vitro* (Lüer *et al.,* 2011). Since breakage of the mucosal barrier allows pathogens to penetrate the mucosa and increase inflammation, the antibacterial property of curcumin could contribute in accelerated healing of OM (Elad *et al.,* 2013). Therefore, topical administration of curcumin on mucosal surfaces is an attractive approach to treat mucositis, since it may down-regulate inflammation and reduce the bacterial load.

A recently published phase I randomized clinical trial assessed the safety dose of two mouthwash formulations containing curcuminoids and *Bidens pilosa L*. extract. None of the healthy volunteers experienced toxicity nor reported adverse reactions, indicating that both formulations were biochemical, cytological and clinically safe (dos Santos Filho *et al.,* 2018). The same research group had already demonstrated the chemoprotective effect of the formulation containing curcuminoids on 5-FU induced toxicity in HaCaT cells and in 5-FU-induced intestinal mucositis in mice (dos Santos Filho *et al.,* 2016; dos Santos Filho *et al.,* 2018). Similarly, Van't Land *et al*. (2004) investigated the effects of curcumin on intestinal mucositis induced by methotrexate in rats and found that inhibition of NF-κB activation with curcumin was effective throughout the entire gastrointestinal tract.

Curcumin mouthwash was also assessed in a case series of pediatric patients undergoing chemotherapy that used curcumin mouthwash to prevent the onset of OM (Elad *et al.,* 2013). The mouthwash was well-tolerated and of easy use, and the severity of OM was relatively low although patients were submitted to high-dose cytotoxic protocol. However, since there was no control group, it could not be concluded if the results were related to efficacy of curcumin or to a low severity in the population.

All these findings are in accordance to the clinical evidence found in this current review, the first systematic review to assess the use of turmeric and curcumin in the management of OM, to the best of our knowledge.

Among all the included studies, the most prevalent formulation was the mouthwash, evaluated in three of the five studies (Patil *et al*., 2015; Rao *et al*., 2014;

Saldanha & Almeida, 2014). This was probably due to its ease of application and better tolerability, since patients are able to swish and spit in a few minutes, without the need to keep the solution in further contact with the mucosa. Although all results were favorable to the use of the mouthwash with turmeric/curcumin, it is important to point out that the concentrations used in the three studies differed from each other, leaving open the possibility of new and well-defined randomized clinical studies to determine the most adequate concentration. A positive aspect was that only patients with head and neck cancer were included in these studies, which made the sample homogeneous as to the location of the cancer and consequently as to the treatment protocol which induces mucositis with similar characteristics.

The curcuma gel formulation was evaluated to treat and to prevent mucositis and both studies were designed as randomized clinical trials (Charantimath, 2016; Mansourian *et al*., 2015). Curcuma gel has been shown to delay the onset of mucositis, as well as to accelerate wound healing and reduce the signs of mucositis and burning sensation. These results provide evidence that topical gel containing curcuma is an effective alternative to treat OM, since it reduces the symptoms due to its anti-inflammatory and antioxidant effects. Yet, it is important to note that, although the formulations are similar, patients underwent radiotherapy in one study (Mansourian *et al*., 2015) and chemoradiotherapy in another (Charantimath, 2016), which usually causes a more severe mucositis due to the association of two sources of toxicity.

Two studies also appraised turmeric effectiveness in a mixture with honey to treat OM and both reported reduced OM scores in the intervention group after application of the solution (Francis & Williams, 2014; Nagarale & Rathod, 2016). However, both studies were excluded from descriptive analysis of the present systematic review since it was recently reported in two meta-analyses of RCT that the incidence of OM in patients who applied honey in the oral mucosa was significantly lower compared to control patients, which proves the anti-inflammatory effect of honey alone (Xu *et al*., 2016; Cho *et al*., 2015). Importantly, these trials did not report turmeric concentration in the solutions, neither reported information about control intervention, form of administration and information about cancer site. It is suggested that studies assessing turmeric/curcumin associated with other compounds, such as honey, should compare it with groups of patients using turmeric/curcumin alone to assess their real effectiveness.

LIMITATIONS

Some methodological limitations of this review should be considered. First, there was a high heterogeneity regarding formulations and period of administration, intervention concentrations and assessment criteria tools used for evaluation of mucositis grade, which made the meta-analysis unfeasible. In addition, only a few studies concerning the use of turmeric/curcumin on OM have been published, which urged the inclusion of both randomized and non-randomized clinical trials, resulting in increased risk of bias of included studies. Furthermore, information about chemo and radiation therapy was not detailed in most studies and other side effects, such as xerostomia and dysphagia, have not been reported.

CONCLUSION

Although only a few studies on the subject are available, current clinical evidence suggests that the main effects of turmeric and curcumin on OM are reduction of pain, erythema intensity, ulceration area and degree of severity. In addition, turmeric and curcumin were effective in delaying the onset of mucositis lesions, suggesting its preventive effect. Thus, further investigation in well-designed clinical trials is required to confirm the promising effects of turmeric and curcumin in OM.

Conflict of Interest

None declared.

Funding and Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Ana Gabriela Normando and Isabela Porto de Toledo are supported by CNPq (Brazilian National Council for Scientific and Technological Development). Amanda Menêses is supported by CAPES

(Coordination for the Improvement of Higher Education Personnel), Ministry of Education, Brazil.

REFERENCES

1. Arantes, D. A. C., Dos Santos Filho, E. X., De Mendonça, E.F., *et al*. (2017). Pp-Safety Assessment of a Phytomedication Based on Bidens Pilosa L. and Curcuma Longa L. for Patients with Oral Mucositis. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, *123*(2), e75.
2. Belcaro, G., Hosoi, M., Pellegrini, L., *et al*. (2014). A controlled study of a lecithinized delivery system of curcumin (Meriva®) to alleviate the adverse effects of cancer treatment. *Phytotherapy research*, *28*(3), 444-450.
3. Borges, G. A., Rego, D. F., Assad, D. X., *et al*. (2017). In vivo and in vitro effects of curcumin on head and neck carcinoma: a systematic review. *Journal of Oral Pathology and Medicine, 46,* 3-20.
4. Charantimath, S. (2016). Use of Curcumin in Radiochemotherapy Induced Oral Mucositis Patients: A Control Trial Study. *World Academy of Science, Engineering and Technology, International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering*, *10*(3), 147-152.
5. Cho, H. K., Jeong, Y. M., Lee, H. S., *et al*. (2015). Effects of honey on oral mucositis in patients with head and neck Cancer: A meta-analysis. *The Laryngoscope*, *125*(9), 2085-2092.
6. Devaraj, S. D., & Neelakantan, P. (2014). Curcumin-pharmacological actions and its role in dentistry. *Asian Journal of Pharmaceutical Research and Health Care*, *6*(1), 19-22.
7. dos Santos Filho, E. X., Ávila, P. H. M., Bastos, C. C. C., *et al*. (2016). Curcuminoids from Curcuma longaL. reduced intestinal mucositis induced by 5-fluorouracil in mice: Bioadhesive, proliferative, anti-inflammatory and antioxidant effects. *Toxicology reports*, *3*, 55-62.
8. dos Santos Filho, E. X., da Silva, A. C. G., de Ávila, R. I., *et al*. (2018). Chemopreventive effects of FITOPROT against 5-fluorouracil-induced toxicity in HaCaT cells. *Life sciences*, *193*, 300-308.

9. dos Santos Filho, E. X., Arantes, D. A. C., Leite, A. F. O., *et al*. (2018). Randomized clinical trial of a mucoadhesive formulation containing curcuminoids (Zingiberaceae) and Bidens pilosa Linn (Asteraceae) extract (FITOPROT) for prevention and treatment of oral mucositis-phase I study. *Chemico-biological interactions, 291, 228-236*.
10. Elad, S., Meidan, I., Sellam, G., *et al*. (2013). Topical curcumin for the prevention of oral mucositis in pediatric patients: case series. *Alternative Therapies in Health and Medicine*, 19(3), 21-4.
11. Elting, L. S., Cooksley, C. D., Chambers, *et al*. (2007). Risk, outcomes, and costs of radiation-induced oral mucositis among patients with head-and-neck malignancies. *International Journal of Radiation Oncology• Biology• Physics*, *68*(4), 1110-1120.
12. Francis, M., & Williams, S. (2014). Effectiveness of Indian Turmeric Powder with Honey as Complementary Therapy on Oral Mucositis: A Nursing Perspective among Cancer Patients in Mysore. *The Nursing journal of India*, *105*(6), 258-260.
13. Ghazi, A., Delavarian, Z., Pakfetrat, A., *et al*. (2015). Effects of curcumin on the prevention and treatment of mucosal inflammation caused by radiation therapy in patients with head and neck cancer. *Avicenna Journal of Phytomedicine*, *5*.
14. Grover, H. S., Deswal, H., & Bhardwaj, A. (2015). Curcumin: A medicinal plant and its effects in medicine and dentistry. *International Journal of Contemporary Dental and Medical Reviews*.
15. Gu, Y., Liu, C., Xia, Y., Xia, Y. (2014). Therapeutic effect of zedoary turmeric oil on erosive esophagitis in mice. *Chinese Journal of Gastroenterology*, 19(3), 161-163
16. Khattry, N., Gawande, J., Patil, P., *et al*. (2012). Curcumin Decreases Cytokine Levels Involved in Mucositis in Autologous Transplant Setting: A Pharmacokinetic-Pharmacodynamic Study. *Blood,* 120(21), 3039.
17. Lalla, R. V., Bowen, J., Barasch, A., *et al*. (2014). MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. *Cancer*, *120*(10), 1453-1461.
18. Lueer, S., Jetter, M., Troller, R., *et al*. (2010). Curcumin inhibits deleterious effects of respiratory tract bacteria on human oropharyngeal cells-potential role

in chemotherapy-induced mucositis? *Swiss Medical Weekly,* 140(21-22), 30S-30S.

