GABRIEL ANTÔNIO DOS ANJOS TOU

EFEITOS DA COLAGEM DO ESPORÃO LINGUAL NO MICROAMBIENTE PERIODONTAL E LIBERAÇÃO DE SUBPRODUTOS DE MATERIAIS RESINOSOS NO TRATAMENTO DE MORDIDA ABERTA ANTERIOR EM CRIANÇAS

Faculdade de Odontologia
Universidade Federal de Minas Gerais
Belo Horizonte
2020

Gabriel Antônio dos Anjos Tou

EFEITOS DA COLAGEM DO ESPORÃO LINGUAL NO MICROAMBIENTE PERIODONTAL E LIBERAÇÃO DE SUBPRODUTOS DE MATERIAIS RESINOSOS NO TRATAMENTO DE MORDIDA ABERTA ANTERIOR EM CRIANÇAS

Dissertação apresentada ao Colegiado de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do grau de Mestre em Odontologia - área de concentração em Clínica Odontológica.

Linha de pesquisa: Propriedades físicas, químicas e biológicas dos materiais odontológicos.

Orientadora: Profª. Drª. Soraia Macari

Coorientadora: Profª. Drª Monica Yamauti

Belo Horizonte
2020

Ficha Catalográfica

T722e 2020 T

Tou, Gabriel Antônio dos Anjos.
Efeitos da colagem do esporão lingual no microambiente periodontal e liberação de subprodutos de materiais resinosos no tratamento de mordida aberta anterior em crianças / Gabriel Antônio dos Anjos Tou. -- 2020.

98 f. : il.

Orientadora: Soraia Macari.
Coorientadora: Monica Yamauti.

Dissertação (Mestrado) -- Universidade Federal de Minas Gerais, Faculdade de Odontologia.

1. Aparelhos ortodônticos. 2. Criança. 3. Mordida aberta. 4. Interleucinas. 5. Líquido do sulco gengival. I. Macari, Soraia. II. Yamauti, Monica. III. Universidade Federal de Minas Gerais. Faculdade de Odontologia. IV. Título.

BLACK - D047

Elaborada por: Sérgio Barbosa dos Santos - CRB: 6/3182.

Biblioteca Faculdade de Odontologia - FAO UFMG

FOLHA DE APROVAÇÃO

EFEITOS DA COLAGEM DO ESPORÃO LINGUAL NO MICROAMBIENTE PERIODONTAL E LIBERAÇÃO DE SUBPRODUTOS DE MATERIAIS RESIONOSOS NO TRATAMENTO DE MORDIDA ABERTA ANTERIOR EM CRIANÇAS

GABRIEL ANTÔNIO DOS ANJOS TOU

Dissertação submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Odontologia, como requisito para obtenção do grau de Mestre, área de concentração Clínica Odontológica.

Aprovada em 28 de fevereiro de 2020, pela banca constituída pelos membros:

Prof(a). Soraia Macari - Orientadora
FO-UFMG

Prof(a). Ivana Marcia Alves Diniz
FO-UFMG

Prof(a). Dauro Douglas Oliveira
PUC Minas

Belo Horizonte, 28 de fevereiro de 2020.

AGRADECIMENTOS

À Profa . Dra. Soraia Macari pela instrução, orientação, dedicação e comprometimento. À Profa . Dra. Monica Yamauti pela instrução, orientação, dedicação e comprometimento. À Profa . Dra. Tarcília Aparecida pela instrução, orientação, dedicação e comprometimento. Aos coordenadores da área de pós-graduação em clínica odontológica Profa . Dra. Cláudia Silami de Magalhães e Prof. Dr. Allyson Nogueira Moreira pela instrução, orientação e incentivo. À chefe do Departamento de Odontologia Restauradora da FO-UFMG, Profa . Dra. Maria Elisa Silva e Souza e toda sua equipe. À Faculdade de Odontologia (FO) da UFMG na pessoa do diretor, Prof. Dr. Henrique Pretti. Ao Colegiado de Pós-Graduação da FO-UFMG na pessoa da coordenadora, Profa . Dra. Isabela de Almeida Pordeus e toda equipe do colegiado. Ao laboratório de Química Analítica da Faculdade Unicamp em Campinas, a Profa . Dra. Carla Bottoli e a Técnica do Laboratório Lucília pela parceria, e orientação.À Fundação Oswaldo Cruz e toda a equipe: Elaine, Daiane e Ana pela colaboração, parceria e orientação. Aos amigos do Mestrado pelos momentos que vivemos juntos, Bruno, Clóvis, Natália Tavares, Natália Gomes, Richard, Elbert. Aos amigos do doutorado pelo apoio durante a pesquisa, Ricardo, Daniel, Ivison, Tassiana, Diandra, Patrícia, Carla, Tuélita. À minha amada Izabela que sempre me apoiou e esteve ao meu lado durante todo esse processo. À minha família e amigos meu agradecimento especial.

“A persistência é o caminho do êxito.”

Charles Chaplin

RESUMO

Introdução: O aparelho ortodôntico passivo esporão é um método utilizado em crianças para correção de mordida aberta anterior. Para realizar a fixação desses acessórios dentes é necessário a utilização de materiais resinosos que podem liberar subprodutos tóxicos como BisGMA e TEGDMA. Além disso, a movimentação dentária ortodôntica pode aumentar a concentração de interleucinas no fluido crevicular gengival (FCG). A liberação de subprodutos resinosos no tratamento de mordida aberta e o comportamento da expressão de interleucinas ainda não estão elucidados na literatura. Objetivo: (1) avaliar a expressão de citocinas no FCG em crianças com mordida aberta que receberam fixação de esporão como tratamento e (2) quantificar o BisGMA e TEGDMA na saliva desses pacientes; e (3) avaliar a migração e viabilidade de quetarinócitos humanos expostos à resina. Métodos: Foram selecionados pacientes da clínica da FO-UFMG que apresentaram mordida aberta anterior. A colagem dos esporões foi realizada e os excessos de resina foram removidos. Foram realizados exames clínico e periodontal nos incisivos superiores e inferiores, coletas do FCG (antes da colagem – *baseline*, 24 h e 7 d após) e saliva (baseline, 30 min, 24 h e 7 d após a colagem). As citocinas do FCG foram analisadas por meio do BD™ Cytometric Bead Array e as amostras de saliva, através do método de cromatografia líquida de alta eficiência. Para as análises *in vitro*, células imortalizadas HaCat foram tratadas com meio de cultura condicionados com incrementos do sistema resinoso em 3 diferentes diluições e posteriormente foram realizados os testes de viabilidade e migração celular. Resultados: O estudo *in vivo* demonstrou que houve aumento do sangramento gengival nos incisivos inferiores após 7d quando comparados ao *baseline* e que este índice estava maior nos dentes inferiores do que nos superiores. Em 24h e 7d após a fixação do esporão, os níveis de IL-8 nos incisivos superiores estavam aumentados. Em 7d, a concentração de IL-1β foi aumentada em comparação ao *baseline* nos dois grupos de dentes. Comparando os incisivos superiores e inferiores, os níveis de IL-8, IL-1β e IL-6 foram maiores nos superiores às 24h. A produção de citocinas pôde ser positivamente correlacionada com o aumento do volume do FCG. Houve aumento dos níveis de BisGMA e TEGDMA na saliva em 30min, com redução dos níveis em 24h e 7d. Os resultados *in vitro* demonstraram aumento da migração celular nos queratinóscitos expostos aos meios condicionados mesmo em baixas concentrações .Conclusão: Foi identificado que após a colagem do esporão lingual ocorreu o aumento na expressão de interleucinas no FCG. Além disso, foi possível identificar a liberação de subprodutos resinosos na saliva das crianças. Embora os mesmos possam interferir na migração celular, não há estudos que quantifiquem a exposição mínima capaz de induzir alterações no indivíduo. Sendo assim, os benefícios da colagem do esporão sobrepõem seus efeitos adversos.

Palavras-chave: Bisfenol-A glicidil metacrilato. Trietilenoglicol dimetacrilato. Interleucinas. Mordida aberta. Esporão.

ABSTRACT

Effects of lingual sporing collage on the periodontal micro-environment and release of by-products of resin materials on the treatment of anterior open bite in children

Introduction: The passive spur orthodontic appliance is a method used in children to correct anterior open bite. To fix these teeth accessories, it is necessary to use resinous materials that can release toxic by-products such as BisGMA and TEGDMA. In addition, orthodontic tooth movement can increase the concentration of interleukins in the gingival crevicular fluid (FCG). The release of resinous by-products in the treatment of open bite and the behavior of interleukin expression are not yet elucidated in the literature. Objective: (1) to evaluate the expression of cytokines in the FCG in children with open bite who received spur fixation as treatment and (2) to quantify the BisGMA and TEGDMA in the saliva of these patients; and (3) evaluate the migration and viability of human ketarinocytes exposed to the resin. Methods: Patients from the FO-UFMG clinic who presented anterior open bite were selected. The spurs were bonded and the excess resin was removed. Clinical and periodontal examinations were performed on the upper and lower incisors, collections of the FCG (before bonding - baseline, 24 h and 7 d after) and saliva (baseline, 30 min, 24 h and 7 d after bonding). The FCG cytokines were analyzed using the BD ™ Cytometric Bead Array and the saliva samples, using the high performance liquid chromatography method. For in vitro analysis, immortalized HaCat cells were treated with culture medium conditioned with increments of the resin system in 3 different dilutions and then the viability and cell migration tests were performed. Results: The in vivo study showed that there was an increase in gingival bleeding in the lower incisors after 7d when compared to the baseline and that this index was higher in the lower than in the upper teeth. At 24h and 7d after spur fixation, IL-8 levels in the upper incisors were increased. At 7d, the concentration of IL-1β was increased compared to the baseline in the two groups of teeth. Comparing the upper and lower incisors, the levels of IL-8, IL-1β and IL-6 were higher in those above 24h. Cytokine production could be positively correlated with increased FCG volume. There was an increase in the levels of BisGMA and TEGDMA in saliva in 30 minutes, with a reduction in levels in 24h and 7d. The in vitro results demonstrated an increase in cell migration in keratinocytes exposed to conditioned media even in low concentrations. Conclusion: It was identified that after the gluing of the tongue spur there was an increase in the expression of interleukins in the FCG. In addition, it was possible to identify the release of resinous by-products in children's saliva. Although they can interfere with cell migration, there are no studies that quantify the minimum exposure capable of inducing changes in the individual. Therefore, the benefits of spur bonding override its adverse effects.

Keywords: Bisphenol-A glycidyl methacrylate. Triethylene glycol dimethacrylate. Interleukins. Open bite. Spur.

LISTA DE FIGURAS

LISTA DE GRÁFICOS

LISTA DE TABELAS

LISTA DE ABBREVIATURAS E SIGLAS

ANOVA	Análise de Variância
Bis-GMA	Bisfenol A Glicidil Metacrilato
DP	Desvio Padrão
FCG	Fluido Crevicular Gengival
g	Grama
h	Hora
HPLC	Cromatografia Líquida de Alta Eficiência
IC	Intervalo de Confiança
IL	Interleucina
LED	Light Emitting Diode (Diodo Emissor de Luz)
min	Minutos
ml	Mililitros
ml/min	Mililitros por Minuto
mm	Milímetro
µg/mm3	Micrograma por Milímetro Cúbico
µl	Microlitro
µm	Micrometro
n	Número de Amostra
ºC	Grau Celsius
rpm	Rotação por Minuto
seg	Segundos
TEGDMA	Trietileno-glicol Dimetacrilato
TNF	Fator de Necrose Tumoral
UDMA	Uretano Dimetracrilato

v	Volume
v/v	Volume por Volume

SUMÁRIO

1 CONSIDERAÇÕES INICIAIS

A mordida aberta anterior é uma maloclusão caracterizada pela falta de contato entre os arcos dentários na região anterior enquanto os dentes posteriores se encontram intercuspidados (ROSA *et al*., 2019). Essa alteração acomete pacientes em todas as dentições, e está associada, principalmente, à quebra do equilíbrio da musculatura peribucal e intrabucal ocasionada por hábitos bucais deletérios, como por exemplo, sucção digital e de chupeta por tempo prolongado (LAMBRECHTS *et al*., 2010; SILVA FILHO *et al.*, 1991). A mordida aberta anterior apresenta prevalência variando de 17% a 18% das crianças na dentição mista (KASPARAVICIENE *et al*., 2014; SILVESTRINI-BIAVATI, 2016; TAUSCHE *et al*., 2004). Quando associada a maus hábitos de sucção, a prevalência aumenta para 36,3% (COZZA *et al*., 2005).

A má oclusão na dentadura decídua pode se autocorrigir caso o hábito seja removido até os 4 anos de idade. A partir dos 5 anos, é necessário que haja interceptação ortodôntica, pois a interposição lingual na deglutição é um fator prejudicial que perdura a má oclusão (GONZALEZ *et al*., 2019; LARA *et al.*, 2009; RUAN *et al*., 2005). Com a persistência da alteração durante o surto de crescimento puberal e crânio-facial, as alterações dentárias poderão se tornar esqueléticas (PHELAN *et al*.,2014).

O tratamento precoce consiste essencialmente na remoção do hábito deletério e da interposição lingual. Existem maneiras diferentes para a realização dessa intervenção, entre elas está a utilização de acessórios ortodônticos denominados esporões, com o intuito de reposição cognitiva da língua (ARTESE, 2011; DIAS *et al*., 2019; MEYER-MARCOTTY, 2007; NOGUEIRA *et al.*, 2005). Esses dispositivos são aderidos aos dentes na face lingual dos incisivos, através de condicionamento ácido, sistemas adesivos e resinas compostas.

Sem a presença do hábito deletério a musculatura peribucal exerce força sobre os dentes estimulando a remodelação óssea e, com auxílio da erupção dentária, a correção da mordida aberta (LING *et al*., 2018; RUAN *et al*., 2005). O processo de remodelação óssea e movimentação dentária são intermediados por interleucinas

(IL) (KIMOTO *et al*., 1999; LI *et al*., 2018; MEAGER, 1999; MUNDY, 1992; SAITO *et al*., 1990; SCHRÖDER *et al*., 2018).

A movimentação dentária está baseada no estímulo mecânico decorrente das forças externas que induzem a remodelação dos tecidos periodontais (LI *et al*., 2019; SCHRÖDER *et al*., 2018; WISE and KING, 2008; YAMAGUCHI *et al*., 2005). Existem mecanismos que transformam a força aplicada em respostas celulares no periodonto, o que leva à liberação local de citocinas (APAJALAHTI *et al*., 2003; LI *et al*., 2018; LI *et al*., 2019; PERINETTI *et al*., 2005; REN *et al*., 2008; SCHRÖDER *et al*., 2018; UEMATSU *et al.,* 1996; VANDEVSKA-RADUNOVIC, 1999; YAMAGUCHI *et al.,* 2005; YAMAGUCHI *et al*., 2006).