19. Lüer, S., Troller, R., Jetter, M., *et al*. (2010). Curcumin protects human oropharyngeal cells against upper respiratory tract bacteria in vitro-potential role for patients with cancer therapy induced mucositis? *Pediatric Blood & Cancer*, *55*(5), 945.
20. Lüer, S., Troller, R., Jetter, C., *et al*. (2011). Topical curcumin can inhibit deleterious effects of upper respiratory tract bacteria on human oropharyngeal cells in vitro: potential role for patients with cancer therapy induced mucositis? *Supportive Care in Cancer*, *19*(6), 799-806.
21. Lüer, S., Goette, J., Troller, R., & Aebi, C. (2012). Curcumin Against Cancer Therapy Induced Mucositis: Comparison Of Pure Synthetic Versus Natural Purified Curcumin In an *In Vitro* Mucositis Model. *Pediatric Blood & Cancer*, *59*(6), 1116-1117.
22. Lüer, S., Troller, R., & Aebi, C. (2012). Antibacterial and antiinflammatory kinetics of curcumin as a potential antimucositis agent in cancer patients. *Nutrition and cancer*, *64*(7), 975-981.
23. Lüer, S. C., Goette, J., Troller, R., & Aebi, C. (2014). Synthetic versus natural curcumin: bioequivalence in an in vitro oral mucositis model. *BMC complementary and alternative medicine*, *14*(1), 53.
24. Mansourian, A., Amanlou, M., Shirazian, S., *et al*. (2015). The effect of "Curcuma Longa" topical gel on radiation-induced oral mucositis in patients with head and neck cancer. *International Journal of Radiation Research,* 13(3), 269-274.
25. Manzi, N. M., Silveira, R.C., dos Reis, P.E., (2016). Prophylaxis for mucositis induced by ambulatory chemotherapy: systematic review. *Journal of Advanced Nursing*, 72(4), 735-746.
26. Moher, D., Liberati, A., Tetzlaff, J., *et al*. PRISMA Group (2010) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *International Journal of Surgery,* 8, 336–341.
27. Nagarale, P., & Rathod, S. (2016). A Quasi Experimental Study To Evaluate The Effectiveness of Indian Turmeric Powder With Honey Mixture on Treatment Induced oral Mucositis of Cancer Patients At Selected Hospital, Kolhapur. *International Journal of Recent Scientific Research,* 7(10), 13525-13529.

28. Nagpal, M., & Sood, S. (2013). Role of curcumin in systemic and oral health: An overview. *Journal of natural science, biology, and medicine*, *4*(1), 3.
29. Normando, A. G. C. N., Meneses, A.M., de Toledo, I.P., *et al* (2018). Effects of curcumin on oral mucositis: a systematic review. PROSPERO 2018: CRD42018083318 Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018083318. Accessed January 08, 2018.
30. Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2017). Rayyan—a web and mobile app for systematic reviews. *Systematic Reviews,* 5(1), 210.
31. Palatty, P. L., Azmidah, A., Rao, S., *et al*. (2014). Topical application of a sandal wood oil and turmeric based cream prevents radiodermatitis in head and neck cancer patients undergoing external beam radiotherapy: a pilot study. *The British journal of radiology*, *87*(1038), 20130490.
32. Patil, K., Guledgud, M. V., Kulkarni, P. K., *et al* (2015). Use of curcumin mouthrinse in radio-chemotherapy induced oral mucositis patients: a pilot study. *Journal of clinical and diagnostic research: JCDR*, *9*(8), ZC59.
33. Rao, S., Dinkar, C., Vaishnav, L. K., *et al*. (2014). The Indian spice turmeric delays and mitigates radiation-induced oral mucositis in patients undergoing treatment for head and neck cancer: an investigational study. *Integrative cancer therapies*, *13*(3), 201-210.
34. Rezvani, M. (2003). Treatment of radiation-induced acute oral mucositis in a rat model. *12th Quadrennial Congress of the International Association for Radiation Research incorporating the 50th Annual Meeting of Radiation Research Society, RANZCR Radiation Oncology Annual Scientific Meeting and AINSE Radiation Science Conference,* (p. 414). Australia
35. Rezvani, M., & Ross, G. A. (2004). Modification of radiation-induced acute oral mucositis in the rat. *International journal of radiation biology*, *80*(2), 177-182.
36. Ruiz-Esquide, G., Nervi, B., Vargas, A. & Maız, A. (2011) Tratamiento y prevencion de la mucositis oral asociada al tratamiento del câncer. *Revista Medica de Chile*, 139, 373–381.
37. Ryan, J. L., Heckler, C. E., Ling, M., *et al* (2013). Curcumin for radiation dermatitis: a randomized, double-blind, placebo-controlled clinical trial of thirty breast cancer patients. *Radiation research*, *180*(1), 34-43.

38. Saldanha, S. P., & Almeida, V. D. (2014). A Comparative Study to Assess the Effectiveness of Turmeric Mouth Wash versus Saline Mouth Wash on Treatment Induced Oral Mucositis (Tiom) in a Selected Hospital at Mangalore. *Journal of Clinical Research & Bioethics*, *5*(6), 1.
39. Scully, C., Epstein, J., & Sonis, S. (2003). Oral mucositis: a challenging complication of radiotherapy, chemotherapy, and radiochemotherapy: part 1, pathogenesis and prophylaxis of mucositis. *Head & Neck*, *25*(12), 1057-1070.
40. Scully, C., Epstein, J., & Sonis, S. (2004). Oral mucositis: a challenging complication of radiotherapy, chemotherapy, and radiochemotherapy. Part 2: diagnosis and management of mucositis. *Head & Neck: Journal for the Sciences and Specialties of the Head and Neck*, *26*(1), 77-84.
41. Sonis, S. T. (2002). The biologic role for nuclear factor-kappa B in disease and its potential involvement in mucosal injury associated with anti-neoplastic therapy. *Critical Reviews in Oral Biology & Medicine,* 13(5), 380-389
42. Sonis, S. T. (2007). Pathobiology of oral mucositis: novel insights and opportunities. *The journal of supportive oncology, 5*(9 Suppl 4), 3-11.
43. Sonis, S. T. (2009). Mucositis: the impact, biology and therapeutic opportunities of oral mucositis. *Oral oncology*, *45*(12), 1015-1020.
44. Sun, J., Chen, F., Braun, C., *et al*. (2018). Role of curcumin in the management of pathological pain. *Phytomedicine, 15*(48), 128-140.
45. The Joanna Briggs Institute. *The Joanna Briggs Institute Reviewer's Manual 2014 Edition:* Meta Analysis of Statistics Assessment and Review Instrument (MAStARI) critical appraisal tools Randomized Control/ Pseudo-randomized Trial. Adelaide, Australia: The Joanna Briggs Institute, 2014.
46. Trotti, A., Bellm, L. A., Epstein, J. B., *et al*. (2003). Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. *Radiotherapy and oncology*, *66*(3), 253-262.
47. Van't Land, B., Blijlevens, N. M. A., Marteijn, J., *et al*. (2004). Role of curcumin and the inhibition of NF-κB in the onset of chemotherapy-induced mucosal barrier injury. *Leukemia*, *18*(2), 276.
48. Vaughn, A. R., Branum, A., & Sivamani, R. K. (2016). Effects of turmeric (Curcuma longa) on skin health: A systematic review of the clinical evidence. *Phytotherapy Research*, *30*(8), 1243-1264.

49. Villa, A., & Sonis, S. T. (2015). Mucositis: pathobiology and management. *Current opinion in oncology*, *27*(3), 159-164.
50. Xu, J. L., Xia, R., Sun, Z. H., *et al*. (2016). Effects of honey use on the management of radio/chemotherapy-induced mucositis: A meta-analysis of randomized controlled trials. *International journal of oral and maxillofacial surgery*, *45*(12), 1618-1625.

Appendix 1. Search strategies with appropriated key words and Mesh terms.

Database	Search (Search date: November 1st, 2017; updated on June 1st, 2018)
Cochrane Library	'curcuma OR curcumas OR curcumin OR curcuminoid OR curcuminoids OR tumeric OR turmeric OR "curcuma longa" OR "curcuma zedoaria" OR zingiberaceae in Title, Abstract, Keywords AND mucositis OR mucositides OR stomatitis OR stomatitides OR "oral mucositis" OR "oral mucositides" OR oromucositis OR oromucositides OR "mouth ulcer" OR "oral mucosa irritation" OR "oral mucosa injury" OR "oral mucosa inflammation" OR "mucosal inflammation" OR "inflamed mucous membranes" OR "radiation induced mucositis" OR "mucosa irritation" OR "mucosal irritation" OR "mucosal barrier injury" OR "MBI" OR "mucosal injury" in Title, Abstract, Keywords
LILACS	(tw:(mucositis OR stomatitis OR "oral mucositis" OR "mucosite oral" OR estomatite OR estomatitis OR mucosite)) AND (tw:(curcuma OR curcumin OR curcumina OR "curcuma longa" OR tumeric OR turmeric OR turmérico))
LIVIVO	TI=(curcuma OR curcumas OR curcumin OR curcuminoid OR curcuminoids OR tumeric OR turmeric OR “curcuma longa” OR “curcuma zedoaria” OR zingiberaceae) AND TI=(mucositis OR mucositides OR stomatitis OR stomatitides OR "oral mucositis" OR "oral mucositides" OR oromucositis OR oromucositides OR "mouth ulcer" OR "oral mucosa irritation" OR "oral mucosa injury" OR "oral mucosa inflammation" OR "mucosal inflammation" OR "inflamed mucous membranes" OR "radiation induced mucositis" OR "mucosa irritation" OR "mucosal irritation" OR "mucosal barrier injury" OR "MBI" OR "mucosal injury")
PubMed	(curcuma[Mesh] OR curcuma OR curcumas OR curcumin OR curcuminoid OR curcuminoids OR tumeric OR turmeric OR “curcuma longa” OR “curcuma zedoaria” OR zingiberaceae) AND ("mucositis"[MeSH] OR mucositis OR mucositides OR "stomatitis"[MeSH] OR stomatitis OR stomatitides OR "oral mucositis" OR "oral mucositides" OR oromucositis OR oromucositides OR "mouth ulcer" OR "oral mucosa irritation" OR "oral mucosa injury" OR "oral mucosa inflammation" OR "mucosal inflammation" OR "inflamed mucous membranes" OR "radiation induced mucositis" OR "mucosa irritation" OR "mucosal irritation" OR "mucosal barrier injury" OR "MBI" OR "mucosal injury")
Scopus	TITLE-ABS-KEY(curcuma OR curcumas OR curcumin OR curcuminoid OR curcuminoids OR tumeric OR turmeric OR "curcuma longa" OR "curcuma zedoaria" OR zingiberaceae) AND TITLE-ABS-KEY (mucositis OR mucositides OR stomatitis OR stomatitides OR "oral mucositis" OR "oral mucositides" OR oromucositis OR oromucositides OR "mouth ulcer" OR "oral mucosa irritation" OR "oral mucosa injury" OR "oral mucosa inflammation" OR "mucosal inflammation" OR "inflamed mucous membranes" OR "radiation induced mucositis" OR "mucosa irritation" OR "mucosal irritation" OR "mucosal barrier injury" OR "MBI" OR "mucosal injury")
Web of Science	(curcuma OR curcumas OR curcumin OR curcuminoid OR curcuminoids OR tumeric OR turmeric OR “curcuma longa” OR “curcuma zedoaria” OR zingiberaceae) AND (mucositis OR mucositides OR stomatitis OR stomatitides OR "oral mucositis" OR "oral mucositides" OR oromucositis OR oromucositides OR "mouth ulcer" OR "oral mucosa irritation" OR "oral mucosa injury" OR "oral mucosa inflammation" OR "mucosal inflammation" OR "inflamed mucous membranes" OR "radiation induced mucositis" OR "mucosa irritation" OR "mucosal irritation" OR "mucosal barrier injury" OR "MBI" OR "mucosal injury")
Google Scholar	(curcuma OR curcumin OR “curcuma longa”) AND (mucositis OR stomatitis OR "oral mucositis")

Open Grey	mucositis
ProQuest	TI,AB(curcuma OR curcumas OR curcumin OR curcuminoid OR curcuminoids OR tumeric OR turmeric OR “curcuma longa” OR “curcuma zedoaria” OR zingiberaceae) AND TI,AB(mucositis OR mucositides OR stomatitis OR stomatitides OR "oral mucositis" OR "oral mucositides" OR oromucositis OR oromucositides OR "mouth ulcer" OR "oral mucosa irritation" OR "oral mucosa injury" OR "oral mucosa inflammation" OR "mucosal inflammation" OR "inflamed mucous membranes" OR "radiation induced mucositis" OR "mucosa irritation" OR "mucosal irritation" OR "mucosal barrier injury" OR "MBI" OR "mucosal injury")

Appendix 2. Excluded articles and reasons for exclusion (n=17).

Reference	Author/Year	Reasons for exclusion
1	Arantes *et al*. (2017)	1
2	Belcaro *et al*. (2014)	6
3	dos Santos *et al*. (2018)	2
4	Elad *et al*. (2013)	2
5	Francis & Williams (2014)	5
6	Ghazi *et al*. (2015)	1
7	Gu *et al*. (2014)	7
8	Khattry *et al*. (2012)	8
9	Lueer *et al*. (2010)	8
10	Lüer *et al*. (2010)	1
11	Lüer *et al*. (2011)	2
12	Lüer *et al*. (2012)	1
13	Lüer *et al*. (2012)	2
14	Lüer *et al*. *(*2014)	2
15	Nagarale & Rathod (2016)	5
16	Rezvani (2003)	8
17	Rezvani & Ross (2004)	2

1- Reviews, letters, conference abstracts, personal opinions, book chapters (n= 4);
2- Observational studies, *in vitro* or *in vivo* animal studies (n= 6);
3- Use of turmeric/curcumin to treat other oral inflammatory diseases (n= 0);
4- Use of turmeric/curcumin on intestinal mucositis (n=0);
5- Use of turmeric/curcumin associated with other compounds (n=2);
6- Data not individualized for oral mucositis (n=1);
7- Language restriction (n= 1);
8- Full paper copy not available (n= 3).

References

1. Arantes, D. A. C., Dos Santos Filho, E. X., De Mendonça, E.F., *et al*. (2017). Pp-Safety Assessment of a Phytomedication Based on Bidens Pilosa L. and Curcuma Longa L. for Patients with Oral Mucositis. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, *123*(2), e75.

2. Belcaro, G., Hosoi, M., Pellegrini, L., *et al*. (2014). A controlled study of a lecithinized delivery system of curcumin (Meriva®) to alleviate the adverse effects of cancer treatment. *Phytotherapy research*, *28*(3), 444-450.

3. dos Santos Filho, E. X., da Silva, A. C. G., de Ávila, R. I., *et al*. (2018). Chemopreventive effects of FITOPROT against 5-fluorouracil-induced toxicity in HaCaT cells. *Life Sciences*.

4. Elad, S., Meidan, I., Sellam, G., *et al*. (2013). Topical curcumin for the prevention of oral mucositis in pediatric patients: case series. *Alternative therapies in health and medicine*, *19*(3), 21.

5. Francis, M., & Williams, S. (2014). Effectiveness of Indian Turmeric Powder with Honey as Complementary Therapy on Oral Mucositis: A Nursing Perspective among Cancer Patients in Mysore. *The Nursing journal of India*, *105*(6), 258-260.

6. Ghazi, A., Delavarian, Z., Pakfetrat, A., *et al*. (2015). Effects of curcumin on the prevention and treatment of mucosal inflammation caused by radiation therapy in patients with head and neck cancer. *Avicenna Journal of Phytomedicine*, *5*.

7. Gu, Y., Liu, C., Xia, Y., Xia, Y. (2014). Therapeutic effect of zedoary turmeric oil on erosive esophagitis in mice. *Chinese Journal of Gastroenterology*, 19(3), 161-163

8. Khattry, N., Gawande, J., Patil, P., *et al*. (2012). Curcumin Decreases Cytokine Levels Involved in Mucositis in Autologous Transplant Setting: A Pharmacokinetic-Pharmacodynamic Study. *Blood,* 120(21), 3039.

9. Lueer, S., Jetter, M., Troller, R., *et al*. (2010). Curcumin inhibits deleterious effects of respiratory tract bacteria on human oropharyngeal cells-potential role in chemotherapy-induced mucositis? *Swiss Medical Weekly,* 140(21-22), 30S-30S.

10. Lüer, S., Troller, R., Jetter, M., *et al*. (2010). Curcumin protects human oropharyngeal cells against upper respiratory tract bacteria in vitro-potential role for patients with cancer therapy induced mucositis? *Pediatric Blood & Cancer*, *55*(5), 945.

11. Lüer, S., Troller, R., Jetter, M., *et al*. (2011). Topical curcumin can inhibit deleterious effects of upper respiratory tract bacteria on human oropharyngeal cells in vitro: potential role for patients with cancer therapy induced mucositis? *Supportive Care in Cancer*, *19*(6), 799-806.

12. Lüer, S., Goette, J., Troller, R., & Aebi, C. (2012). Curcumin Against Cancer Therapy Induced Mucositis: Comparison Of Pure Synthetic Versus Natural Purified Curcumin In an *In Vitro* Mucositis Model. *Pediatric Blood & Cancer*, *59*(6), 1116-1117.

13. Lüer, S., Troller, R. & Aebi, C. (2012) Antibacterial and Antiinflammatory Kinetics of Curcumin as a Potential Antimucositis Agent in Cancer Patients. *Nutrition and Cancer*, 64(7), 975-981.

14. Lüer, S. C., Goette, J., Troller, R., & Aebi, C. (2014). Synthetic versus natural curcumin: bioequivalence in an in vitro oral mucositis model. *BMC complementary and alternative medicine*, *14*(1), 53.

15. Nagarale, P., & Rathod, S. (2016). A Quasi Experimental Study To Evaluate The Effectiveness of Indian Turmeric Powder With Honey Mixture on Treatment Induced oral Mucositis of Cancer Patients At Selected Hospital, Kolhapur. *International Journal of Recent Scientific Research*7(10), 13525-13529.

16. Rezvani, M. (2003). Treatment of radiation-induced acute oral mucositis in a rat model. *12th Quadrennial Congress of the International Association for Radiation Research incorporating the 50th Annual Meeting of Radiation Research Society, RANZCR Radiation Oncology Annual Scientific Meeting and AINSE Radiation Science Conference,* (p. 414). Australia

17. Rezvani, M., & Ross, G. A. (2004). Modification of radiation-induced acute oral mucositis in the rat. *International journal of radiation biology*, *80*(2), 177-182.