Citocinas como, IL-1β, IL-6 e fator de necrose tumoral alfa (TNF-α), são liberadas no fluido crevicular gengival (FCG) e possuem papel fundamental no início de uma série de processos bioquímicos que estimulam as atividades celulares durante as alterações inflamatórias na movimentação dentária e remodelação óssea (ALHASHIMI *et al*., 2001; UEMATSU *et al*., 1996; YAMAGUCHI *et al.,* 2005). A ação desses biomarcadores está diretamente relacionada com a estimulação local de osteoclastos e *turnover* do osso alveolar. (AZUMA *et al*., 2000; DAVIDOVITCH *et al*., 1988; DAVIDOVITCH *et al*., 1991; LI *et al*., 2018; MEIKLE, 2006; PALMQUIST *et al*., 2008; SCHRÖDER *et al*., 2018).

A presença de interleucinas no FCG se torna interessante para ortodontia já que reflete o microambiente da região em que as forças são exercidas (DAVIDOVITCH *et al*., 1988; GARCIA-LOPEZ *et al.,* 2005; RUBIN *et al.,* 2002). Estudos têm observado diferenças na concentração de interleucinas, como IL-1β, IL-6 e TNF-α, no FCG durante a movimentação ortodôntica e identificado o aumento desses componentes nos períodos iniciais da movimentação (JAYAPRAKASH *et al*., 2019; REN *et al*., 2002).

Um estudo em modelo animal identificou que a expressão de citocinas, como IL-3, IL-8, TNF-a, IL-1β e IL-6, aumentou durante a aplicação de forças ortodônticas em 24 horas (TEIXEIRA *et al*., 2005). Resultados similares foram observados nos estudos em humanos nos quais identificaram, além do aumento do volume do FCG, que a movimentação ortodôntica foi capaz de induzir o aumento da liberação de IL-8

e IL-6 no FCG em 24 horas e após uma semana (JAYAPRAKASH *et al*., 2019; TUNCER *et al*., 2005; UEMATSU *et al*., 1996). Esse comportamento está relacionado com o papel dessas interleucinas nos momentos iniciais da movimentação.

O aumento do volume do fluído crevicular tem sido considerado um fator de indicação de inflamação periodontal e está relacionado ao aumento da presença de interleucinas (SOCRANSKY e HAFFAJEE, 2005). Da mesma forma acontece na movimentação ortodôntica. O aumento do volume FCG tem relação direta com o aumento da presença de interleucinas (BASARAN *et al.,* 2006; GRANT *et al.*, 2013).

Outros estudos com aplicação de forças através da movimentação ativa, também detectaram a presença de IL-1β em 24 horas e após uma semana no FCG, em todos os tempos avaliados e o aumento de IL-1β foi significativo quando comparado ao controle (DUDIC *et al*., 2006; GRANT *et al.,* 2013; JAYAPRAKASH *et al*., 2019; TEXIEIRA *et al.,* 2005). Apesar de a literatura elucidar o papel das interleucinas na movimentação ortodôntica ativa, poucos trabalhos têm focado no tratamento passivo.

Outra discussão envolvendo o tratamento ortodôntico tem sido levantada em estudos recentes. Trata-se da biocompatibilidade dos materiais resinosos aplicados para a colagem dos acessórios (GUPTA *et al*., 2012; VAN LANDUYT *et al*., 2011). Com a introdução do condicionamento ácido na superfície do esmalte e a evolução industrial na produção de polímeros resinosos (BUONOCORE, 1955), tornou-se rotina a colagem de acessórios ortodônticos com compósitos de resina à base de metacrilato (GOTO, *et al*., 2019; MALKIEWICZ, *et al*., 2015). Esses materiais consistem em uma matriz polimérica, geralmente um dimetacrilato, partículas de carga silanizadas e substâncias que promovem ou modulam a reação de polimerização. O monômero de base da matriz orgânica é o bisfenol-A glicidil metacrilato (BisGMA), que devido à sua alta viscosidade é misturado com outros dimetacrilatos, como dimetacrilato de trietileno glicol (TEGDMA) e dimetacrilato de uretano (UDMA) (FERRACANE, 2011).

Estudos indicam que componentes individuais das resinas compostas (como TEGDMA e BisGMA) são capazes de lesionar diversos tipos celulares. Dependendo

do período de exposição, esses componentes induzem a apoptose ou necrose em células humanas primárias, podem suprimir a diferenciação de células pulpares humanas e interferir na liberação de IL-1β e TNF-α (BOLLING *et al*., 2013; SPAGNUOLO *et al*., 2004). Kuan *et al*., (2012) afirmaram que o BisGMA, de maneira dose dependente, apresentou citotoxicidade e capacidade de estimular a produção de fator de necrose tumoral (TNF-α), a liberação das citocinas IL-1β e IL-6 e a produção de óxido nítrico e derivados do oxigênio.

A IL-1β é uma citocina que atua diretamente na reabsorção óssea e na inibição da formação óssea. Em conjunto com outros mediadores inflamatórios têm um papel importante na regulação da resposta inflamatória dos tecidos periodontais (BORONAT-CATALÁ, *et al*., 2014). Boronat-Catalá, *et al*., (2014) afirmaram que há evidência suficiente de que a citocina IL-1β na saliva ou fluido crevicular gengival pode ser usada como marcador para o grau de inflamação da gengivite. A citocina IL-8 é uma quimiocina liberada por monócitos, macrófagos, fibroblastos, queratinócitos e células endoteliais, sendo que o aumento de IL-8, juntamente com IL-6 e TNF-α foram detectados em pacientes expostos a materiais restauradores (CELIK, *et al*., 2003). Entretanto, poucos estudos clínicos foram realizados utilizando sistemas adesivos ortodônticos, principalmente em crianças.

Em vista da importância da biocompatibilidade dos materiais resinosos à base de metacrilatos aplicados para a colagem dos acessórios ortodônticos, faz se necessário melhor compreensão de sua liberação (GUPTA *et al*., 2012; VAN LANDUYT *et al*., 2011). Esses materiais são susceptíveis a degradação quando aplicados clinicamente e, por consequência, pode ocorrer liberação de subprodutos no meio bucal (FERRACANE, 1994; FERRACANE, 2011; POLYDOROU *et al*., 2007; ZIMMERMAN-DOWNS *et al*., 2010). Os principais monômeros que compõem a matriz resinosa e estão susceptíveis a liberação no meio bucal são o BisGMA, o TEGDMA e UDMA (FERRACANE, 2011).

Há relatos na literatura de que a liberação desses monômeros são potencialmente perigosos (GUPTA *et al*., 2012; VAN LANDUYT *et al*., 2011), com efeitos em nível local na mucosa bucal, gengiva e polpa dentária (PUTZEYS *et al*., 2017) e em níveis sistêmicos (SCHWENGBERG *et al*., 2005). Os produtos da decomposição do BisGMA são capazes de simular a função do estrogênio no

organismo (GAO *et al*., 2015), além de existir estudos associando a exposição desses compostos com a maturação sexual precoce em crianças (HOWDESHELL *et al*., 1999), infertilidade (AL-HIYASAT *et al*., 2006) aumento do risco de câncer de mama e próstata (MAFFINI *et al*., 2006) e alterações nas funções imunológicas (SAWAI *et al*., 2003). Além disso, esses produtos são citotóxicos para diversos tipos celulares, incluindo fibroblastos gengivais e queratinócitos humanos e são capazes de interferir na proliferação e migração celular (ISSA *et al*., 2004; THEILIG *et al*., 2000).

Apesar de existirem muitos estudos avaliando a presença de interleucinas na movimentação dentária ativa e a liberação de monômeros a partir de resinas utilizadas na colagem de acessórios ortodônticos, poucos trabalhos tem dado atenção à presença dos marcadores biológicos no tratamento ortodôntico passivo nos períodos iniciais de movimentação. Não há nenhum trabalho que avalie o papel das interleucinas no tratamento de mordida aberta anterior com auxílio dos esporões. Além disso, não há relato na literatura de análise da liberação de monômeros em crianças que apresentam essa alteração.

2 OBJETIVOS

2.1. Objetivos gerais

Avaliar os efeitos da colagem de esporão lingual em crianças com mordida aberta anterior na liberação de biomarcadores inflamatórios no fluido crevicular gengival e a liberação de subprodutos resinosos na saliva desses pacientes.

2.2. Objetivos específicos

1) Identificar e quantificar a presença de citocinas inflamatórias IL-1β, IL-6, IL-8, IL-10, IL-12p70 e TNF no fluido crevicular gengival dos incisivos superiores e inferiores nos períodos de 0 hora, 24 horas e 7 dias após a colagem dos esporões linguais.
2) Correlacionar a resposta clínica periodontal com a expressão de interleucinas no fluido crevicular.
3) Identificar e quantificar a liberação de BisGMA e TEGDMA na saliva nos tempos de 0 hora, 30 minutos, 24 horas e 7 dias após a colagem dos esporões linguais nestes pacientes.
4) Avaliar *in vitro* a migração e viabilidade de quetarinócitos humanos expostos ao meio condicionado com o sistema resinoso em concentrações similar ao encontrado clinicamente.

3 METODOLOGIA EXPANDIDA

3.1. Desenho do estudo

A pesquisa corresponde a um estudo clínico longitudinal prospectivo, simples cego, quali- e quantitativo, *in vivo.* Os procedimentos clínicos foram executados na Clínica de Ortodontia e as análises experimentais no Laboratório de Patologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais (UFMG), na Fundação Oswaldo Cruz (FIOCRUZ), no Laboratório de cultura de células da Universidade Federal de Minas Gerais (UFMG) e Instituto de Química da Universidade de Campinas (UNICAMP).

3.2. Considerações éticas

O projeto foi submetido e aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais (Resolução CNS 466/2012 - CAAE: 87714218.0.0000.5149), além de ser registrado na base do Registro Brasileiro de Estudos Clínicos (REBEC). A população do estudo foi selecionada na clínica de Ortodontia da Faculdade de Odontologia da Universidade Federal de Minas Gerais através de triagem. Foram realizados exame clínico, anamnese e solicitação de documentação ortodôntica. Todos os procedimentos foram executados no mesmo local e por operadores previamente treinados (Kappa Interexaminador: 0,83 Intraexaminador: 0,91). Foram selecionadas crianças de 6 a 11 anos de idade, independentemente do sexo, cuja necessidade de tratamento se enquadrava nos critérios de inclusão. Os participantes receberam os devidos esclarecimentos e explicação sobre os objetivos do estudo junto com os responsáveis. Aqueles que decidiram participar do estudo assinaram o Termo de Consentimento Livre e Esclarecido (TCLE)(ANEXO 1) e Termo de Assentimento Livre Esclarecido (TALE) (ANEXO 2).

3.3 Materiais utilizados

O sistema resinoso utilizado para a colagem dos acessórios foi Transbond XT (3M Unitek, Monrovia, Califórnia)(FIGURA 1). Para a realização do condicionamento

ácido foi utilizado o ácido fosfórico 37% (Fusion-Duralink, Angelus, Brazil)(Tabela1). Todos os procedimentos de fotoativação foram realizados utilizando uma lâmpada LED (Bluephase N, Ivoclar Vivadent Inc., Amherst, NY, EUA. 1000mW/cm^2).

Tabela 1 – Materiais utilizados no estudo

Material (Fabricante)	Composição básica	Instruções do fabricante
(Fusion-Duralink, Angelus, Brazil)	Solução aquosa de ácido fosfórico 37%; sílica coloidal	Condicionar o esmalte por 30 segundos, lavar e secar.
3M Unitek Transbond XT Primer (Figura 1B)	Bisphenol a diglycidyl ether dimethacrylate (45 – 55 %), Triethylene glycol dimethacrylate (45 – 55), Ttriphenylantimony (< 1%), 4-(dimethylamino)-benzeneethanol (< 0.5%), dl-camphorquinone (< 0.3%), Hydroquinone (< 0.03%) .	Secar o dente completamente com jato de ar. Aplicar uma pequena quantidade de primer Transbond XT. Aplicar uma camada uniforme de primer na superfície do dente a receber o aparelho.
3M Unitek Transbond XT Light Cure Adhesive (Figura 1 A)	Silane treated quartz (70-80%), Bisphenol a diglycidyl ether dimethacrylate (10-20%), Bisphenol a bis(2-hydroxyethyl ether) dimethacrylate (5-10%) , Silane treated silica (< 2 %)	Aplicar uma pequena quantidade de Transbond XT na base do bráquete. Use moderadamente. Limpar a ponta da seringa e recolocar a tampa.

Fonte: Do autor, 2020

Figura 1 – Resina 3M Unitek Transbond XT

(A = Light Cure Adhesive) (B = Primer)

Fonte: Do autor, 2020

3.4. Ensaio Clínico

3.4.1. Cálculo amostral

O cálculo amostral foi realizado de acordo com a fórmula descrita em Sampaio (2007), cap. 5 e p. 32-34, segundo a qual número de amostras por tratamento é (r)= (t.S/IC)^².

Em que t é um valor tabelado (em função do grau de liberdade dos tratamentos e probabilidade do erro tipo I - usual 1,96 para 90%). S é o desvio padrão que foi obtido no artigo (BASARAN, *et al*., 2006) no qual foi selecionada a dose de IL-6 no período de 6 meses (= 0,528). A média (x) foi obtida no artigo e foi usada para calcular o IC conforme calculo abaixo. (média dos níveis de IL-6 no período de 6 meses = 2,32).

Dessa forma:

IC= intervalo de confiança (10% - calculado com base na média e margem de erro de 2% sobre a média = (x+5%)-(x-5%).

IC= (2,32+5%)-(2,32-5%) = 2,436 - 2,204 = 0,232

(r)= (t.S/IC)^².

r = (1,96 x 0,528/0,232)²= r ˜19,89 crianças, o valor final foi arredondado para 20 participantes.

3.4.2. Seleção dos participantes

Foram selecionados participantes apresentando os seguintes critérios:

- Critérios de inclusão:

Foram incluídos no estudo indivíduos: sistemicamente saudáveis; com boa higiene bucal; com presença de mordida aberta anterior; periodonto normal: os parâmetros periodontais adotados para avaliação do estado de normalidade serão aqueles estabelecidos por Lindhe, Lang e Karring (2014) e Carranza e Sznajder

(1996). Estes parâmetros são Profundidade Sondagem (PS), Índice de Placa Visível (IPV), e Índice de Sangramento à Sondagem (SS).

- Critérios de exclusão*:*

Foram excluídos do estudo indivíduos: com alteração sistêmica grave; em uso de antibióticos e anti-inflamatórios nos últimos três meses; com periodonto anormal; que realizaram restaurações nos últimos 12 meses; sítios periodontais que apresentaram sangramento durante a coleta do fluido crevicular ou sítios que impedirem a coleta adequada dos parâmetros clínicos.