Appendix 3. Risk of bias assessed by Meta Analysis of Statistics Assessment and Review Instrument (MAStARI)[1] critical appraisal tools. Risk of bias was categorized as **High** when the study reaches up to 49% score "yes", **Moderate** when the study reached 50% to 69% score "yes", and **Low** when the study reached more than 70% score "yes".

MAStARI critical appraisal tools for Randomized Control / Pseudo-randomized Trial

Question	*Answer**				
	Charantimath *et al.* (2016)	*Mansourian et al.* (2015)	Patil *et al.* (2015)	Rao *et al.* (2014)	Saldanha & Almeida (2014)
1. Was the assignment to treatment groups truly random?	U	Y	U	Y	N
2. Were participants blinded to treatment allocation?	U	Y	U	Y	N
3.Was allocation to treatment groups concealed from the allocator?	U	Y	U	Y	N
4. Were the outcomes of people who withdrew described and included in the analysis?	U	U	U	Y	U
5. Were those assessing outcomes blind to the treatment allocation?	U	Y	U	Y	U
6. Were the control and treatment groups comparable at entry?	Y	Y	Y	Y	Y
7. Were groups treated identically other than for the named interventions?	Y	Y	Y	Y	Y
8. Were outcomes measured in the same way for all groups?	Y	Y	Y	Y	Y
9. Were outcomes measured in a reliable way?	Y	Y	Y	Y	Y
10. Was appropriate statistical analysis used?	Y	Y	Y	Y	Y
% yes/risk	50% M	90% L	50% M	100 % L	50% M

*Y=Yes, N=No, U=Unclear, M=Moderate, H=High, L=Low.

[1]Meta Analysis of Statistics Assessment and Review Instrument (MAStARI). Joanna Briggs Institute Reviewers Manual. Australia: The Joanna Briggs Institute, 2014.

5. CONSIDERAÇÕES GERAIS E PERSPECTIVAS

Pacientes em QT e RT estão em risco elevado de desenvolver MO, uma vez que trata-se de um dos efeitos adversos mais prevalentes. A ingestão oral de alimentos é comumente prejudicada devido à náusea induzida pela quimioterapia, e esse problema ainda é agravado quando os pacientes sofrem de mucosite, influenciando na absorção de nutrientes e resultando em perda de peso. Além disso, a mucosite causa um impacto econômico e na qualidade de vida dos pacientes, já que o atendimento ambulatorial precisa ser prolongado para fornecimento de terapia analgésica opióide, nutrição parenteral total, inserção de sonda de alimentação e controle de febre e infecção. Também comum e igualmente relevante, a mucosite pode ser responsável por reduções de dose ou interrupções da terapia do câncer (Villa & Sonis, 2016; Berger *et al*., 2018).

Fica evidente a necessidade de entender a fisiopatologia da mucosite para desenvolver novas estratégias preventivas e terapêuticas que tenham como alvo moléculas ou vias envolvidas no desenvolvimento da lesão (Cinausero *et al*., 2017). O presente estudo objetivou estabelecer um modelo *in vitro* que simulasse em laboratório parcialmente o que acontece na mucosa oral de pacientes irradiados, para, posteriormente, investigar potenciais terapias-alvo que sejam eficazes no controle da condição, tais como a cúrcuma e a curcumina.

O desenvolvimento de um modelo a partir de fibroblastos gengivais humanos mostrou-se uma estratégia viável e bastante efetiva de se simular a mucosa oral. Além disso, pouco se sabe sobre os efeitos da radiação ionizante e do microbioma oral nas células da mucosa, incluindo os fibroblastos, as quais são as primeiras células a sofrerem apoptose quando atingidas pelas terapias antineoplásicas (Sonis, 2004). A utilização de componentes de dois tipos bacterianos diferentes possibilitou observar que determinadas citocinas são mais estimuladas com *Pg* do que com *E. coli* e vice-versa, e que associação com radioterapia aumentou a expressão gênica dos mediadores quando comparado a células apenas irradiadas. Entretanto, foi possível observar que o tempo de 6h após estimulo pareceu ser o mais efetivo para análise da expressão de todas as citocinas tanto para a *Pg* quanto para a radiação. Além disso, estímulo com *Pg* e *E. coli* foi capaz de gerar um aumento na expressão do NF-κB, que é um dos

mediadores inflamatórios mais importantes e mais estudados na fisiopatologia da mucosite.

O presente trabalho também demonstrou por meio de revisão sistemática da literatura, que representa o ápice da pirâmide da evidência científica, o potencial da cúrcuma e da curcumina no controle da mucosite oral. As evidências clínicas atuais sugerem que ambos os compostos são capazes de reduzir dor, intensidade do eritema, área de ulceração, gravidade e retardar o aparecimento de lesões de mucosite (Charantimath, 2016; Mansourian *et al.,* 2015; Patil *et al.,* 2015; Rao *et al.,* 2014; Saldanha & Almeida, 2014). Entretanto, ainda são necessários esclarecimentos quanto ao mecanismo de ação desses agentes em âmbito laboratorial.

Sabe-se que a curcumina, principal polifenol da cúrcuma, é um produto natural derivado da dieta com potencial de inibir a via de sinalização do mTOR direta ou indiretamente (Beevers *et al*., 2010). A inibição da via do mTOR, por sua vez, demonstrou causar uma acentuada redução no acúmulo de ROS em queratinócitos orais, sendo, portanto, um mecanismo efetivo para proteção das células nos casos de mucosite radio-induzida (Iglesias-Bartolome *et al*., 2012). Além disso, foi evidenciado que a cúrcuma inibe a ativação do *NF-κB* que está diretamente relacionado com a produção de citocinas inflamatórias, demonstrando o efeito anti-inflamatório que a cúrcuma tem associado ao efeito anti-oxidante (Kim *et al*., 2012).

Diante do exposto, as perspectivas incluem aprimoramento do modelo *in vitro* aqui proposto e desenvolvido, ao testar os estímulos também em linhagem de queratinócitos humanos. Além disso, o modelo foi idealizado para servir como medida de eficácia de produtos naturais derivados da dieta que inibem a via do mTOR, tais como a curcumina, o resveratrol e o EGCG. Portanto, faz-se necessário explorar o mecanismo de ação, citoxicidade e potencial anti-oxidante e anti-inflamatório desses compostos sobre linhagens de fibroblastos humanos e queratinócitos estimulados por radiação e carga bacteriana. A hipótese é de que tais compostos serão capazes de reduzir a expressão dos mediadores inflamatórios do modelo de mucosite *in vitro.* Caso a hipótese se confirme, saberemos que os inibidores naturais de mTOR podem ser efetivos no controle da inflamação, podendo ser futuramente aplicados na prática clínica em associação

a outras terapias, como a fotobiomodulação, já utilizadas no controle da mucosite oral.

6. CONCLUSÕES

A partir das perguntas de pesquisa e hipóteses estabelecidas e dos resultados encontrados, pode-se concluir que:

- A cultura primária de fibroblastos gengivais humanos é uma estratégia efetiva e relativamente simples para se estabelecer um modelo *in vitro* de mucosite oral.
- O estímulo inflamatório com LPS de *E. coli* foi capaz de induzir aumento mais acentuado na expressão de *IL-1*β e *TNF-*α, enquanto que estímulo com extrato proteico de *Pg* induziu maior expressão de *NF-*κ*B* e *IL-6*.
- A dose de radiação de 12 Gy aumentou a expressão de todos mediadores inflamatórios quando comparado às células não irradiadas, porém sem significado estatístico.
- A associação de radiação com extrato de *Pg* causou maior expressão dos mediadores inflamatórios do que em células estimuladas apenas com radiação.
- Com base na evidência científica existente, a cúrcuma e a curcumina reduzem dor, intensidade de eritema, área de ulceração e gravidade da mucosite, sendo boas estratégias terapêuticas.
- A revisão sistemática realizada mostrou que a cúrcuma e a curcumina retardam o aparecimento de lesões de mucosite, sendo boas estratégias preventivas.

7. REFERÊNCIAS BIBLIOGRÁFICAS

1. Al-Ansari, S., Zecha, J. A. E. M., Barasch, A., *et al* (2015). Oral mucositis induced by anticancer therapies. *Curr. Oral Health Rep.* 2, 202–211.
2. Al-Dasooqi, N., Gibson, R. J., Bowen, J. M., *et al* (2010). *Matrix metalloproteinases are possible mediators for the development of alimentary tract mucositis in the dark agouti rat. Experimental Biology and Medicine, 235(10), 1244–1256.*
3. Algharably, E. A. H., Kreutz, R., & Gundert-Remy, U. (2019). Importance of in vitro conditions for modeling the in vivo dose in humans by in vitro–in vivo extrapolation (IVIVE). *Archives of Toxicology*, 2019 Jan 2.
4. Basso, F. G., Pansani, T. N., Soares, D. G., *et al* (2015). Biomodulation of inflammatory cytokines related to oral mucositis by low-level laser therapy. *Photochemistry and photobiology*, *91*(4), 952-956.
5. Beevers, C. S., Chen, L., Liu, L., *et al* (2009). Curcumin disrupts the Mammalian target of rapamycin-raptor complex. *Cancer research*, *69*(3), 1000-1008.
6. Berger, K., Schopohl, D., Bollig, A., *et al* (2018). Burden of Oral Mucositis: A Systematic Review and Implications for Future Research. *Oncology research and treatment*, *41*.
7. Bossi, P., Bergamini, C., Miceli, R., *et al* (2016). Salivary cytokine levels and oral mucositis in head and neck cancer patients treated with chemotherapy and radiation therapy. *International Journal of Radiation Oncology* Biology* Physics*, *96*(5), 959-966.
8. Bray, F., Ferlay, J., Soerjomataram, I., *et al* (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *68*(6), 394-424.
9. Carvalho, C. G., Medeiros-Filho, J. B., & Ferreira, M. C. (2018). Guide for health professionals addressing oral care for individuals in oncological treatment based on scientific evidence. *Supportive Care in Cancer*, 1-11.
10. Chaitanya, N. C., Muthukrishnan, A., Babu, D. B. G., *et al* (2017). Role of Vitamin E and Vitamin A in Oral Mucositis Induced by Cancer Chemo/Radiotherapy-A Meta-analysis. *Journal of clinical and diagnostic research: JCDR*, *11*(5), ZE06.

11. Charantimath, S. (2016). Use of Curcumin in Radiochemotherapy Induced Oral Mucositis Patients: A Control Trial Study. *World Academy of Science, Engineering and Technology, International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering*, *10*(3), 147-152.
12. Chaudhry, H. M., Bruce, A. J., Wolf, R. C., *et al* (2016). The incidence and severity of oral mucositis among allogeneic hematopoietic stem cell transplantation patients: a systematic review. *Biology of Blood and Marrow Transplantation*, *22*(4), 605-616.
13. Chaveli-López, B. (2014). Oral toxicity produced by chemotherapy: A systematic review. *Journal of clinical and experimental dentistry*, *6*(1), e81.
14. Colley, H. E., Eves, P. C., Pinnock, A., *et al* (2013). Tissue-engineered oral mucosa to study radiotherapy-induced oral mucositis. *International journal of radiation biology*, *89*(11), 907-914.
15. Curra, M., Junior, S., Valente, L. A., *et al* (2018). Chemotherapy protocols and incidence of oral mucositis. An integrative review. *Einstein (São Paulo)*, *16*(1).
16. Denham, J. W., & Hauer-Jensen, M. (2002). *The radiotherapeutic injury – a complex "wound." Radiotherapy and Oncology, 63(2), 129–145.*
17. Devaraj, S. D., & Neelakantan, P. (2014). Curcumin-pharmacological actions and its role in dentistry. *Asian Journal of Pharmaceutical Research and Health Care*, *6*(1), 19-22.
18. Donetti, E., Bedoni, M., Capone, P., *et al* (2009). An in vitro model of human oral explants to study early effects of radiation mucositis. *European journal of oral sciences*, *117*(2), 169-174.
19. Dos Reis, P. E. D., Ciol, M. A., de Melo, N. S., *et al* (2016). Chamomile infusion cryotherapy to prevent oral mucositis induced by chemotherapy: a pilot study. *Supportive Care in Cancer*, *24*(10), 4393-4398.
20. Epstein, J. B., Thariat, J., Bensadoun, R. J., *et al* (2012). Oral complications of cancer and cancer therapy: from cancer treatment to survivorship. *CA: a cancer journal for clinicians*, *62*(6), 400-422.
21. Gruber, S., Bozsaky, E., Roitinger, E., *et al* (2017). Early inflammatory changes in radiation-induced oral mucositis. *Strahlentherapie und Onkologie*, *193*(6), 499-507.

22. Iglesias-Bartolome, R., Patel, V., Cotrim, A., *et al* (2012). mTOR inhibition prevents epithelial stem cell senescence and protects from radiation-induced mucositis. *Cell stem cell*, *11*(3), 401-414.
23. Kim, J. H., Gupta, S. C., Park, B., *et al* (2012). Turmeric (Curcuma longa) inhibits inflammatory nuclear factor (NF)-κB and NF-κB-regulated gene products and induces death receptors leading to suppressed proliferation, induced chemosensitization, and suppressed osteoclastogenesis. *Molecular nutrition & food research*, *56*(3), 454-465.
24. Kwon, Y. (2016). Mechanism-based management for mucositis: option for treating side effects without compromising the efficacy of cancer therapy. *OncoTargets and therapy*, *9*, 2007.
25. Lacouture, M., & Sibaud, V. (2018). Toxic Side Effects of Targeted Therapies and Immunotherapies Affecting the Skin, Oral Mucosa, Hair, and Nails. *American journal of clinical dermatology*, 1-9.
26. Lalla, R. V., Bowen, J., Barasch, A., *et al* (2014). MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. *Cancer*, *120*(10), 1453-1461.
27. Lalla, R. V., Saunders, D. P., & Peterson, D. E. (2014). Chemotherapy or radiation-induced oral mucositis. *Dental Clinics*, *58*(2), 341-349.
28. Lambros, M. P., Kondapalli, L., Parsa, C., *et al* (2015). Molecular signatures in the prevention of radiation damage by the synergistic effect of N-acetyl cysteine and qingre liyan decoction, a traditional chinese medicine, using a 3-dimensional cell culture model of oral mucositis. *Evidence-Based Complementary and Alternative Medicine*, *2015*.
29. Logan, R.M., Stringer, A.M., Bowen, J.M., *et al* (2008). Serum levels of NF-κB and pro-inflammatory cytokines following administration of mucotoxic drugs. *Cancer biology & therapy*, *7*(7), 1139-1145.
30. Mansourian, A., Amanlou, M., Shirazian, S., *et al*. (2015). The effect of "Curcuma Longa" topical gel on radiation-induced oral mucositis in patients with head and neck cancer. *International Journal of Radiation Research,* 13(3), 269-274.
31. Mayo, B. J., Stringer, A. M., Bowen, J. M., *et al* (2017). Irinotecan-induced mucositis: the interactions and potential role of GLP-2 analogues. *Cancer chemotherapy and pharmacology*, *79*(2), 233-249.

32. McGuire, D. B., Fulton, J. S., Park, J., *et al* (2013). Systematic review of basic oral care for the management of oral mucositis in cancer patients. *Supportive Care in Cancer*, *21*(11), 3165-3177.
33. Migliorati, C., Hewson, I., Lalla, R. V., *et al* (2013). Systematic review of laser and other light therapy for the management of oral mucositis in cancer patients. *Supportive Care in Cancer*, *21*(1), 333-341.
34. Migliorati, C. A., Seneda, L. M., & Burton, E. L. (2015). Oral Complications of Cancer Therapy: A Summary Guide for the Clinician. *The Journal of the Tennessee Dental Association*, *95*(1), 24-32.
35. Moura, J. F., Mota, J. M. S. C., Leite, C. A. V., *et al* (2015). A novel model of megavoltage radiation-induced oral mucositis in hamsters: Role of inflammatory cytokines and nitric oxide. *International journal of radiation biology*, *91*(6), 500-509.
36. Nagi, R., Patil, D.J., Rakesh, N., *et al* (2018). Natural agents in the management of oral mucositis in cancer patients-systematic review. *J Oral Biol Craniofac Res*., 8(3):245-254.
37. Nagpal, M., & Sood, S. (2013). Role of curcumin in systemic and oral health: An overview. *Journal of natural science, biology, and medicine*, *4*(1), 3.
38. Nicolatou-Galitis, O., Sarri, T., Bowen, J., *et al* (2013). Systematic review of anti-inflammatory agents for the management of oral mucositis in cancer patients. *Supportive Care in Cancer*, *21*(11), 3179-3189.
39. Oberoi, S., Zamperlini–Netto, G., Beyene, J., *et al* (2014). Effect of prophylactic low level laser therapy on oral mucositis: a systematic review and meta-analysis. *PLoS one*, *9*(9), e107418.
40. Oton-Leite, A. F., Silva, G. B. L., Morais, M. O., *et al* (2015). Effect of low-level laser therapy on chemoradiotherapy-induced oral mucositis and salivary inflammatory mediators in head and neck cancer patients. *Lasers in surgery and medicine*, *47*(4), 296-305.
41. Patil, K., Guledgud, M. V., Kulkarni, P. K., *et al* (2015). Use of curcumin mouthrinse in radio-chemotherapy induced oral mucositis patients: a pilot study. *Journal of clinical and diagnostic research: JCDR*, *9*(8), ZC59.
42. Peterson, D. E., Boers-Doets, C. B., Bensadoun, R. J., & Herrstedt, J. (2015). Management of oral and gastrointestinal mucosal injury: ESMO Clinical

Practice Guidelines for diagnosis, treatment, and follow-up. *Annals of oncology*, *26*(suppl_5), v139-v151.

43. Peterson, D. E., O'Shaughnessy, J. A., Rugo, H. S., *et al* (2016). Oral mucosal injury caused by mammalian target of rapamycin inhibitors: emerging perspectives on pathobiology and impact on clinical practice. *Cancer medicine*, *5*(8), 1897-1907.
44. Pico, J. L., Avila-Garavito, A., & Naccache, P. (1998). Mucositis: its occurrence, consequences, and treatment in the oncology setting. *The oncologist*, *3*(6), 446-451.
45. Rambod, M., Pasyar, N., & Ramzi, M. (2018). The effect of zinc sulfate on prevention, incidence, and severity of mucositis in leukemia patients undergoing chemotherapy. *European Journal of Oncology Nursing*, *33*, 14-21.
46. Rao, S., Dinkar, C., Vaishnav, L. K., *et al*. (2014). The Indian spice turmeric delays and mitigates radiation-induced oral mucositis in patients undergoing treatment for head and neck cancer: an investigational study. *Integrative cancer therapies*, *13*(3), 201-210.
47. Saldanha, S. P., & Almeida, V. D. (2014). A Comparative Study to Assess the Effectiveness of Turmeric Mouth Wash versus Saline Mouth Wash on Treatment Induced Oral Mucositis (Tiom) in a Selected Hospital at Mangalore. *Journal of Clinical Research & Bioethics*, *5*(6), 1.
48. Sanders, K., Moran, Z., Shi, Z., *et al* (2016). Natural products for cancer prevention: Clinical update 2016. *Seminars in oncology nursing*, 32(3), 215-240
49. Shapiro, C. L. (2016). Highlights of recent findings on quality-of-life management for patients with cancer and their survivors. *JAMA Oncology.* 2, 1401–1402.
50. Shin, Y. S., Shin, H. A., Kang, *et al* (2013). Effect of epicatechin against radiation-induced oral mucositis: in vitro and in vivo study. *PLoS One*, *8*(7), e69151.
51. Sonis, S. T., Lindquist, L., Van Vugt, A., *et al* (1994). Prevention of chemotherapy-induced ulcerative mucositis by transforming growth factor β3. *Cancer research*, *54*(5), 1135-1138.