3.4.3. Equipe de trabalho

A equipe foi composta por dois pesquisadores examinadores que ficaram encarregados de selecionar os pacientes e um pesquisador operador treinado e calibrado (Kappa Interexaminador: 0,83 Intraexaminador: 0,91) que foi responsável por treinar a higienização dos pacientes, avaliar os dentes em estudo durante os 3 períodos de acompanhamento, executar a colagem dos esporões e coletar os dados clínicos, saliva e fluido crevicular gengival.

3.4.4. Coletas de saliva e do fluido crevicular gengival

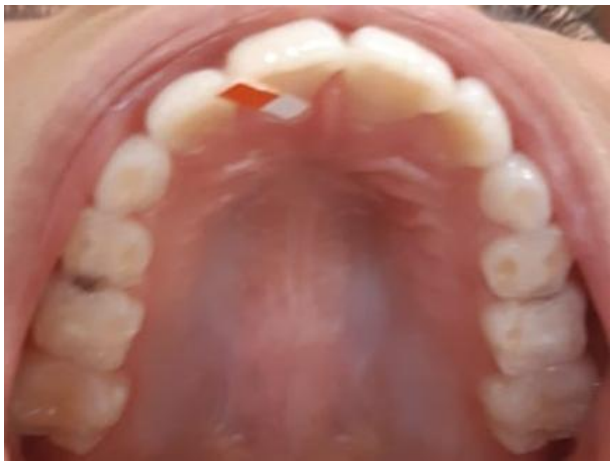
Foi coletado 1 mL de saliva não estimulada de cada voluntário no período da manhã. Aos pacientes foi recomendado permanecer sentado confortavelmente, com a cabeça levemente inclinada para baixo, deixando acumular saliva na boca, para, em seguida, coletá-la num frasco de vidro (Figura 2). A saliva coletada foi imediatamente armazenada em gelo e em seguida a -80ºC até o momento da análise.

Figura 2 – Frascos de armazenamento amostras de saliva.

Fonte: Do autor, 2020.

Para a coleta das amostras do fluido crevicular gengival, todo biofilme detectável clinicamente foi removido, sem tocar na gengiva para minimizar a contaminação dos filtros de papel absorvente com sangue. Depois de isolado com rolete de algodão, a gengiva foi delicadamente seca com seringa de ar e o fluido crevicular periodontal dos quatro incisivos inferiores e dos dois incisivos centrais superiores permanentes foi coletado utilizando uma fita de papel absorvente (Periopaper®, Oralflow, New York, NY, USA). Esta foi introduzida no sulco, por lingual, até encontrar resistência do tecido e mantido no local por 30 segundos (Figura 3), as amostra contaminada visivelmente com sangue foram descartadas. (ERTUGRUL *et al*., 2013; ENGEBRETSON *et al*., 2002; DEINZER *et al*., 2007 e ARIAANS *et al*., 2015). Os periopapers foram inseridos em eppendorfs estéreis e imersos em gelo até o seu armazenamento em freezer -80ºC para análise. As leituras do Periotron® 8000 (FIGURA 4) foram convertidas para o volume em microlitros empregando-se uma curva padrão de soro humano (GRÁFICO 1).

Figura 3 – Inserção do periopaper no sulco gengival



Fonte: Do autor, 2020.

Figura 4 – Materiais utilizados para coleta do fluido crevicular gengival

(A = Periotron® 8000) (B = Periopaper®) (C= Leitura do Periopaper®

Fonte: Do autor, 2020.

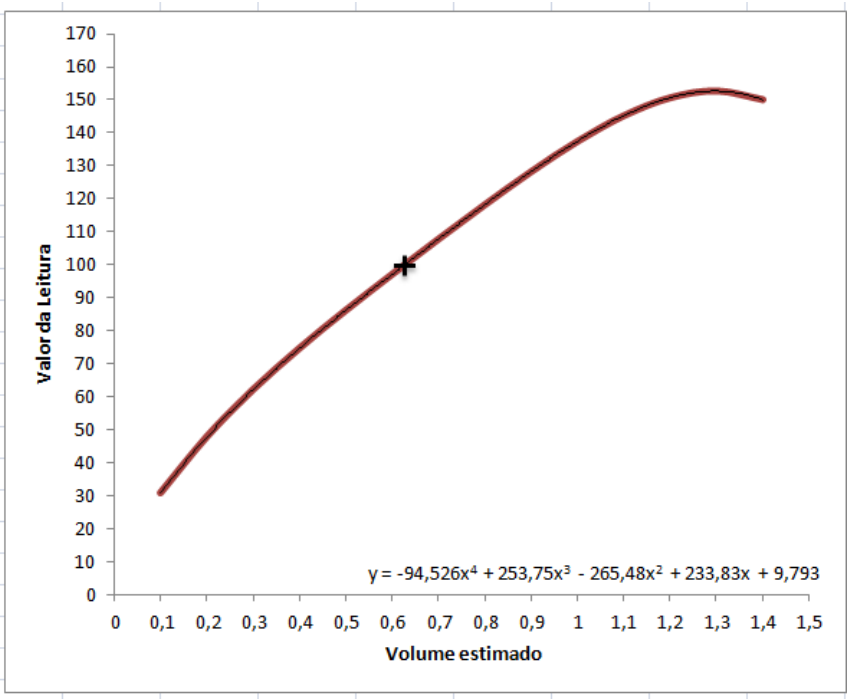


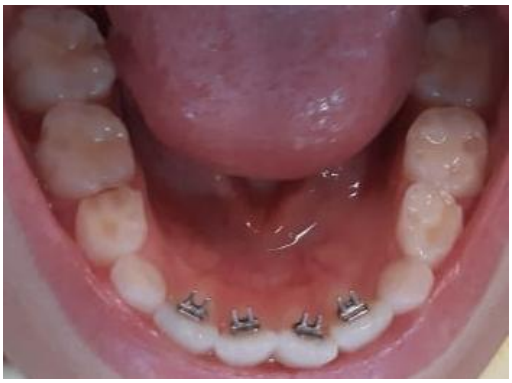
Gráfico 1 - Curva padrão de soro humano.
Fonte: Do autor, 2020.

3.4.5. Procedimentos operatórios

Para os pacientes selecionados, sete dias antes da colagem dos esporões foi executada profilaxia para remover a placa dentária visível e realizar instruções de higiene bucal. No dia da colagem dos esporões, foi realizada uma avaliação periodontal (controle inicial – baseline), seguida da coleta de saliva e fluido crevicular gengival dos quatro dentes que receberam a colagem e dos dois dentes superiores. Foi realizada a limpeza dentária desses dentes para retirar a película salivar e qualquer placa dental residual, seguido de lavagem e secagem. Em seguida foi realizado isolamento relativo, condicionamento ácido, aplicação do kit de sistema adesivo Transbond XT (3M, Unitek, Monrovia, CA, USA) e colagem dos esporões (Morelli, Sorocaba, SP, Brasil) na lingual dos incisivos centrais inferiores (FIGURA 5). A resina composta foi adicionada à base dos esporões, em seguida os esporões foram centralizados no terço médio da lingual dos incisivos inferiores. Foi realiazada pressão no centro dos esporões para que houvesse menor espessura da camada do sistema resinoso na base do acessório e os excessos foram removidos. Cada

acessório recebeu duas fotopolimerizações de 40 segundos, uma por lingual e um por incisal.

Figura 5 – Aspecto clínico após a colagem dos esporões



Fonte: Do autor, 2020.

3.4.6. Avaliação da resposta periodontal

Os parâmetros periodontais adotados para avaliação do estado de normalidade do periodonto foram aqueles estabelecidos por Lindhe, Lang e Karring (2014) e Carranza e Sznajder, 1996. A resposta periodontal inicial foi avaliada antes da colagem (*baseline*). Posteriormente, foram feitas avaliações da resposta periodontal 30 minutos, 24 horas e 1 semana após a colagem do esporão. Para a realização dos exames periodontais foi utilizado sonda milimetrada com marcação a laser. (Carolina do Norte -15- Hu Friedy). Para análise de sangramento gengival e profundidade de sondagem foram avaliados os quatros sítios dos elementos dentários (vestibular, lingual, mesial e distal). Já na análise do índice de placa visível foram avaliados os sítios vestibular e lingual.

3.5. Análise laboratorial

3.5.1. Análise de interleucinas no fluido crevicular gengival

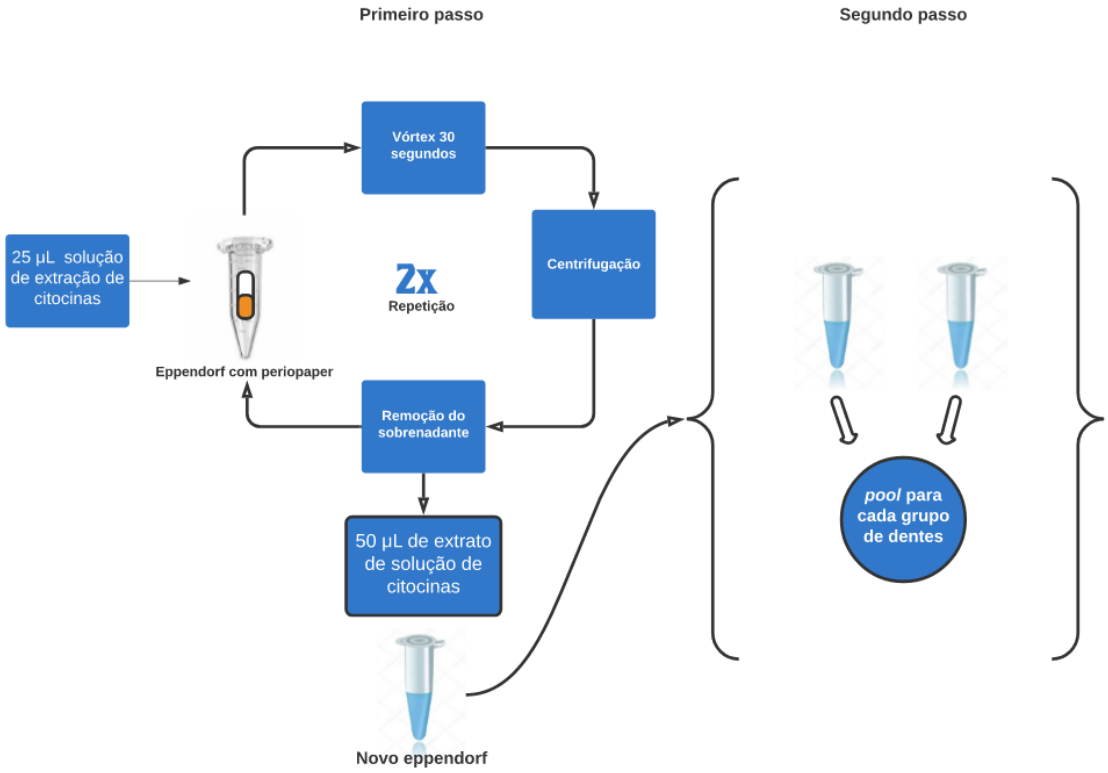
Quantificação do volume das amostras do fluido crevicular gengival (FCG):

O FCG foi quantificado no aparelho Periotron® 8000 (Oralflow, PlainView, New York, NY, USA) imediatamente após cada coleta, a fim de se obter maior

precisão. Em seguida, as fitas foram armazenadas em tubos de plástico do tipo Eppendorf apropriados e armazenadas a -80°C até serem analisadas. Para a determinação do volume do FCG foi realizada a calibração do Periotron.

Processamento laboratorial das amostras de FCG para análise de citocinas IL-1β, IL-6, IL-8, IL-12p70 e TNF:

A extração das amostras de fluido crevicular gengival foi realizada em dois passos. Foram adicionados 25 µL de solução de extração de citocinas [PBS (0,4 mM NaCl, 10 mM NaPO4) com inibidores de proteases (0,1 mM PMSF, 0,1 mM Benzethonium clorídrico, 10 mM EDTA e 0,01 mg/mL Aprotinina A, pH 7.4) e Tween 20 (0,05%)], em cada eppendorf contendo o periopaper. Em seguida os tubos foram levados ao vórtex por 30 segundos e depois centrifugados por 10 min em 10.000 RPM a 4ºC. O sobrenadante foi retirado e armazenado em um novo eppendorf e o mesmo processo foi repetido mais uma vez. No final um total de 50 µL foi obtido de cada periopaper, como mostra o esquema a seguir:



Fonte: Do autor, 2020.

Os sobrenadantes foram unidos em um único eppendorf e passados em vortex. Foi realizado um pool para os grupos de dentes superiores e inferiores de todos os tempos de coleta. Em seguida, foram separados 50 µL do sobrenadante para análise de citocinas por CBA e as demais amostras armazenadas em freezer a -80ºC.

As análises do FCG foram determinadas utilizando o kit BD™ CBA Human Inflammatory Cytokines Kit para humano (BD Biosciences, San Diego, CA, EUA) e analisados em BD FACSVerse Flow Cytometer (Becton Dickinson, San Jose, CA, EUA), seguindo as orientações do fabricante.

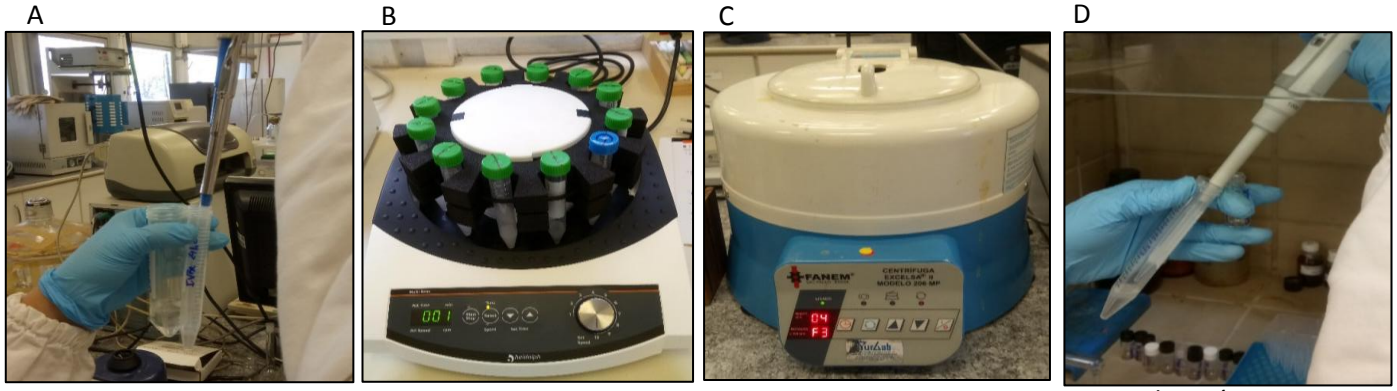
3.5.2. Análise das amostras de saliva quanto a presença de metacrilatos

Preparo das amostras:

O procedimento de preparo de amostra foi adaptado de Michelsen *et al*., (2012) e realizado da seguinte forma:

1 mL da amostra e 1 mL de acetato de etila grau HPLC foram transferidos para um tubo Falcon® de 15 mL (FIGURA 6A). Agitou-se o tubo por 1 minuto em vórtex (Figura 6B) e centrifugou-se por 4 minutos a 3000 rpm (FiGURa 6C). Retirou-se 0,5 mL do sobrenadante e transferiu-se para um frasco de vidro (FIGURA 6D). Repetiu-se esse procedimento 3 vezes. Ao final, obteve-se 1,5 mL do extrato em acetato de etila, evaporou-se sob fluxo de nitrogênio esse volume e ressuspendeu-se o extrato seco em 0,5 mL de fase móvel. A amostra foi filtrada em filtro de seringa de 0,45 µm para um vial e analisada via HPLC-MS/MS.

Figura 6 – Preparo das amostras de saliva para análise no HPLC



Fonte: Instituto de Química UNICAMP, 2020.

Legenda: A - Transferência das amostras para tubo Falcon. B - Vórtex utilizado para preparo das amostras. C - Processo de centrifugação das amostras D - Transferência para o frasco de vidro.

Preparo dos padrões:

Foram preparadas soluções estoque dos padrões de BIS-GMA e TEG-DMA (SIGMAALDRICH, St. Louis, EUA), em acetonitrila grau HPLC, na concentração de 10,0 µg/mL e a partir dessas, realizou-se as seguintes diluições para se obter as soluções de trabalho:

Tabela 2 – Diluições Bis-GMA e TEG-DMA

BIS-GMA (µg/mL)	TEG-DMA (µg/mL)
0,10	0,10
0,25	0,25
0,50	0,50
0,75	0,75
1,00	1,00

Fonte: Instituto de Química UNICAMP, 2020.

Estas soluções foram filtradas em filtro de seringa de 0,45 µm para um vial e injetadas no HPLC-MS/MS. Construiu-se a curva analítica utilizada para a quantificação das amostras:

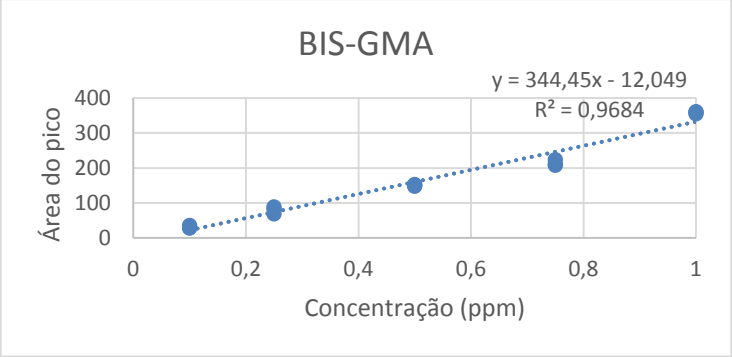


Gráfico 2 - Curva analítica do BIS-GMA.

Fonte: Instituto de Química UNICAMP, 2020.

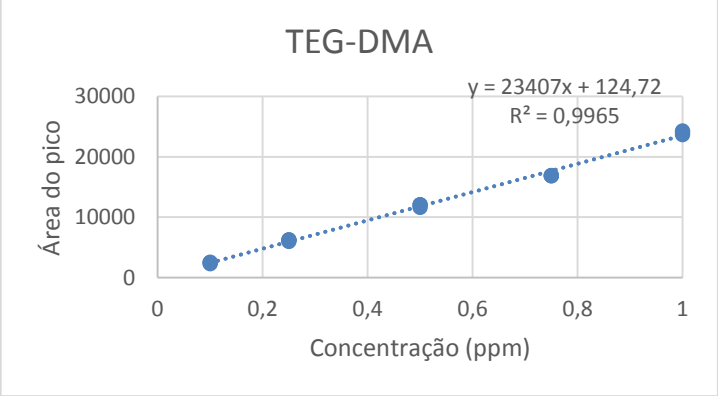


Gráfico 3 - Curva analítica do TEG-DMA.

Fonte: Instituto de Química UNICAMP, 2020.

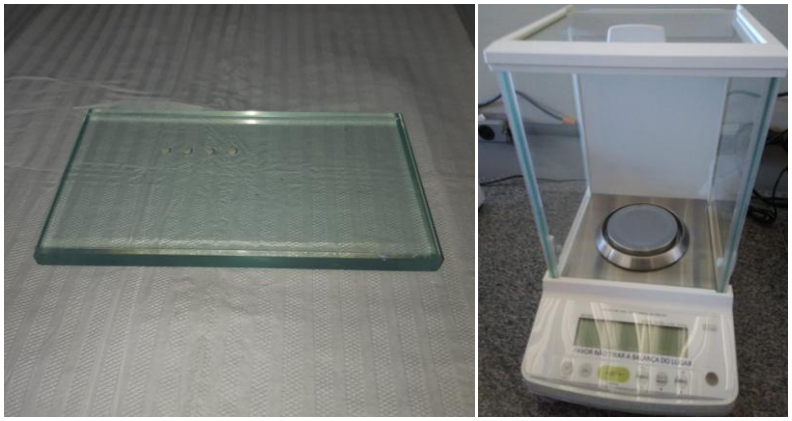
O método cromatográfico utilizado foi através de coluna NovaPack C18 Waters 3,9 mm x 150 mm x 4 µm com modo de eluição isocrático, volume de injeção 10 µL e vazão 0,5 mL/min e para a fase móvel acetonitrila foi utilizado acetato de amônio 10 mM 65:35 (v/v).

As condições do espectrômetro de massas foram BIS-GMA (modo MRM): Íon precursor (m/z) 513,19 Da, íon filho (m/z) 143,0 Da, voltagem do cone 35 V e energia de colisão 18 eV. Para TEG-DMA (modo MRM): Íon precursor (m/z) 287,10 Da, íon filho (m/z) 112,9 Da, voltagem do cone 20 V e energia de colisão 12 eV.

3.6. Ensaio laboratorial *in vitro*

Foram realizados testes de cultura celular para avaliar a migração e viabilidade celular na presença de subprodutos da resina ortodôntica. Para isso foi realizado a padronização de quatro incrementos de resina (Figura 7) com balança de precisão (7,5 mg para cada incremento) e, após a polimerização (40s), foram adicionados à 3 diluições diferentes de meio de cultivo celular por 30 minutos, à 37ºC. Segundo Moura *et al*., (2008) o fluxo salivar de uma criança até 12 anos de idade é de aproximadamente 0,7366 mL/min. Os incremento foram adicionados a 3 diluições diferentes, considerando o fluxo salivar e tempo de incubação de 30 min. Para avaliar o efeitos de diferentes concentrações as diluições foram realizadas da seguinte maneira, uma com o volume de 5,5 ml (Diluição 1 -D1), outra com 11 ml (Diluição 2 - D2) e outra com o volume de 22 ml (Diluição 3 - D3). Sendo esta última referente ao volume salivar produzido por crianças de 6 – 12 anos no período de 30 minutos. As outras diluições testadas tiveram o objetivo de aumentar a concentração dos subprodutos.

Figura 7 – Preparo para teste de cultura celular.



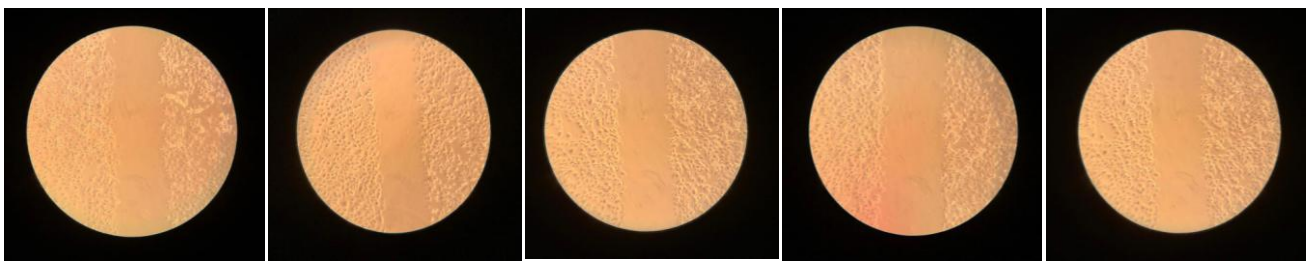
(A = Incrementos de Resina) (B = Balança de precisão)

Fonte: Do autor, 2020.

Os queratinócitos (HaCat) foram plaqueados em quadruplicata à densidade de 1 x 10^4 células/poço em placas de 96 poços para o ensaio de Viabilidade MTT {brometo de [3-(4,5-dimetiltiazol-2yl)-2,5-difenil tetrazolium]} no qual foram avaliados os tempos de 24h, 48h e 120h. Além disso, queratinócitos (HaCat) foram plaqueados em triplicata à densidade de 5 x 10^5 células/poço em placas de 6 poços para o teste de migração - Cell Scratch – e avaliados nos tempos de 0h, 24h e 48h. Após 24h, as três diluições de meio de cultura foram colocados em contato com as células. Um grupo cultivado em condições ideais foi utilizado como controle.

No tempo 0h do ensaio de migração foi realizada uma ferida com ponteira P200 em cada poço. Foram feitas 5 imagens (FIGURA 8) de cada grupo e tempo experimental para quantificação da área de fechamento no software Image J (National Institute of Health, Bethesda, MD, USA).

Figura 8 – Fotos realizadas no tempo 0 horas



Fonte: Do autor, 2020.

3.7. Análise estatística

Os resultados foram expressos como média ± desvio padrão (DP). Os conjuntos de dados apresentaram distribuição não normal (teste de normalidade omnibus de D'Agostino & Pearson e teste de normalidade de Shapiro-Wilk). Na análise de interleucinas as diferenças entre os grupos foram analisadas pelo teste não paramétrico de Wilcoxon em cada ponto no tempo. O teste de Mann-Whitney foi utilizado para verificar a diferença entre os incisivos inferiores e superiores. Na análise dos metacrilatos as diferenças entre os grupos foram analisadas pelo teste não-paramétrico de Kruskal-Wallis, seguido pelo teste de comparação múltipla de Dunn. As diferenças entre os grupos nos experimentos *in vitro* foram analisadas pela análise de variância bidirecional seguida pelo teste post-hoc de Bonferroni. As correlações foram determinadas usando o teste de correlação de Pearson. $P < 0,05$ foi considerado estatisticamente significante.

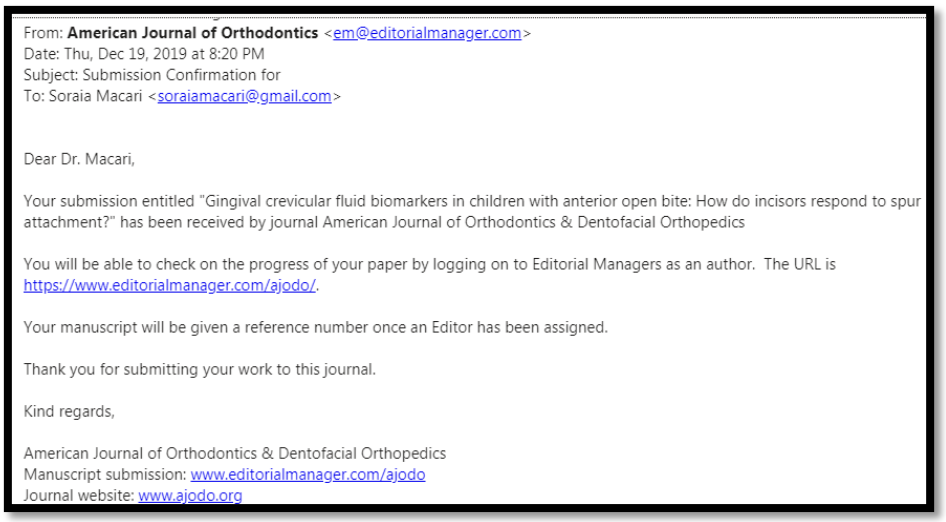
4 RESULTADOS

Os resultados e discussão serão apresentados na forma de dois artigos científicos.

4.1. Artigos

4.1.1 Artigo 1 – Resposta ao objetivo específico 1

- Artigo submetido ao periódico “American Journal of Orthodontics” (Qualis A1). O trabalho está sobre revisão da revista e as correções e sugestões da banca serão adicionadas. O texto se encontra no formato padronizado pela revista.



From: **American Journal of Orthodontics** <em@editorialmanager.com>
Date: Thu, Dec 19, 2019 at 8:20 PM
Subject: Submission Confirmation for
To: Soraia Macari <soraiamacari@gmail.com>

Dear Dr. Macari,

Your submission entitled "Gingival crevicular fluid biomarkers in children with anterior open bite: How do incisors respond to spur attachment?" has been received by journal American Journal of Orthodontics & Dentofacial Orthopedics

You will be able to check on the progress of your paper by logging on to Editorial Managers as an author. The URL is https://www.editorialmanager.com/ajodo/.

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

Thank you for submitting your work to this journal.

Kind regards,

American Journal of Orthodontics & Dentofacial Orthopedics
Manuscript submission: www.editorialmanager.com/ajodo
Journal website: www.ajodo.org

Gingival crevicular fluid biomarkers in children with anterior open bite: How do incisors respond to tongue spur attachment?

Gabriel Antônio dos Anjos Tou[1], Marcus Vinicius Lucas Ferreira[1], Ricardo Alves de Mesquita[2], Tarcília Aparecida Silva[2], Soraia Macari[1]

[1] Department of Restorative Destistry, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

[2] Department of Oral Pathology and Surgery, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Corresponding author: Soraia Macari. Mailing address: Departamento de Odontologia Restauradora, Faculdade de Odontologia, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos 6627, CEP 31.270-901, Belo Horizonte, Minas Gerais, Brasil. E-mail: soraiamacari@gmail.com.

Declarations of interest: none

ABSTRACT

Introduction: The anterior open bite is a malocclusion characterized by the lack of contact between the dental arches. The use of a passive orthodontic appliance, spur, is a well-accepted method used in children. Despite the orthodontic tooth movement induced by mechanical load can increase the concentration of interleukins in gingival crevicular fluid (GCF), no studies showing the effects of passive accessories are reported. The purpose of this study was to evalute GCF pro-inflammatory cytokines

in children with anterior open bite receiving spur attachment as orthodontic treatment. **Methods:** Twenty children were included in this study. GCF samples were collected before spur attachment (baseline), 24 hours (h) and 7 days (d) after bonding the lower and upper central incisors. Cytokines analysis was performed using the cytometric bead array. **Results:** 24 h and 7 days after the spur attachment, IL-8 levels in the upper incisors were enhanced compared to baseline. At day 7, the concentration of IL-1β was enhanced compared to baseline in both teeth groups. Comparing upper to lower incisors, levels of IL-8, IL-1β and IL-6 were higher in upper incisors at 24h. Cytokine production was not correlated to gingival bleeding, probing depth and visible plaque. **Conclusion:** Differential expression of IL-8, IL1β and IL-6 were observed in GCF in the upper and lower incisors of children with open bite using spur as orthodontic treatment.