52. Sonis, S. T., Peterson, R. L., Edwards, L. J., *et al* (2000). Defining mechanisms of action of interleukin-11 on the progression of radiation-induced oral mucositis in hamsters. *Oral Oncology*, *36*(4), 373-381.
53. Sonis, S. T. (2002). The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with anti-neoplastic therapy. *Critical Reviews in Oral Biology & Medicine*, *13*(5), 380-389.
54. Sonis, S. T. (2004). The pathobiology of mucositis. *Nature Reviews Cancer*, *4*(4), 277.
55. Sonis, S. T. (2007). Pathobiology of oral mucositis: novel insights and opportunities. *J Support Oncol*, *5*(9 Suppl 4), 3-11.
56. Sonis, S. T. (2009). Mucositis: the impact, biology and therapeutic opportunities of oral mucositis. *Oral oncology*, *45*(12), 1015-1020.
57. Stringer, A. M., and Logan, R. M. (2015). The role of oral flora in the development of chemotherapy-induced oral mucositis. *J. Oral Pathol. Med.* 44, 81–87.
58. Tobita, T., Izumi, K., & Feinberg, S. E. (2010). Development of an in vitro model for radiation-induced effects on oral keratinocytes. *International journal of oral and maxillofacial surgery*, *39*(4), 364-370.
59. Tra, W. M. W., Tuk, B., van Neck, J. W., *et al* (2013). Tissue-engineered mucosa is a suitable model to quantify the acute biological effects of ionizing radiation. *International journal of oral and maxillofacial surgery*, *42*(8), 939-948.
60. Trotti, A., Bellm, L. A., Epstein, J. B., *et al*. (2003). Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: a systematic literature review. *Radiotherapy and oncology*, *66*(3), 253-262.
61. Tsujimoto, T., Yamamoto, Y., Wasa, M., *et al* (2015). L-glutamine decreases the severity of mucositis induced by chemoradiotherapy in patients with locally advanced head and neck cancer: a double-blind, randomized, placebo-controlled trial. *Oncology reports*, *33*(1), 33-39.
62. Vigarios, E., Epstein, J. B., & Sibaud, V. (2017). Oral mucosal changes induced by anticancer targeted therapies and immune checkpoint inhibitors. *Supportive Care in Cancer*, *25*(5), 1713-1739.

63. Villa, A., & Sonis, S. T. (2015). Mucositis: pathobiology and management. *Curr. Opin. Oncol.* 27, 159–164.
64. Villa, A., & Sonis, S. (2016). Toxicities associated with head and neck cancer treatment and oncology-related clinical trials. *Current problems in cancer*, *40*(5), 244-257.
65. Vuyyuri, S. B., Hamstra, D. A., Khanna, D., *et al* (2008). Evaluation of D-methionine as a novel oral radiation protector for prevention of mucositis. *Clinical Cancer Research*, *14*(7), 2161-2170.
66. Zecha, J. A., Raber-Durlacher, J. E., Nair, R. G., *et al* (2016). Low-level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 2: proposed applications and treatment protocols. *Supportive Care in Cancer*, *24*(6), 2793-2805.
67. Zhang, Q. Y., Wang, F. X., Jia, K. K., & Kong, L. D. (2018). Natural product interventions for chemotherapy and radiotherapy-induced side effects. *Frontiers in pharmacology*, *9*.
68. Zhou, H., Luo, Y., & Huang, S. (2010). Updates of mTOR inhibitors. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *10*(7), 571-581.

ANEXOS

ANEXO A – APROVAÇÃO PELO COMITÊ DE ÉTICA EM PESQUISA, CAAE Nº 78679717.6.0000.0030

UNB - FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Modelo in vitro de mucosite oral induzida por radiação
Pesquisador: ANA GABRIELA COSTA NORMANDO
Área Temática:
Versão: 3
CAAE: 78679717.6.0000.0030
Instituição Proponente: FACULDADE DE SAÚDE - FS
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.544.428

Apresentação do Projeto:
Resumo: "Dentre os diversos efeitos colaterais decorrentes da terapia antineoplásica, a mucosite oral é um dos mais debilitantes, acometendo mais de 80% dos pacientes submetidos à radioterapia de cabeça e pescoço. Possíveis alvos preventivos e terapêuticos são a interrupção das cascatas inflamatórias e redução da carga bacteriana. Produtos naturais com ações antioxidantes e anti-inflamatórias são uma maneira promissora de radioproteção. Os polifenóis apresentam atividade anti-inflamatória, anti-proliferativa e próapoptótica,
e por isso têm sido sugeridos para prevenir e tratar condições inflamatórias. Com a finalidade de melhor compreensão da expressão gênica de citocinas inflamatórias, este estudo tem como objetivo estabelecer modelo in vitro de mucosite oral. Para cumprir o objetivo proposto, será estabelecida cultura primária de tecidos gengivais humanos, obtidos de cirurgias de exodontia ou instalação de implantes. Será realizada avaliação da viabilidade celular utilizando o teste de MTT (Ensaio de Atividade Mitocondrial), análise de morfologia celular, teste de cicatrização (Scratch assay), teste de migração (Transwell Migration Assay), análise da expressão gênica de citocinas pró-inflamatórias, tratamento com produtos naturais e por fim a análise estatística dos dados obtidos. Espera-se que a pesquisa permita formular um modelo in vitro de mucosite oral para melhor compreensão da expressão gênica de citocinas pro-inflamatórias de tecidos gengivais tratados com produtos naturais tais como os polifenóis curcumina, resveratrol e EGCG, no intuito de estabelecer novas medidas terapêuticas para o uso baseado em evidências científicas,

Endereço: Faculdade de Ciências da Saúde - Campus Darcy Ribeiro
Bairro: Asa Norte **CEP:** 70.910-900
UF: DF **Município:** BRASILIA
Telefone: (61)3107-1947 **E-mail:** cepfsunb@gmail.com

proporcionando melhora na qualidade de vida dospacientes oncológicos. O projeto está vinculado ao Mestrado no programa de Pós-graduação em Ciências da Saúde da Universidade de Brasília."
Proposta: "O uso de medicamentos fitoterápicos ainda é muito restrito no manejo de ucosite oral. Até o presente momento, os efeitos dos polifenóis em lesões inflamatórias de mucosa, como a mucosite, foram descritos na literatura de forma escassa. Com a finalidade de melhor utilização desses medicamentos como fármacos, este estudo tem como objetivo estabelecer modelo in vitro de mucosite oral induzida por radiação, assim como avaliar os efeitos de medicamentos fitoterápicos tais como EGCG, Curcumina, Resveratrol e outros na expressão de citocinas inflamatórias, para, assim, oferecer uma nova abordagem terapêutica segura e eficaz para o tratamento da mucosite oral."

Tamanho da Amostra: 20 participantes de 18 a 25 anos.

CRITÉRIOS DE INCLUSÃO
"Pacientes de ambos os gêneros; Pacientes com idade entre 18 e 25 anos; Fragmento de gengiva integra, sem inflamação, removida durante extração de dentes terceiros molares
inclusos ou não com indicação de exodontia; Fragmento de gengiva sem inflamação removida durante extração de dentes pré-molares com indicação ortodôntica de exodontia;
Fragmento de gengiva sem inflamação removida durante cirurgia para instalação de implantes dentários osseointegrados;
Estar de acordo e assinar o Termo de Consentimento Livre e Esclarecido."

CRITÉRIOS DE EXCLUSÃO:
"Gengiva com aspecto clínico de inflamação."

Objetivo da Pesquisa:
Objetivo Geral:
"Desenvolver modelo in vitro de mucosite oral induzida por radiação ionizante e lipopolissacarídeos (LPS)."

Objetivos Específicos:
"Estabelecer modelo in vitro de mucosite oral por meio de cultura celular, usando cultura primáriade células gengivais humanas, tratadas com LPS e/ou radiação; Analisar a expressão

gênica de citocinas próinflamatórias após tratamento com LPS e/ou radiação; Avaliar a viabilidade e a morfologia celular após tratamento com medicamentos fitoterápicos; Avaliar o potencial de migração e cicatrização celular após tratamento com medicamentos fitoterápicos; Investigar o efeito antiinflamatório de medicamentos fitoterápicos na expressão gênica de citocinas próinflamatórias."

Avaliação dos Riscos e Benefícios:

Segundo a pesquisadora:

Riscos:" Os fragmentos gengivais utilizados na pesquisa serão aqueles obtidos quando da extração de dentes por razões terapêuticas não estando, portanto, o procedimento cirúrgico vinculado ao projeto. Para os pacientes que aceitarem cederem amostras de gengiva para utilização no projeto, o risco poderia ser vincular a amostra a sua identidade. Nesse sentido, a confidencialidade do participante será garantida por meio da codificação das células com números."