Key words: Open bite. Cytokines. Spur. Biomarkers.

Highlights

1. Increased gingival crevicular fluid biomarkers were seen after 24 hours of spur bonding.
2. The upper incisors exhibited higher cytokines production compared to lower the incisors.
3. Spur attachment yield increased gingival bleeding at day 7 in the lower incisors.
4. Cytokines in GCF were not correlated to gingival bleeding, probing depth and visible plaque, regardless tooth position.

INTRODUCTION

The anterior open bite is a malocclusion characterized by the lack of contact between anterior teeth (Rosa, Quinzi *et al*., 2019) and have negative impact on children's oral health–related quality of life (Pithon, Magno *et al*., 2019). This change affects patients in the deciduous and mixed dentition, and has multifactorial origin with combination of skeletal, dental and soft tissue (Lambrechts, De Baets *et al.,* 2010).

The open bite can be associated with the breakdown of the peribucal and intraoral muscles balance caused by deleterious oral habits, such as prolonged digital and pacifier sucking habits resulting in tongue thrust during both rest and swallowing(Lambrechts, De Baets *et al*., 2010; Ling, Sum *et al.*, 2018; Rosa, Quinzi *et al.*, 2019). This specific malocclusion in the deciduous dentition may correct itself if the habit is removed until 4 years of age. From the age of 5, orthodontic interception is necessary, as lingual interposition between incisors is a harmful factor that endures malocclusion(Ruan, Chen *et al.*, 2005; Lambrechts, De Baets *et al*., 2010; Valentim, Furlan *et al.*, 2016; Gonzalez, MartInez *et al.*, 2019). Treatment consists essentially of the removal of deleterious habit and lingual interposition(Lambrechts, De Baets *et al.*, 2010). There are different ways to perform this intervention; among them is the use of orthodontic accessory called tongue spur, which is a passive orthodontic appliance. The spur´s main purpose is the tongue replacement backwards thus avoiding the tongue interposition between the lower and upper incisors(Dias, Assis Urnau *et al*., 2019). These devices adhere to teeth on the lingual face of incisors through acid etching, bonding systems and composite resins(Dias, Assis Urnau *et al.*, 2019). Without the presence of deleterious habit and tongue interposition, the lips musculature exerts force on the vestibular surface of the

incisors, stimulating bone remodeling and tooth eruption for open bite correction(Ruan, Chen *et al*., 2005; Ling, Sum *et al*., 2018).

The mechanical induced orthodontic tooth movement are mediated by interleukins yielded in the local microenvironment of the periodontal tissue, which can be released to the gingival crevicular fluid (Ren and Vissink 2008; Wise and King 2008; Brylka and Schinke 2019). These biological markers are usually expressed in response to local factors and can proceed locally as autocrine or paracrine intercellular signals in adjacent tissues (Ren and Vissink 2008; Smuthkochorn, Palomo *et al*., 2017). The presence of cytokines in gingival crevicular fluid (GCF) is useful for monitoring teeth response to orthodontics as it reflects the microenvironment where forces are exerted (Tuncer, Ozmeric *et al.*, 2005; Madureira, da Silva *et al.*, 2015).

Studies have observed differences in the concentration of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, in GCF during active orthodontic movement and identified the increase of these components in the initial periods of tooth movement(Uematsu, Mogi *et al*., 1996; Tuncer, Ozmeric *et al.*, 2005; Basaran, Ozer *et al*., 2006; Dudic, Kiliaridis *et al.*, 2006; Teixeira, Khoo *et al.*, 2010; Grant, Wilson *et al.*, 2013; Madureira, da Silva *et al.*, 2015; Bergamo, Nelson-Filho *et al.*, 2016). However, few studies have analyzed the presence of biological markers in passive orthodontic treatment in the initial periods of movement and there is none study evaluating the role of cytokines in the treatment of anterior open bite with the aid of spurs. The purpose of this study was to investigate GCF cytokine expression in response of tongue spur attachment in children with anterior open bite.

MATERIAL AND METHODS

The study group consisted of 20 patients based on the following inclusion criteria: presence of anterior open bite; oral hygiene and periodontal health. The exclusion criteria were occurrence of systemic diseases, use of antibiotics and anti-inflammatory drugs in the last three months, bleeding during the collection of crevicular fluid and use of any orthodontic accessory. The consent form was collected from all participants. The study was approved by the Research Ethics Committee of the Federal University of Minas Gerais (CAAE: 87714218.0.0000.5149).

The periodontal parameters recorded were probing depth, percentage of visible plaque and probing bleeding(Madureira, da Silva et al. 2015).

Clinical examination and device bonding was performed by a single trained and calibrated researcher (Kappa Inter-examiner = 0.91 and Intra-examiner = 0.83). Seven days before the first day of sample collection, the participants received hygiene instructions and dental prophylaxis was performed. Periodontal evaluation and crevicular fluid collection were performed before the appliance was placed (baseline) and within 24 hours (24h) and 7 days (7d) after the device bonding.

Relative isolation was performed, phosphoric acid (Fusion-Duralink, Angelus, Londrina, Brazil) was applied for 30 seconds on the enamel surface, application of the Transbond XT adhesive system kit (3M, Unitek, Monrovia, California, USA) according to the manufacturer's recommendations , and the bonding of the spurs (Morelli, Sorocaba, SP, Brazil) were performed on the lingual surface of the lower incisor, applying pressure in the center of the accessory so that the composite resin could leak out on the spur sides, these excesses were removed. Two 40-second

photoactivations were performed with an LED lamp (Bluephase N, Ivoclar Vivadent Inc., Amherst, NY, USA. 1000mW / cm2), one by lingual and the other by incisal.

Gingival crevicular fluid (GCF) samples were collected from the lower (31 and 41) and upper (11 and 21) central incisors. All clinically detectable biofilm was removed without touching the gum to minimize contamination of the absorbent paper filters with blood. After being isolated with a cotton roller, the gum was air-dried and periodontal crevicular fluid collected using an absorbent paper tape (Periopaper®, Oralflow, New York, NY, USA). This was introduced into the gingival sulcus by lingual until it found tissue resistance and held in place for 30 seconds; samples visibly contaminated with blood were discarded. The GCF was quantified on the Periotron® 8000 apparatus (Oralflow, PlainView, New York, NY, USA) immediately after each collection in order to obtain greater accuracy. The periopapers were inserted into sterile eppendorfs and immersed in ice until stored in -80°C freezer for further analysis. Periotron® 8000 readings were converted to volume in microliters using a standard human serum curve.

Extraction of gingival crevicular fluid samples was performed in two steps. 25 µL of cytokine extraction solution was added [PBS (0.4 mM NaCl, 10 mM NaPO4) with protease inhibitors (0.1 mM PMSF, 0.1 mM Hydrochloric Benzethonium, 10 mM EDTA and 0.01 mg / mL Aprotinin A, pH 7.4) and Tween 20 (0.05%)], in each eppendorf containing the periopaper. The tubes were then vortexed for 30 seconds and then centrifuged for 10 min at 10,000 RPM at 4°C. The supernatant was removed and stored in a new eppendorf and the same process repeated one more time. At the end a total of 50 µL was obtained from each periopaper. The supernatants total volume were added to a single eppendorf and vortexed. A pooled sample was performed for the upper and lower incisors teeth groups for each of the

collection times and 50 µL of the supernatant was separated for CBA cytokine analysis and the other samples stored in -80ºC freezer(Madureira, da Silva et al. 2015).

GCF analysis were determined using the BD ™ CBA Human Inflammatory Cytokines Human Kit reference number 551811 (BD Biosciences, San Diego, CA, USA) and analyzed on BD FACSCalibur ™ flow cytometer (Becton Dickinson, San Jose, CA, USA) following manufacturer's guidelines. This kit is able to measure interleukin 8 (IL-8) protein levels, interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor (TNF) and 12p70 interleukin (IL-12p70) in a single human sample. The values of cytokines concentration in GCF were corrected by total volume of fluid obtained.

The results were expressed in picograms per milliliter (pg/mL).

Statistical analysis

Results were expressed as mean ± standard deviation (S.D.). Data sets presented a non-normal distribution (D'Agostino & Pearson omnibus normality test and Shapiro-Wilk normality test). The differences among groups were analyzed by Wilcoxon nonparametric test at each timepoint. The Mann-Whitney test was used to verify the difference between lower and upper incisor. Correlations were determined using Pearson's correlation test. $P < 0.05$ was considered statistically significant.

RESULTS

Spur attachment yield increase gingival bleeding at day 7 in the lower incisors

Twenty children (mean age 8.85 years-old) with anterior open bite were included in the study. Demographic information is shown in Table I. The appliances

were well tolerated. The spur attachment did not yield changes in the probing depth, visible plaque and in the volume of the gingival crevicular fluid at the different timepoints (baseline, 24 hours and 7 days) (Table I). On the other hand, except by the increase of gingival bleeding in the lower incisors in comparison to baseline in the same teeth group and compared to the upper incisors at day 7, no difference was verified in the gingival bleeding among the groups considering the timepoints and teeth group (Table I). There was a significant positive correlation between the gingival bleeding and the visible plaque at day seven in the lower incisors (Table II). Visible plaque was also positively correlated to the volume of gingival crevicular fluid only 24 hours after the spur bonding in the upper and lower incisors (Table II).

Table I. Demographic distribution and clinical data of the patients. Values are mean ± S.D.; $p<0.05$; letters means difference between timepoints; *different from lower incisors

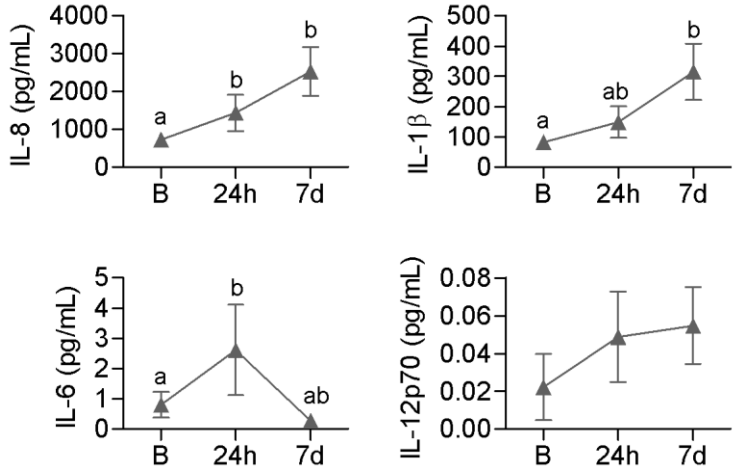
Timepoint	Age (years)	Gender	N	Probing depth (mm)		Gengival bleeding (%)		Visible plaque (%)		GCF volume (µL)	
				Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Baseline	8.85 ± 1.19	7 M / 13 F	20	1.29 ± 0.24	1.48 ± 0.28	0 ± 0[a]	0 ± 0	33.1 ± 0.34	17.5 ± 0.28	0.13 ± 0.21	0.14 ± 0.17
24h			20	1.3 ± 0.23	1.51 ± 0.29	1.2 ± 0.03[ab]	0.6 ± 0.02	28.7 ± 0.36	17.5 ± 0.28	0.19 ± 0.22	0.15 ± 0.18
7d			20	1.35 ± 0.21	1.55 ± 0.34	2.2 ± 0.04[b]	0 ± 0*	33.7 ± 0.36	18.7 ± 0.29	0.26 ± 0.24	0.18 ± 0.17

Table II. Correlation between the clinical data at the different timepoints baseline, 24 hours (h) and 7 days (d). Pearson's correlation test.* $p < 0.05$ was considered statistically significant

	Clinical data											
	Probing depth (mm)				**Gingival bleeding (%)**				**GCF volume (µL)**			
	Lower Incisors		**Upper Incisors**		**Lower Incisors**		**Upper Incisors**		**Lower Incisors**		**Upper Incisors**	
	r^2	P	r^2	P	r^2	P	r^2	p	r^2	p	r^2	p
Probing depth (mm)												
Baseline	-	-	-	-	-	-	-	-	-	-	-	-
24 hours	-	-	-	-	-	-	-	-	-	-	-	-
7 days	-	-	-	-	-	-	-	-	-	-	-	-
Gingival bleeding (%)												
Baseline	0,006062	0,5543	0,0001	0,9568	-	-	-	-	-	-	-	-
24 hours	0,01056	0,6664	0	0,9667	-	-	-	-	-	-	-	-
7 days	0,03838	0,4078	0,0008	0,9492	-	-	-	-	-	-	-	-
Visible Plaque (%)												
Baseline	0,005485	0,7563	0,00049	0,9257	0,04472	0,1048	0,02245	0,2795	0,1074	0,1583	0,01637	0,5909
24 hours	0,01166	0,6505	0,00006	0,9736	0,00561	0,7535	0,0362	0,2965	**0,4591**	**0.001***	**0,5129**	**0.0004***
7 days	0,007246	0,7212	0,03391	0,4371	**0,296**	**0.0131***	0,07111	0,2698	0,00194	0,8538	0,00808	0,7062
GCF volume (µL)												
Baseline	0,001675	0,864	0,00698	0,7262	0,000560	0,8567	0,00042	0,8759	-	-	-	-
24 hours	0,04415	0,3739	0,06039	0,2963	0,08575	0,2102	0,00173	0,8614	-	-	-	-
7 days	0,06666	0,2718	0,01228	0,6418	0,0437	0,3764	0,00562	0,8974	-	-	-	-

Global increased of cytokines were detected in GCF after 24 hour of spur bonding

After 24 hours of the spur attachment there was an increase of IL-8 and IL-6 levels in the GCF in the global incisors compared to baseline, while only IL-8 and IL-1β were progressively augmented at day 7 compared to baseline. Regardless the cytokines, no differences were seen comparing the 24 hours and 7 days time points. IL-12p70 concentration was not modified after spur attachment (Figure 1). The levels of IL-10 and TNF were not detected at any timepoint and group of teeth.



[

Figure 1. Time course of IL-8, IL-1β, IL-6 and IL-12p70 cytokines production in the GCF (n = 20 patients per timepoint) at the different timepoints baseline, 24 hours (h) and 7 days (d).. The data are expressed as mean ± SD. Letters means difference between timepoints. Wilcoxon nonparametric test was used at each timepoint. $p < 0.05$.

Upper incisors exhibited higher cytokines production than lower incisors

To better understand the effects of the attachment of the spur in the GCF during the orthodontic treatment of open bite in children we analyzed the levels of cytokines in the lower and upper incisors separately. After 24 hours of the spur attachment the levels of IL-8, IL-1β and IL-6 were increased in the GCF of the upper incisors in comparison to the lower incisors, while at day 7 no difference between the groups of teeth was found (Figure 2).

In addition, 24 hours after the spur attachment IL-8 levels in the upper incisors were enhanced compared to baselines levels but similar to 7 day (Figure 2). In the group 7 days after spur bonding the levels of IL-8 and IL-1β were augmented in the superior and inferior incisors when compared to baseline levels. In other hand, IL-1β and IL-6 levels in the upper incisor were similar and decreased compared to 24 hours, respectively (Figure 2). No statistical difference was verified in IL-6 levels in lower incisors and in IL-12p70 in lower and upper incisors along the different timepoints (Figure 2).

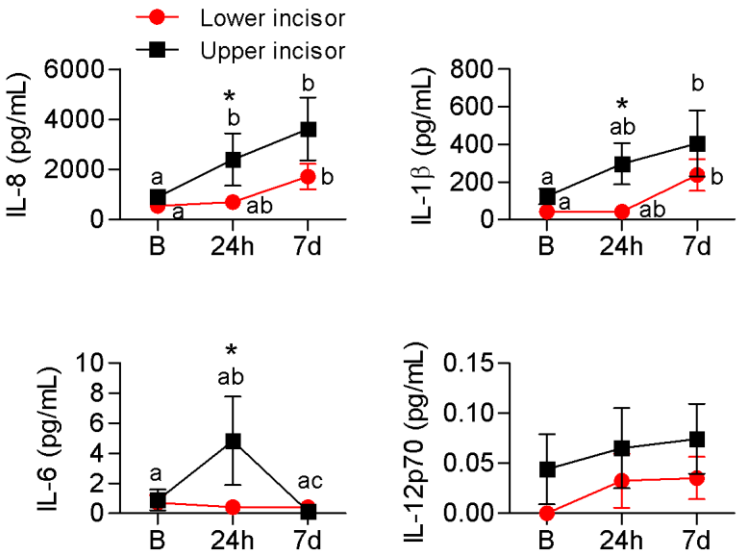


Figure 2. Cytokines levels in the GCF of lower and upper incisor. (n = 20 patients per timepoint) at the different timepoints baseline, 24 hours (h) and 7 days (d). The data

are expressed as mean ± standard deviation (SD). Letters means difference between timepoints. *different from lower incisors. Wilcoxon nonparametric test was used at each timepoint and Mann-Whitney test was used to compare differences between lower and upper incisors. $p < 0.05$.

Due to the statistical difference found in the gingival bleeding in the lower incisor at day 7, the correlation between all clinical in this period and the cytokines were performed. No correlation was found between the lower and upper incisors and the probing depth, gingival bleeding and visible plaque measurements (Table III). On the other hand, a positive correlation was verified between the levels of IL-8, IL-1β and IL-6 and the volume of gingival crevicular fluid (Table III).

Table III. Correlation between the cytokines level and the clinical data at day 7 after spur attachment. Pearson's correlation test.* p < 0.05 was considered statistically significant

	Interleukins versus clinical data															
	Probing depth (mm)				**Gingival bleeding (%)**				**Visible Plaque (%)**				**GCF volume (µL)**			
	Lower Incisor		**Upper Incisor**		**Lower Incisor**		**Upper Incisor**		**Lower Incisor**		**Upper Incisor**		**Lower Incisor**		**Upper Incisor**	
pg/mL	r^2	P	r^2	P	r^2	p	r^2	p	r^2	p	r^2	P	r^2	p	r^2	p
IL-8																
7 days	0,03142	0,4961	0,0132	0,6829	0,0285	0,5175	0,02077	0,6083	0,0745	0,2892	0,186	0,109	**0,7572**	**< 0.0001*** **	**0,7193**	**< 0.0001*** **
IL-1β																
7 days	0,03141	0,4962	0,00006	0,9774	0,012	0,6752	0,00141	0,8901	0,1016	0,2123	0,0357	0,5	**0,8017**	**< 0.0001*** **	**0,3654**	**0.017***
IL-6																
7 days	0,02907	0,4724	0,0915	0,1948	0,0246	0,5087	0,00626	0,7402	0,0036	0,8007	0,5233	0,023	**0,3368**	**0.0068***	**0,1291**	**0.0096****
IL-12p70																
7 days	0,04539	0,3671	0,1785	0,0635	0,034	0,4363	0,0861	0,9283	0,0983	0,1783	0,0228	0,525	0,0414	0,3898	0,0031	0,8157

DISCUSSION

This study analyzed the effect of a passive orthodontic appliance on the lower and upper incisors 24 hour and 7 days after its bonding. The main findings demonstrate timepoint fluctuations in the production of IL-8, IL-1β and IL-6. The levels of cytokines in GCF were higher in upper compared to lower incision at the same time point. Except by the volume of GCF, none other clinical feature were correlated to the clinical data analyzed.

The production of biomarkers in crevicular fluid during early periods of passive orthodontic tooth movement was evaluated in this study. Although treatment for anterior open bite with spurs is considered a passive movement(Dias, Assis Urnau *et al.*, 2019), the removal of deleterious oral habits allows the peribucal muscles to exert force on the teeth(Ruan, Chen *et al.*, 2005; Lambrechts, De Baets *et al.*, 2010) and thus induce bone remodeling and biomarker release(Ren, Hazemeijer *et al.*, 2007). In addition, with the use of orthodontic appliances attached to the teeth, it is possible to change the periodontal clinical parameters and, therefore, periodontal inflammation should be considered(van Gastel, Quirynen *et al.* 2011; Grant, Wilson *et al.*, 2013; Bergamo, Nelson-Filho *et al.* 2016).

Our findings showed that the increase in gingival bleeding rate after 7 days after bonding the orthodontic spur in the lower incisors was correlated to the increased plaque percentage. This result is consistent with the study conducted by Pejda *et al.*(Pejda, Varga *et al.* 2013), who identified a significant increase in gingival bleeding in patients treated with a fixed appliance after bracket bonding. Other studies confirm these findings by demonstrating that the presence of orthodontic accessories contributes to the worsening of periodontal clinical parameters, such as plaque percentage, which results in increased inflammation and gingival

bleeding(Naranjo, Trivino *et al*. 2006). The results found in this study demonstrate that even with the hygiene instruction carried out with the participants, there was a worsening of clinical parameters, this change may be related to the presence of the accessory as a physical barrier that hinders hygiene and facilitates the accumulation of plaque. This change may remain until the end of orthodontic treatment when accessories are removed(van Gastel, Quirynen *et al.* 2011).

Besides that, other findings in the literature have shown that in addition to gingival bleeding, plaque and crevicular fluid volume have increased in patients undergoing orthodontic treatment(Naranjo, Trivino *et al.* 2006; Bergamo, Nelson-Filho *et al.* 2016). Our results showed increase in dental plaque percentage is correlated to the increase in crevicular fluid volume 24 hours after spur attachment. Similar results were obtained by Grant et al.(Grant, Wilson *et al.* 2013) demonstrating the positive correlation between the plaque and the GFC volume in sites that suffered orthodontic forces action.

Although there is a relationship between increase in dental plaque in patients with orthodontic appliances and release of pro inflammatory markers(Grant, Wilson *et al.* 2013), no correlation was found in our results with the increase of the interleukin and the clinical data analyzed, reinforcing that changes in cytokines of GCF were directly related to spur attachment and not to an inflammatory process.

Several studies have also shown the presence of biomarkers in GFC during orthodontic movement due to the forces applied on the teeth (Tuncer, Ozmeric *et al.* 2005; Ren and Vissink 2008). In present study, was possible to identify the significant increase of IL-8, IL-6 and IL-1β over time. IL-8 showed a significant increase in 24 hours and 7 days, similar results were observed by Tuncer et al.(Tuncer, Ozmeric *et*

al. 2005) who identified, besides the increase in GFC volume, orthodontic movement was able to induce increased release of IL-8 in CFGs at 24 hours and 6 days.

The IL-6 release profile found in this study also agrees with other results in the literature (Uematsu, Mogi *et al*. 1996) in which an increase in IL-6 was identified in the first 24 hours after the application of orthodontic forces However, in our findings these values decreased significantly after 7 days. This behavior is probably related to the role of these interleukins in the initial moments of movement. Dudic *et al*.(Dudic, Kiliaridis *et al*. 2006) identified the presence of IL-1β in GFC after force application on the separator dental elements at 1 hour, 24 hours and 7 days, at all times the increase in IL-1β was significant when compared to the control. Other studies with force application through active movement also detected the presence of IL-1β within 24 hours (Teixeira, Khoo *et al*. 2010; Grant, Wilson *et al.* 2013). In the present study, IL-1β presented significant values only from 7 days in the lower and upper incisors, with increased levels in the upper incisor compared to the lowers 24 hours after spur attachment. However, it is necessary to consider that the amount of force applied to teeth and the speed of tooth movement is directly related to the amount of interleukins released(Grant, Wilson *et al.* 2013) and in our study we used a passive appliance.

Most studies in the literature have evaluated and quantified the expression of interleukins in orthodontic movement through the Enzyme-Linked Immunosorbent Assay. In our study, the evaluation method used to identify interleukins was the cytometric bead array, which presents great sensitivity and has low standard deviation.

Increased volume of crevicular fluid has also been considered an indication factor for periodontal inflammation and is related to the increased presence of

interleukins(Socransky and Haffajee 2005). The same is true for orthodontic movement as seen in Basaran et al. study(Basaran, Ozer *et al.* 2006) who identified the relationship between increased GFC volume and increased presence of IL-8 after 7 days in orthodontic treatment. Similar results were identified in canine distalization through orthodontic forces and IL-1β release after 4 hours and 7 days(Tuncer, Ozmeric *et al*. 2005; Grant, Wilson *et al*. 2013). In accordance with the previous studies, in the present study there was a positive correlation was identified between the increase in interleukins IL-8, IL-6, IL-1β and the increase in GFC volume.

Different devices can be used for early anterior open bite correction and most of them promotes dental changes, especially in the anterior region, and contribute to anterior bite reduction(Rossato, Fernandes *et al*. 2018). In our study the lingual spur were used(Dias, Assis Urnau *et al.* 2019) and accepted by the patients. After the spur attachment the previous thrusted tongue (Figure 3A) is forced to a backward position (Figure 3B). Usually patients with open bite also exhibit open lip relationship and impaired tongue positions(Lambrechts, De Baets *et al*. 2010; Gonzalez, MartInez *et al.* 2019). In subjects with an open lip and open bite the lip pressure is lower when compared with the tongue interposition pressure(Lambrechts, De Baets *et al.* 2010). In our study, the spur bonding prevented lingual interposition what may result in the increase of lip pressure on the upper incisors in an initial moment what may have activated alveolar bone remodeling and incisors eruption with the increase of the interleukins levels in the GCF after 24 hour and 7 days after the beginning of the treatment.

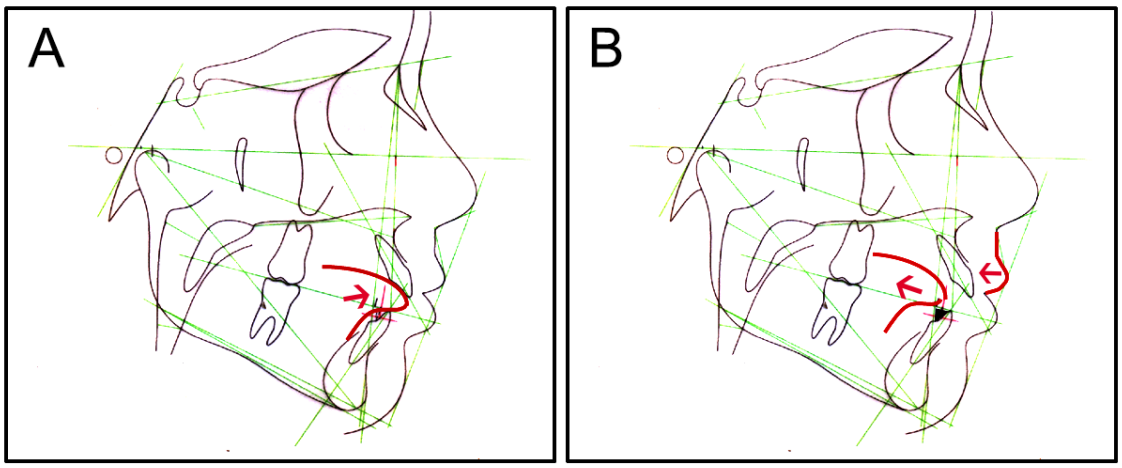


Figure 3. A. Representative image of the tongue interposition between lower and upper incisors in a child with anterior open bite. B. After the attachment of the spur (black triangle bonded in the lingual surface of the lower incisor) the tongue is moved backwards and the superior lip exerts its load on the anterior incisor with effects in the alveolar bone remodeling and tooth eruption 24 hour after the attachment.

CONCLUSION

Differential expression of IL-8, IL1β and IL-6 were observed in GCF in the upper and lower incisors of children with open bite using spur as orthodontic treatment.

Acknowledgments

This study was supported by: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

The authors thank the Program for Technological Development in Tools for Health-RPT-FIOCRUZ for use of its facilities; especial thanks to Elaine Speziali de Faria e Dayane Andriotti Otta for their helpful knowledge in CBA analysis.

The authors declare no conflict of interest. All co-authors are aware of, and in agreement with the submission.

REFERENCES

Basaran, G., T. Ozer, et al. (2006). "Interleukins 2, 6, and 8 levels in human gingival sulcus during orthodontic treatment." American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics **130**(1): 7 e1-6.

Bergamo, A. Z., P. Nelson-Filho, et al. (2016). "Gingival crevicular fluid volume and periodontal parameters alterations after use of conventional and self-ligating brackets." Journal of orthodontics **43**(4): 260-267.

Brylka, L. J. and T. Schinke (2019). "Chemokines in Physiological and Pathological Bone Remodeling." Frontiers in immunology **10**: 2182.

Dias, F. A., F. D. Assis Urnau, et al. (2019). "Stability of early treatment of anterior open bite: clinical performance of bonded lingual spurs." Journal of orthodontics **46**(1): 68-73.

Dudic, A., S. Kiliaridis, et al. (2006). "Composition changes in gingival crevicular fluid during orthodontic tooth movement: comparisons between tension and compression sides." European journal of oral sciences **114**(5): 416-422.

Gonzalez, P., M. B. MartInez, et al. (2019). "Tongue position assessment during oral phase deglutition in children with anterior open bite and normal vertical overbite." Journal of the Indian Society of Pedodontics and Preventive Dentistry **37**(2): 167-171.

Grant, M., J. Wilson, et al. (2013). "Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement." European journal of orthodontics **35**(5): 644-651.

Lambrechts, H., E. De Baets, et al. (2010). "Lip and tongue pressure in orthodontic patients." European journal of orthodontics **32**(4): 466-471.

Ling, H. T. B., F. Sum, et al. (2018). "The association between nutritive, non-nutritive sucking habits and primary dental occlusion." BMC oral health **18**(1): 145.

Madureira, D. F., J. M. da Silva, et al. (2015). "Cytokine measurements in gingival crevicular fluid and periodontal ligament: Are they correlated?" American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics **148**(2): 293-301.