Benefícios:"A pesquisa não oferece benefícios diretos e imediatos aos participantes, mas como benefícios futuros, espera-se que a pesquisa permita uma melhor compreensão da expressão gênica de citocinas proinflamatórias de tecidos gengivais tratados com produtos naturais tais como curcumina, resveratrol e EGCG, no intuito de estabelecer novas medidas terapêuticas para o uso baseado em evidências científicas, proporcionando melhora na qualidade de vida dos pacientes oncológicos."

Comentários e Considerações sobre a Pesquisa:

Trata-se de um projeto de mestrado do Programa de Pós-Graduação em Ciências da Saúde da discente Ana Gabriela Costa Normando, orientado pela Profa. Dra. Eliete Neves da Silva Guerra. A pesquisa será desenvolvida na Universidade de Brasília (UnB) no Laboratório de Histopatologia Bucal do Departamento de Odontologia da Faculdade de Ciências da Saúde, com apoio do Laboratório de Farmacologia Molecular (FARMOL). Na Unidade de Alta Complexidade em Oncologia do Hospital Universitário de Brasília (UNACON -HUB) que possui todos os equipamentos necessários para auxiliar na pesquisa. Apresenta uma equipe de 5 pesquisadores, além da discente, composta por professores pesquisadores e alunos de pós-graduação e graduação.

Orçamento no valor total de R$ 5.372,50, consistindo de materiais de consumo.

Considerações sobre os Termos de apresentação obrigatória:

Documentos analisados para emissão do presente parecer:

1) "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1005221.pdf", postado em 21/02/2018.

2) Carta de resposta às pendências em "Carta_resposta2.doc", postado em 21/02/2018.

3) "TCLE_modificado2.doc", postado em 21/02/2018.

Recomendações:

Não se aplicam.

Conclusões ou Pendências e Lista de Inadequações:

Análise das resposta às pendências apontadas no Parecer Consubstanciado no.: 2.504.202

1) O armazenamento de material biológico para o estudo caracteriza formação de biorrepositório. Dessa forma, documentos para adequação à Resolução CNS 441/2011, Portaria MS 2.201/11 e Norma Operacional CNS 001/2013 deverão ser apresentados. Ou, se for o caso, apresentar declaração informando a destruição das amostras ao final do estudo. Tal informação deverá constar também no TCLE.

RESPOSTA: 1) Documentos para adequação à Resolução CNS 441/2011, Portaria MS 2.201/11 e Norma Operacional CNS 001/2013, em relação ao armazenamento de material biológico foram anexados: Justificativa de Necessidade para Utilização Futura de Amostra Armazenada e Declaração de Submissão ao Sistema CEP/CONEP em caso de novos estudos. Tal informação também foi adicionada ao TCLE (Página 1, parágrafo 3 e Página 2, parágrafo 1). Foi também incluído ao projeto o item sobre armazenamento e uso futuro de material biológico armazenado (Página 9,item 6).

ANÁLISE: A pesquisadora realizou as adequações e incluiu uma justificativa assinada - JUSTIFICATIVA DE NECESSIDADE PARA UTILIZAÇÃO FUTURA DE AMOSTRA ARMAZENADA. "O Projeto de Pesquisa "Modelo in vitro de mucosite oral induzida por radiação" será realizado com fragmentos gengivais obtidos em cirurgias orais tais como extrações dentárias e instalação de implantes e cedidos pelos pacientes ou pelos responsáveis. Durante a realização dos experimentos e após sua conclusão, as células serão armazenadas em congelador à -80°C, sem identificação do doador. A autorização do participante para o armazenamento das células encontra-se em Termo de Consentimento Livre e Esclarecido. Dependendo dos resultados obtidos no presente estudo, o material armazenado poderá ser utilizado em pesquisas futuras. Por

isso, há a necessidade de armazenamento dos tecidos coletados, para não precisar de uma nova coleta em outros indivíduos. De acordo com o cronograma do projeto, a pesquisa terá duração de 1(um) ano, tempo necessário para que os tecidos dentários e células cultivadas sejam armazenados. As renovações da autorização de armazenamento serão solicitadas pelo pesquisador responsável, ao CEP, acompanhada de justificativa e relatório das atividades de pesquisa desenvolvidas com o material."
PENDÊNCIA ATENDIDA

2) Apresentar orçamento detalhado conforme exigido no item 3.3, item "e" da Norma Operacional CNS 001 de 2013. Apresentar previsão de ressarcimento de despesas do participante e seus acompanhantes, quando necessário, tais como transporte e alimentação e exames (se for o caso).
RESPOSTA: Orçamento detalhado foi anexado e previsão de ressarcimento de despesas do participante e seus acompanhantes, quando necessário, tais como transporte e alimentação foram adicionados ao TCLE (Página 1, parágrafo 4).
ANÁLISE: A pesquisadora declarou o orçamento conforme solicitado e ainda informou Recursos próprios do laboratório, obtidos em diversos editais (CNPq, FAPDF, CAPES-COFFECUB). O laboratório tem apoio financeiro do projeto Chamada MCTI/CNPq/FNDCT Ação Transversal - Redes Regionais de Pesquisa em Ecossistemas, Biodiversidade e Biotecnologia N o 79/2013.
PENDÊNCIA ATENDIDA.

3) Solicita-se atualizar e unificar os cronogramas prevendo o início da pesquisa para período posterior à aprovação pelo CEP, apresentar um cronograma de pesquisa e não de desenvolvimento do mestrado. Ressalta-se que cabe ao pesquisador responsável aguardar a decisão de aprovação ética, antes de iniciar a pesquisa (Res. CNS 466/2012, item XI.2.a). Tal alteração deverá ser realizada no projeto da plataforma Brasil e no projeto detalhado.
RESPOSTA: 3)O cronograma foi atualizado, prevendo o início da pesquisa para período posterior à aprovação pelo CEP.
ANÁLISE: terá uma duração de 1(um) ano e será iniciado no mês seguinte à aprovação do Comitê de Ética com obtenção em Março.
PENDÊNCIA ATENDIDA.

4) Nas Informações Básicas do Projeto, apresenta um estudo piloto no cronograma, ressalta-se que estudo piloto faz parte da pesquisa e, portanto, não pode ter ser realizado sem aprovação do

CEP. De acordo com o item XI.2, subitem a, Res. CNS 466/2012, que trata das obrigações do pesquisador de aguardar a aprovação do CEP para iniciar a pesquisa. Solicita-se ao pesquisador responsável que os dados do estudo piloto não sejam utilizados e que apresente uma declaração explicitando que tais dados não serão utilizados no estudo.
RESPOSTA: 4)O estudo piloto citado no projeto faz parte da pesquisa e será feito apenas após aprovação pelo CEP, uma vez que serão testadas diferentes doses de radiação e concentrações de LPS nos tecidos gengivais para termos a informação de qual dose de radiação/concentração de LPS induz mais inflamação.
ANÁLISE: Ficou clara a metodologia proposta e que só iniciar estudos após a aprovação.
PENDÊNCIA ATENDIDA

5)TCLE: Esclarecemos que "II.21 - ressarcimento - compensacao material, exclusivamente de despesas do participante e seus acompanhantes, quando necessario, tais como transporte e alimentacao". Solicita-se a inclusão desse item no TCLE.
5.1)Solicita-se no que se refere a pendência 1, deste parecer,incluir no TCLE informação sobre biorrepositório.
RESPOSTA: 5.1 – Foi incluído no TCLE o item sobre ressarcimento, exclusivamente de despesas do participante e seus acompanhantes, quando necessário, tais como transporte e alimentação (Página 1, parágrafo 4).
5.2 - Foi incluído no TCLE informação sobre biorrepositório (Página 2, parágrafo 1).
ANÁLISE: Alteração realizada. Lê-se " (...) Ao assinar esse termo, o senhor (a) autoriza a coleta, o depósito, o armazenamento e a utilização do material associado a esse projeto de pesquisa."
PENDÊNCIA ATENDIDA.

6) Solicita-se incluir termos: Termo de Ciência Instituição coparticipante e Declaração de Instituição proponente, os documentos apresentados como Termo de Concordancia é da Coparticipante e não há termo da Instituição Proponente UnB/FS. Veja modelo dos documentos para submissão no seguinte endereço: http://fs.unb.br/cep/index.php/modelos-de-documentos
RESPOSTA: Foram incluídos o Termo de Ciência da Instituição Coparticipante da UNACON-HUB e Declaração de Instituição Proponente envolvendo os dois laboratórios que serão utilizados ao longo da pesquisa: o Laboratório de Histopatologia Bucal e o Laboratório de Farmacologia Molecular. ANÁLISE: Conforme solicitado. Assinado pela Profa. Dra. Ana Carolina Acevedo Poppe do Laboratório de Histopatologia Bucal e do Superintendente do hospital universitário de Brasília,

UNB - FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA

informando estar de acordo com a realização da pesquisa.
PENDÊNCIA ATENDIDA

7) Quanto ao TCLE, solicita-se que o termo "ceder", "cedente" e "cessão" sejam substituídos por "conceder", "concedente" e "concessão".
Resposta: No TCLE, os termos "ceder" e "cedendo" foram substituídos por "conceder" e "concedendo". As alterações encontram-se na primeira página, terceiro e quarto parágrafos respectivamente.
Analise: Alteração realizada.
PENDÊNCIA ATENDIDA.