Naranjo, A. A., M. L. Trivino, et al. (2006). "Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement." American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics **130**(3): 275 e217-222.

Pejda, S., M. L. Varga, et al. (2013). "Clinical and microbiological parameters in patients with self-ligating and conventional brackets during early phase of orthodontic treatment." The Angle orthodontist **83**(1): 133-139.

Pithon, M. M., M. B. Magno, et al. (2019). "Oral health-related quality of life of children before, during, and after anterior open bite correction: A single-blinded randomized controlled trial." American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics **156**(3): 303-311.

Ren, Y., H. Hazemeijer, et al. (2007). "Cytokine profiles in crevicular fluid during orthodontic tooth movement of short and long durations." Journal of periodontology **78**(3): 453-458.

Ren, Y. and A. Vissink (2008). "Cytokines in crevicular fluid and orthodontic tooth movement." European journal of oral sciences **116**(2): 89-97.

Rosa, M., V. Quinzi, et al. (2019). "Paediatric Orthodontics Part 1: Anterior open bite in the mixed dentition." European journal of paediatric dentistry **20**(1): 80-82.

Rossato, P. H., T. M. F. Fernandes, et al. (2018). "Dentoalveolar effects produced by different appliances on early treatment of anterior open bite: A randomized clinical trial." The Angle orthodontist **88**(6): 684-691.

Ruan, W. H., M. D. Chen, et al. (2005). "Muscular forces exerted on the normal deciduous dentition." The Angle orthodontist **75**(5): 785-790.

Smuthkochorn, S., J. M. Palomo, et al. (2017). "Gingival crevicular fluid bone turnover biomarkers: How postmenopausal women respond to orthodontic activation." American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics **152**(1): 33-37.

Socransky, S. S. and A. D. Haffajee (2005). "Periodontal microbial ecology." Periodontology 2000 **38**: 135-187.

Teixeira, C. C., E. Khoo, et al. (2010). "Cytokine expression and accelerated tooth movement." Journal of dental research **89**(10): 1135-1141.

Tuncer, B. B., N. Ozmeric, et al. (2005). "Levels of interleukin-8 during tooth movement." The Angle orthodontist **75**(4): 631-636.

Uematsu, S., M. Mogi, et al. (1996). "Interleukin (IL)-1 beta, IL-6, tumor necrosis factor-alpha, epidermal growth factor, and beta 2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement." Journal of dental research **75**(1): 562-567.

Valentim, A. F., R. M. Furlan, et al. (2016). "Relationship between perception of tongue position and measures of tongue force on the teeth." CoDAS **28**(5): 546-550.

van Gastel, J., M. Quirynen, et al. (2011). "Longitudinal changes in microbiology and clinical periodontal parameters after removal of fixed orthodontic appliances." European journal of orthodontics **33**(1): 15-21.

Wise, G. E. and G. J. King (2008). "Mechanisms of tooth eruption and orthodontic tooth movement." Journal of dental research **87**(5): 414-434.

4.1.2 Artigo 2 - Resposta ao objetivo específico 2 e 3

- Artigo ainda não submetido aguardando as correções e sugestões da banca.

Methacrylates release in the saliva of children with anterior open bite treated with tongue spur

Gabriel Antônio dos Anjos Tou[1], Luiza Rinco [1], Mônica Yamauti[1], Fabiane Pires[2], Marcela Schimidt[2], Carla Botoli[2], Soraia Macari[1]

[1] Department of Restorative Destistry, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

[2] Department of Analytical chemistry, Chemistry Institute, Federal University of Campinas. Campinas, São Paulo, Brazil

Corresponding author: Soraia Macari. Mailing address: Departamento de Odontologia Restauradora, Faculdade de Odontologia, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos 6627, CEP 31.270-901, Belo Horizonte, Minas Gerais, Brasil. E-mail: soraiamacari@gmail.com.

Declarations of interest: none

ABSTRACT

Introduction: The anterior open bite is a malocclusion characterized by the lack of contact between the dental arches. In order bond these accessories for instance a tongue spur on dental elements, it is necessary to use resinous materials. Products released by methacrylate resins have been linked to changes at the local and systemic level. Such as early sexual maturation in children, infertility and changes in immune functions. The aim of this study was to evaluate the release of by-products of the composite resin used in the bonding of spurs applied in the treatment of children with anterior open bite and its effects on human keratinocytes. **Methods:** Standardized resin increments were added to 3 different dilutions of cell culture medium were prepared by incubation at 37ºC, for 30 minutes. Keratinocytes (HaCat) were cultivated in the conditioned media and evaluated for the Cell Viability (MTT) and scratch assay. For methacrylates study twenty children were included. Salive samples were collected before spur attachment (baseline), 30 minutes (m), 24 hours (h) and 7 days (d) after bonding. Analysis was performed using the High performance liquid chromatography. **Results:** The levels of BisGMA and TEGDMA in the saliva were increased in the period of 30 minutes in comparison to baseline, 24 hours and 7 days with no difference between these aforementioned groups. The *in vitro* results showed that the viability has increased along the different timepoints of 24, 48 and 120 hours. The percentage of the wound area was decreased after 24h and 48h in when compared to 0h. **Conclusions:** Resin composites used to attach spurs in children with anterior open bite during the orthodontic treatment releases methacrylate after polymerization and are able to influence the behavior of human keratinocytes in cell culture, even in very low concentration.

Key words: Methacrylate. Bisphenol-A glycidyl methacrylate. Triethylene glycol dimethacrylate. Keratinocytes. Open bite.

INTRODUCTION

Methacrylate-based resin composites have been commonly used in bonding various accessories in orthodontic treatment (Malkiewicz, Turlo *et al*. 2015; Goto, Hasegawa *et al*. 2019). These materials consist of a polymeric matrix, often a dimethacrylate, silanized filler particles and substances that promote or modulate the polymerization reaction. The base monomer of the organic matrix is the bisphenol-A glycidyl methacrylate (BisGMA), which due to its high viscosity, is mixed with other dimethacrylates such as triethylene glycol dimethacrylate (TEGDMA) and urethane dimethacrylate (UDMA) or other monomers (Ferracane 2011).

The release of composite resin monomers are potentially hazardous (Van Landuyt, Nawrot *et al*. 2011; Gupta, Saxena *et al*. 2012) with systemic (Schwengberg, Bohlen *et al*. 2005) and local effects on the oral mucosa, gum and dental pulp (Putzeys, Cokic *et al*. 2017). BisGMA breakdown products are able to simulate estrogen function in the body (Gao, Yang *et al*. 2015). In addition, there are studies linking exposure of these compounds to early sexual maturation in children (Howdeshell, Hotchkiss *et al*. 1999), infertility (Al-Hiyasat, Darmani *et al*. 2004) increased risk of breast and prostate cancer (Maffini, Rubin *et al*. 2006), and changes in immune functions (Sawai, Anderson *et al*. 2003). These products are cytotoxic to several cell types, including human gingival fibroblasts and keratinocytes, and interfere with cell proliferation and migration (Theilig, Tegtmeier *et al*. 2000; Issa, Watts *et al*. 2004). TEGDMA exhibits an excellent viscosity and copolymerization behavior and also revealed a considerable cytotoxic potency (Geurtsen and Leyhausen 2001).

Methacrylate-based dental materials are susceptible to degradation when applied clinically and, as a consequence, by-products may be released into the oral

environment (Ferracane 1994; Polydorou, Trittler *et al*. 2007; Zimmerman-Downs, Shuman *et al*. 2010; Ferracane 2011). Among these compounds are those applied for bonding orthodontic accessories in the treatment of adults and also children and pre-adolescents patients (Kotyk and Wiltshire 2014).

However, no study has analyzed methacrylate release after bonding of orthodontic fixtures in children. The aim of this study is to analyze the release of BisGMA and TEGDMA at different time-points in the saliva of children after bonding spurs used to treat anterior open bite and to simulate its effects on human keratinocytes (HaCat) in cell cultures.

MATERIAL AND METHODS

Participants

For the methacrylate quantification, patients with indication for interceptive orthodontic treatment and who had anterior open bite were recruited. A total of 22 participants agreed to participate in the study, 8 males and 14 females. The consent form was collected from all participants. The study was approved by the Research Ethics Committee of the Federal University of Minas Gerais (CAAE: 87714218.0.0000.5149).

The study included patients with no systemic conditions, with good oral hygiene, with presence of anterior open bite and healthy periodontal status. The periodontal parameters used to evaluate the state of normality of the central upper and lower incisor in this study were probing depth, visible plaque index, and probing bleeding index.

The study excluded individuals with severe systemic alterations, using antibiotics and anti-inflammatory drugs in the last three months, with abnormal

periodontal status, who underwent restorations in the last 12 months, or any orthodontic accessory that has come loose.

The clinical examination and the bonding of the device was performed by a single trained and calibrated researcher (Kappa inter-examiner = 0.91 and intra-examiner = 0.83). Seven days before the first day of sample collection, the participants received hygiene instructions, oral conditioning and dental cleaning. Periodontal evaluation and saliva collection were performed before the device was placed (baseline) and within 30 minutes (min), 24 hours (h) and 7 days (d) after the device was bonded.

Relative isolation was performed, phosphoric acid (Fusion-Duralink, Angelus, Brazil) was applied for 30 seconds on the enamel surface, application of the Transbond XT adhesive system kit (3M, Unitek, Monrovia, California, USA) according to the manufacturer's recommendations , and the bonding of the spurs (Morelli, Sorocaba, SP, Brazil) were performed on the lingual surface of the lower incisor, applying pressure in the center of the accessory so that the composite resin could leak out on the spur sides, these excesses were removed. Two 40-second photoactivations were performed with an LED lamp (Bluephase N, Ivoclar Vivadent Inc., Amherst, NY, USA. 1000mW / cm2), one by lingual and the other by incisal.

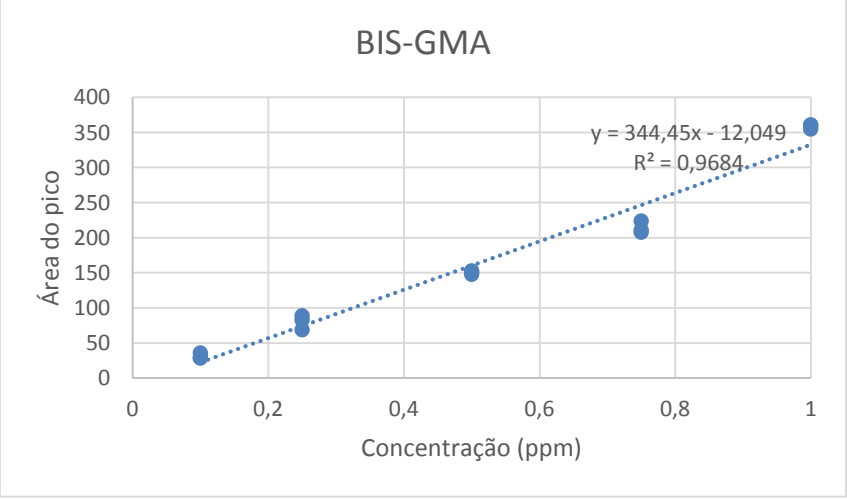
Saliva collection

To collect unstimulated saliva, volunteers were advised to sit comfortably with their heads slightly tilted down, allowing saliva to accumulate in their mouth, and then collect it in a glass vial. The collected saliva was immediately stored on ice and then at -80ºC until the moment of analysis.

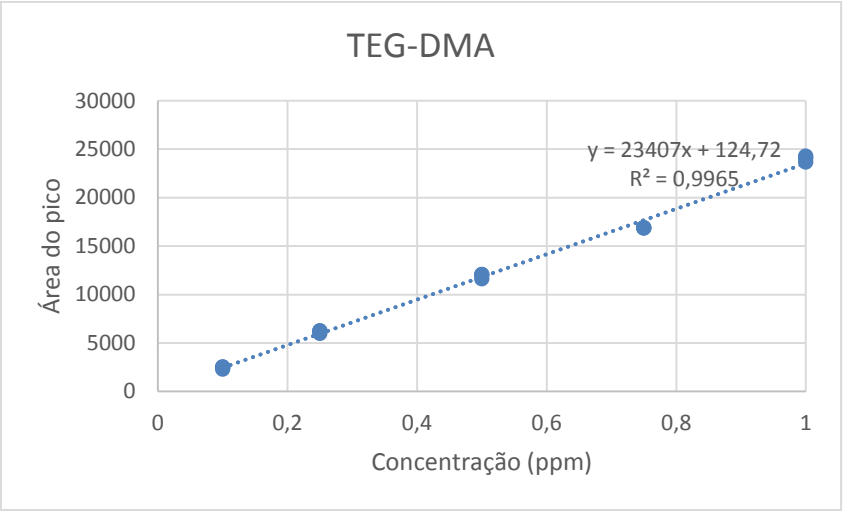
BisGMA and TEGDMA analysis

The elution assay was adapted from Michelsen *et al*. (2012) and carried out as follows: 1 ml of the sample and 1 ml of high performance liquid chromatography (HPLC) grade ethyl acetate were transferred to a 15 mL vial, vigorously agitated for 1 minute and centrifuged for 4 minutes at 3000 rpm. 0.5 mL of the supernatant was removed and transferred to a glass vial. This procedure was repeated 3 times. At the end, 1.5 mL of the ethyl acetate extract was obtained, the volume was evaporated under nitrogen flow and the dried extract was resuspended in 0.5 mL of mobile phase. The sample was filtered through a 0.45 µm syringe filter into a vial and placed in the chromatograph sampler. Stock solutions of the BisGMA and TEGDMA standards were prepared in HPLC grade acetonitrile at the concentration of 10 µg/mL and from these the following dilutions were performed to obtain working solutions.

These solutions were filtered on a 0.45 µm syringe filter into a vial, wrapped in the chromatograph sampler and injected. The analytical curve used for the quantification of the samples was constructed (Supplemental figure 1 and Supplemental figure 2).



Supplementary Figure 1. Analytical curve constructed for the quantification of BisGMA samples.



Supplementary Figure 2. Analytical curve constructed for the quantification of TEGDMA samples.

The chromatographic method used was from NovaPack Column (Waters Corporation, Milford, USA) (C18 3.9 mm x 150 mm x 4 µm) with isocratic elution mode, 10 µL injection volume, flow rate 0.5 mL/min and mobile phase acetonitrile: ammonium acetate 10 mm 65:35 (v/v). Mass spectrometer conditions for BisGMA (MRM mode): precursor ion (m/z) 43 513.19 Da, son ion (m/z) 142.9 Da, cone voltage 25 V, collision energy 20 eV. For the TEGDMA (MRM mode): precursor ion (m/z) 286.97 Da, son ion (m/z) 112.9 Da, cone voltage 25 V, collision energy 12 eV.

In vitro experiment

To mimic the oral microenvironment in the condition of spur attachment, cell culture tests were performed to evaluate cell viability (MTT assay) and cell migration (cell scratch experiment) in the presence of orthodontic composite realized products.