Considerações Finais a critério do CEP:
De acordo com a Resolução CNS 466/12, itens X.1.- 3.b. e XI.2.d, os pesquisadores responsáveis deverão apresentar relatórios parcial semestral e final do projeto de pesquisa, contados a partir da data de aprovação do protocolo de pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1005221.pdf	21/02/2018 21:51:03		Aceito
Outros	Carta_resposta2.pdf	21/02/2018 21:46:18	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Carta_resposta2.doc	21/02/2018 21:45:44	ANA GABRIELA COSTA NORMANDO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_modificado2.pdf	21/02/2018 21:44:38	ANA GABRIELA COSTA NORMANDO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_modificado2.doc	21/02/2018 21:44:14	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Manuseio Material Biológico /	Justificativa_Armazenamento.pdf	28/12/2017 23:44:59	ANA GABRIELA COSTA NORMANDO	Aceito

UNB - FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA

Continuação do Parecer: 2.544.428

Biorepositório / Biobanco	Justificativa_Armazenamento.pdf	28/12/2017 23:44:59	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Justificativa_Armazenamento.docx	28/12/2017 23:44:45	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Declaracao_novos_estudos.pdf	28/12/2017 23:44:25	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Declaracao_novos_estudos.docx	28/12/2017 23:42:00	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Instituição e Infraestrutura	Termo_Concord_HPB.pdf	28/12/2017 23:41:04	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Instituição e Infraestrutura	Termo_Concord_HPB.doc	28/12/2017 23:40:51	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Instituição e Infraestrutura	Termo_Concord_FarMol.pdf	28/12/2017 23:40:39	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Instituição e Infraestrutura	Termo_Concord_FarMol.doc	28/12/2017 23:40:25	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Instituição e Infraestrutura	Termo_coparticipacaoHUB.pdf	28/12/2017 23:38:43	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Instituição e Infraestrutura	Termo_coparticipacaoHUB.doc	28/12/2017 23:38:26	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Instituição e Infraestrutura	termo_concordancia_hub.pdf	28/12/2017 23:37:55	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Instituição e Infraestrutura	termo_concordancia_hub.doc	28/12/2017 23:37:12	ANA GABRIELA COSTA NORMANDO	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_Pesquisa_modificado.docx	28/12/2017 23:32:57	ANA GABRIELA COSTA NORMANDO	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_Pesquisa_modificado.pdf	28/12/2017 23:32:40	ANA GABRIELA COSTA NORMANDO	Aceito

Endereço: Faculdade de Ciências da Saúde - Campus Darcy Ribeiro
Bairro: Asa Norte **CEP:** 70.910-900
UF: DF **Município:** BRASILIA
Telefone: (61)3107-1947 **E-mail:** cepfsunb@gmail.com

Orçamento	Orcamento_modificado.pdf	28/12/2017 23:31:56	ANA GABRIELA COSTA NORMANDO	Aceito
Orçamento	Orcamento_modificado.doc	28/12/2017 23:31:43	ANA GABRIELA COSTA NORMANDO	Aceito
Cronograma	Cronograma_modificado.docx	28/12/2017 23:29:51	ANA GABRIELA COSTA NORMANDO	Aceito
Cronograma	Cronograma_modificado.pdf	28/12/2017 23:29:38	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Termo_de_responsabilidade.doc	09/10/2017 16:58:16	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Instituição e Infraestrutura	Termo_de_concordancia_institucional.doc	09/10/2017 16:57:35	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Carta_de_apresentacao_CEP.doc	09/10/2017 16:54:57	ANA GABRIELA COSTA NORMANDO	Aceito
Folha de Rosto	Folha_de_rosto_assinada.pdf	04/10/2017 19:44:53	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Termo_de_responsabilidade.pdf	03/10/2017 10:45:24	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Curriculo_Paula.pdf	03/10/2017 10:44:07	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Curriculo_Gabriel.pdf	03/10/2017 10:43:40	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Curriculo_Eliete.pdf	03/10/2017 10:42:51	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Curriculo_Claudio.pdf	03/10/2017 10:42:36	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Curriculo_Caroline.pdf	03/10/2017 10:42:00	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Curriculo_AnaGabriela.pdf	03/10/2017 10:40:10	ANA GABRIELA COSTA NORMANDO	Aceito
Outros	Carta_de_apresentacao_CEP.pdf	03/10/2017 10:38:50	ANA GABRIELA COSTA NORMANDO	Aceito
Declaração de Instituição e Infraestrutura	Termo_de_concordancia_institucional.pdf	03/10/2017 10:36:12	ANA GABRIELA COSTA NORMANDO	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

APÊNDICES

APÊNDICE A – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Universidade de Brasília
Pós-Graduação em Ciências da Saúde

Termo de Consentimento Livre e Esclarecido - TCLE

Convidamos o(a) Senhor(a) a participar voluntariamente do projeto de pesquisa "**Modelo *in vitro* de mucosite oral induzida por radiação"**, sob a responsabilidade da pesquisadora Ana Gabriela Costa Normando. A mucosite oral é um efeito colateral que atinge cerca de 80% de pacientes submetidos a radioterapia e/ ou quimioterapia, diminuindo significativamente a qualidade de vida dos pacientes com câncer. Assim, o presente estudo tem como objetivo desenvolver um modelo laboratorial de mucosite oral para melhor compreender o processo inflamatório e o reparo da mucosa oral tratada com produtos naturais. O modelo será desenvolvido a partir de fragmentos gengivais obtidos em cirurgias orais tais como extrações dentárias e instalação de implantes. Com esta pesquisa, espera-se verificar a possibilidade ou não de se utilizar produtos naturais como medicamentos no controle da mucosite oral.

O(a) senhor(a) receberá todos os esclarecimentos necessários antes e no decorrer da pesquisa e lhe asseguramos que seu nome não aparecerá, sendo mantido o mais rigoroso sigilo pela omissão total de quaisquer informações que permitam identificá-lo(a).

Ao assinar este termo, o senhor (a) estará concedendo uma amostra de gengiva em região com indicação de extração dentária ou instalação de implante para contribuir com esta pesquisa, consciente de que este(s) procedimento(s) tiveram indicação terapêutica, para melhoria da sua saúde, não estando, portanto, a cirurgia oral vinculada ao projeto em questão. Cabe ressaltar que o procedimento cirúrgico será realizado independente do aceite ou não em participar da pesquisa. Ao assinar esse termo, o senhor (a) autoriza a coleta, o depósito, o armazenamento e a utilização do material associado a esse projeto de pesquisa.

O senhor (a) não terá nenhum custo e não receberá nenhuma remuneração ao participar desta pesquisa. A sua participação ao conceder a gengiva não implica despesas, mas caso haja, será feita compensação material, exclusivamente de suas despesas e seus acompanhantes, tais como transporte e alimentação. Os riscos que este estudo oferece seriam os de vincular as células da gengiva a sua identidade. Porém, as amostras serão codificadas com números, garantindo, assim, a sua confidencialidade (seu nome não será revelado). Caso haja algum dano direto ou indireto decorrente de sua participação na pesquisa, você deverá buscar ser indenizado, obedecendo-se as disposições legais vigentes no Brasil. Em qualquer momento, o senhor (a) poderá ter acesso aos resultados e eles poderão ser publicados em eventos e revistas científicas sempre mantendo o sigilo da sua participação.

A pesquisa não oferece benefícios diretos e imediatos ao senhor(a), mas como benefícios futuros, espera-se que a pesquisa permita uma melhor compreensão dos eventos relacionados com os processos

inflamatórios e de reparo da mucosa oral, bem como o desenvolvimento de novas estratégias terapêuticas buscando a melhoria da saúde bucal.

Durante a realização dos experimentos e após sua conclusão, as células serão armazenadas e dependendo dos resultados obtidos no presente estudo, poderão ser utilizadas em pesquisa futura. Por isso, há a necessidade de armazenamento dos tecidos coletados em biorrepositório, para não precisar de uma nova coleta em outros indivíduos. No caso de pesquisa futura, o senhor(a) será contatado para novo consentimento específico referente ao novo projeto de pesquisa e os novos projetos serão submetidos para aprovação do Comitê de Ética em Pesquisa e, se for o caso, da Comissão Nacional de Ética em Pesquisa (CONEP).

O(a) Senhor(a) pode se recusar ou desistir de participar da pesquisa em qualquer momento sem nenhum prejuízo para o(a) senhor(a), retirando o consentimento de utilização e armazenamento de suas células e pedindo a destruição e descarte do material. Para isso, o senhor(a) deve formalizar a retirada do consentimento por manifestação escrita e assinada.

Se o(a) Senhor(a) tiver qualquer dúvida em relação à pesquisa, por favor telefone para a pesquisadora responsável, Ana Gabriela Costa Normando, pelo telefone 61 99995-1617, disponível inclusive para ligação a cobrar, ou por e-mail (gabinormando@gmail.com), ou com a professora orientadora, Eliete Neves da Silva Guerra, pelo telefone 61 99668-4988 ou por e-mail (elieteneves.unb@gmail.com).

Este projeto foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Ciências da Saúde (CEP/FS) da Universidade de Brasília. O CEP é composto por profissionais de diferentes áreas cuja função é defender os interesses dos participantes da pesquisa em sua integridade e dignidade e contribuir no desenvolvimento da pesquisa dentro de padrões éticos. As dúvidas com relação à assinatura do TCLE ou os direitos do participante da pesquisa podem ser esclarecidos pelo telefone (61) 3107-1947 ou pelo e-mail cepfs@unb.br ou cepfsunb@gmail.com, horário de atendimento de 10:00hs às 12:00hs e de 13:30hs às 15:30hs, de segunda a sexta-feira. O CEP/FS se localiza na Faculdade de Ciências da Saúde, Campus Universitário Darcy Ribeiro, Universidade de Brasília, Asa Norte.

Caso concorde em participar, pedimos que assine este documento que foi elaborado em duas vias, uma ficará com o pesquisador responsável e a outra com o Senhor (a).

_____
Assinatura do participante

_____
Assinatura do Pesquisador Responsável

Brasília, ___ de _____de _____.