To simulate the amount of byproducts realized in the mean oral of 3o min, four resin increments were weighted (7.5 mg) and immediately after polymerization (40 seconds) were added to 3 different dilutions of cell culture medium, 5,5, 11 and 22 mL. The dilutions of 22 mL (Diluition 3 – D3) correspond to the volume of salive produced by a child up to 12 years old, which is approximately 0.7366 mL/min (MOURA *et al*., 2008). Two other different concentrations of conditional medium were used for comparations, 5.5 mL (Dilution 1 – D1) and 11 mL (Dilution 2 – D2). A group grown under ideal conditions was used as a control.

Human keratinocytes (HaCat) were plated in quadruplicate at a cell density of 1 x 10^4 cells/well in 96-well plates the MTT assay (3- (4,5-dimethylthiazol-2yl) -2,5-diphenyl bromide) tetrazolium]} was performed according to the manufacture tehn asses of contact with the conditional media, 24, 48 and 120 hours (h).

For cell scratch migration test the HaCat were plated in triplicate at a density of 5 x 10^5 cells/well in 6-well plates and were evaluated at 0, 24 and 48 hours (h). At time 0h, P200 tip wound was performed in each well. Five images of each group were made and the quantification of the percentage (%) of the wound area was measured using Image J software (National Institute of Health, Bethesda, MD, USA).

Statistical analysis

Results were expressed as mean ± standard deviation (S.D.). Data sets from the *in vivo* experiments presented a non-normal distribution (D'Agostino & Pearson omnibus normality test and Shapiro-Wilk normality test). The differences among groups were analyzed by Kruskal-Wallis test nonparametric test followed by Dunn's Multiple Comparison Test. The differences among groups in the *in vitro* experiments were analyzed by two-way analysis of variance followed by the Bonferroni post hoc

test. Correlations were determined using Pearson's correlation test. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic information of the participants and the periodontal evaluation is shown in Table I. Statistical increase of the gingival bleeding was exhibited in the lower incisors after 7 days of spur attachment in comparison to baseline. No other difference was verified in the clinical variables among the different timepoints.

Table I. Demographic distribution and clinical data of the patients. Values are mean ± S.D.; p<0.05; *different from baseline in the same group of teeth

N = 20	**Age(Years) =** 8.85 ± 1.19						**Gender:** 7 M / 13 F	
	Timepoint							
	Baseline		**30 min**		**24h**		**7d**	
Clinical variables	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**
Probing depth (mm)	1.48 ± 0.28	1.29 ± 0.24	1.48 ± 0.28	1.29 ± 0.24	1.51 ± 0.29	1.3 ± 0.23	1.55 ± 0.34	1.35 ± 0.21
Gengival bleeding (%)	0 ± 0	0 ± 0	0 ± 0	1,25 ± 0.05	0.6 ± 0.02	1.2 ± 0.03	0 ± 0	2.2 ± 0.04*
Visible plaque (%)	17.5 ± 0.28	33.1 ± 0.34	7.5 ± 0.16	18.75 ± 0.30	17.5 ± 0.28	28.7 ± 0.36	18.7 ± 0.29	33.7 ± 0.36

Table I. Demographic distribution and clinical data of the patients. Values are mean ± S.D.; p<0.05; *different from baseline in the same group of teeth

The levels of BisGMA and TEGDMA in the saliva were increased in the period of 30 minutes in comparison to baseline, 24 hours and 7 days with no difference between these aforementioned groups (Fig 1A). The increase of BisGMA was negatively correlated to the plaque index in the upper incisors in the period of 30 minutes (Fig 1B).

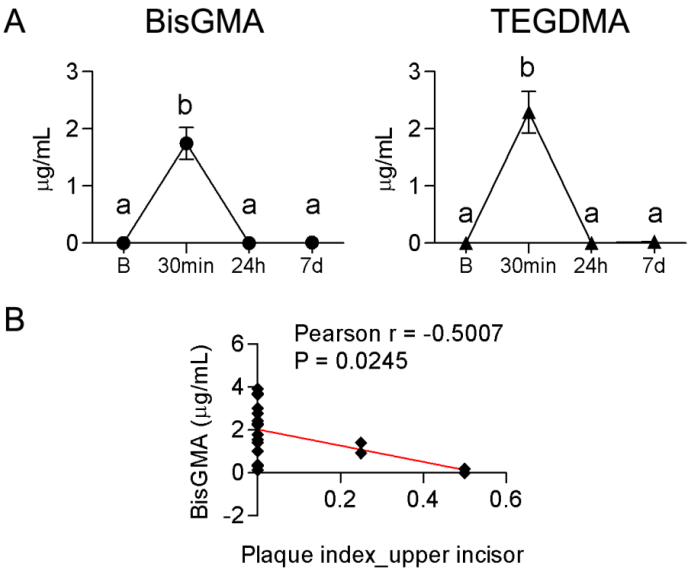


Figure 1. A - Analysis of level of BisGMA and TEGDMA in the saliva after spur attachment in children using spur as treatment for treatment to anterior open bite. B – Correlation between BisGMA levels and plaque index in the upper incisors. 20 children participate in this study. Kruskal-Wallis test nonparametric test followed by Dunn's Multiple Comparison Test and Pearson's correlation test. $p < 0.05$ was considered statistically significant.

The *in vitro* results showed that the viability has increased along the different timepoints of 24, 48 and 120 hours, with exception of the D1 - 48h that was similar to D1 – 24h. No difference among the dilutions was verified with the same timepoint

(Fig. 2A). The percentage of the wound area decreased significantly behavior 24h and 48h in D1, D2 and D3 when compared to 0h (Fig. 2B-C). Within the same period of 24h the dilution groups (D1, D2 and D3) demonstrated decreased wound percentage compared to the control (Fig. 2B-C).

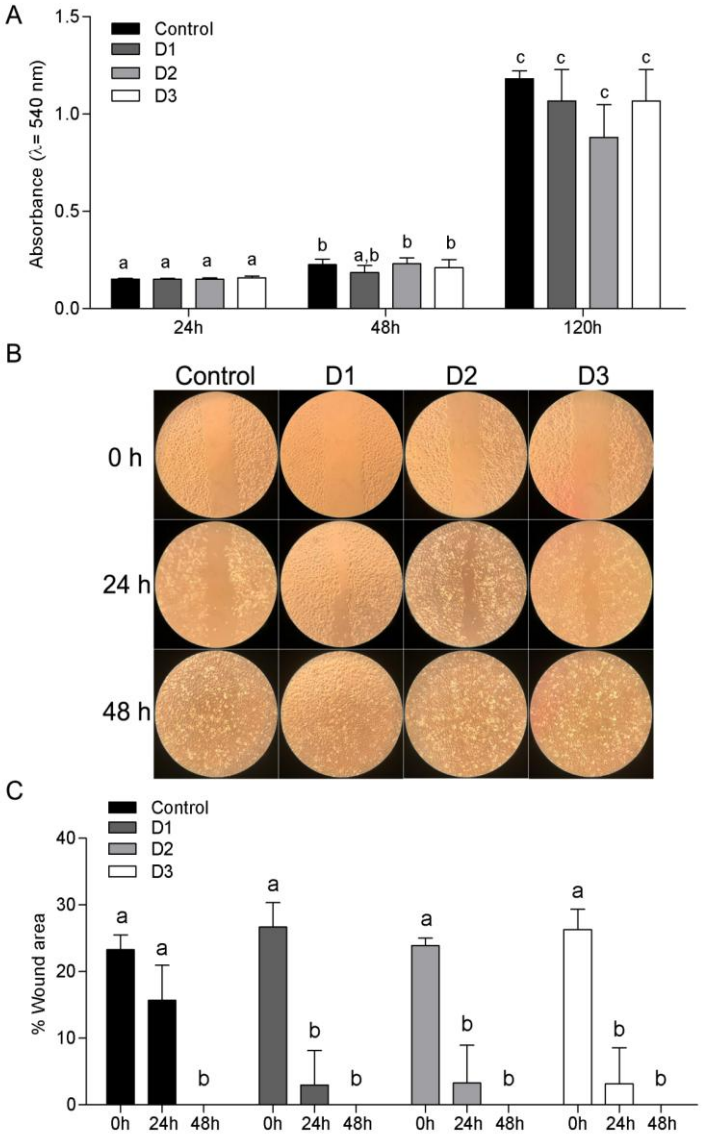


Figure 2. *In vitro* experiment with resin increments added to 3 different dilutions of cell culture: 5.5 mL (Dilution 1 – D1), 11 mL (Dilution 2 – D2) and 22 mL (Dilution 3 – D3) of cell culture medium respectively. A group grown under ideal conditions was used as a control. A – Viability assay using hHuman keratinocytes (HaCat) plated in quadruplicate for the MTT assay (3- (4,5-dimethylthiazol-2yl) -2,5-diphenyl bromide)

tetrazolium]} for 24, 48 and 120 hours (h). B - Cell scratch migration test with HaCat cells plated in and evaluated at 0, 24 and 48 hours (h). ANOVA 2-way test followed by Bonferroni posttests. $p < 0.05$ was considered statistically significant.

DICUSSION

The release of monomers from a resinous compound used in bonding orthodontic spur in children with anterior open bite was evaluated *in vivo* and *in vitro*. It has been identified that BisGMA and TEGDMA are released into the oral environment after 30 minutes after polymerization and are able to increase queratinocytes migration in human cultured cells even in very low concentrations.

The presence of BisGMA and TEGDMA in the saliva of children over time was analyzed. HPLC is the most widely accepted method for identifying and quantifying products from resinous dental materials because of its high efficiency in the evaluation of these compounds (Van Landuyt, Nawrot *et al.* 2011; MacAulay, Tam *et al.* 2017; Putzeys, Cokic *et al.* 2017). However, the operational process and the techniques used for the extraction of sample compounds are varied (Van Landuyt, Nawrot *et al.* 2011), which may generate different findings among the studies. In addition, the amount and size of samples may vary from study to study, including *in vitro* to *in vivo* analysis, so standardization of the samples may be challenging (Ferracane 1994; Polydorou, Trittler *et al.* 2007; Ferracane 2011). The difference in the trademarks used may also lead to differences in the amount of components released by the materials (Polydorou, Trittler *et al.* 2007). Our study was based on the experimental protocol of (Moreira, Matos *et al.* 2017) in order to reduce risk of error, thus increasing the capability of comparison among other studies.

According to Ferracane *et al.* (1994) most components, 50-70% depending on the environment, are released within the first three to six hours after polymerization and a rate of 80-100% is released within the first 24 hours. An *in vitro* study quantified during one year the elution of compounds from resin-based dental composites and verified that BisGMA, HEMA and UDMA were able to continuously elute from the materials, up to 52 weeks after initial immersion (Putzeys, Nys *et al.* 2019). In the present study, it was possible to identify the significant presence, when compared to the baseline, of BisGMA and TEGDMA at the 30 minutes after polymerization. Other studies have also identified component release within the first minutes after polymerization (Nathanson, Lertpitayakun *et al*. 1997; Komurcuoglu, Olmez *et al.* 2005). In corroboration to our study Polydorou *et al.* (2007), in an *in vitro* study, identified the presence of BisGMA and TEGDMA at 24 hours and 7 days after polymerization.

The concern with the release of resin composites by products is due to the fact that these components have been shown to be toxic to cells and tissues (Geurtsen and Leyhausen 2001). Depending on the type of cell used and the flowability of the resinous material the results may vary (Thonemann, Schmalz *et al*. 2002; Al-Hiyasat, Darmani *et al*. 2005; Ausiello, Cassese *et al.* 2013). Issa *et al.* (2004) evaluated the cytotoxicity of the monomers present in resinous materials by MTT in human gingival fibroblasts and found that all monomers used in the manufacture of these materials, including BisGMA and TEGDMA, showed significant cytotoxicity, however in this study the monomers were applied directly to the culture. Different results were found in a study with odontoblast-like MDPC-23 cells, in which no cytotoxic effect was identified when the monomers had undergone polymerization (de Souza Costa,

Hebling *et al*. 2003; Aranha, Giro *et al.* 2010). Similar results were found in the present study, where there was no change in cell viability after polymerization.

On the other hand, studies have shown the effect on component-induced cell migration released from composite resins. Theilig *et al.* (2000) evaluated the effect of BisGMA and TEGDMA on the induction of cell migration and proliferation of human fibroblasts and keratinocytes. It was identified that the presence of BisGMA, but not TEGDMA, was able to significantly induce cell migration. Similar results were found in the present study in which cell cultures with released component dilutions presented higher cell migration rate when compared to the control group. The levels of BisGMA and TEGDMA were not measured in the cell culture medium in the different experimental timepoints and dilution in the present study what demonstrate a limitation of our results. However, more experimental data are necessary before these important aspects can be elucidated.

The increased level of BisGMA in the saliva after 30 minutes of the resin polymerization was negatively correlated to the plaque index in the upper incisors, which indicates that as less dental plaque present greater will be the presence of BisGMA in the saliva. Although plaque index did not exhibited statistical difference among the timepoints, there was a tendency of decrease at the period of 30 minutes probably due to the dental cleaning that the children received before spur bonding. As this result is the first to correlate clinical data with the release levels of methacrylate in the saliva of patients, the comparison to others studies was not possible. One hypothesis is that the BisGMA released can be retained by the dental biofilm, which would lead to its decrease in saliva, on the other hand, in lower indices of dental plaque a greater amount of BisGMA would be found in the saliva

CONCLUSION

Resin composites used to attach spurs in children with anterior open bite during the orthodontic treatment releases methacrylate after polymerization and are able to influence the behavior of human keratinocytes in cell culture even at low concentration.

References

Al-Hiyasat, A. S., H. Darmani, et al. (2004). "Leached components from dental composites and their effects on fertility of female mice." European journal of oral sciences **112**(3): 267-272.

Al-Hiyasat, A. S., H. Darmani, et al. (2005). "Cytotoxicity evaluation of dental resin composites and their flowable derivatives." Clinical oral investigations **9**(1): 21-25.

Aranha, A. M., E. M. Giro, et al. (2010). "Effects of light-curing time on the cytotoxicity of a restorative composite resin on odontoblast-like cells." Journal of applied oral science : revista FOB **18**(5): 461-466.

Ausiello, P., A. Cassese, et al. (2013). "Cytotoxicity of dental resin composites: an in vitro evaluation." Journal of applied toxicology : JAT **33**(6): 451-457.

de Souza Costa, C. A., J. Hebling, et al. (2003). "Effects of light-curing time on the cytotoxicity of a restorative resin composite applied to an immortalized odontoblast-cell line." Operative dentistry **28**(4): 365-370.

Ferracane, J. L. (1994). "Elution of leachable components from composites." Journal of oral rehabilitation **21**(4): 441-452.

Ferracane, J. L. (2011). "Resin composite--state of the art." Dental materials : official publication of the Academy of Dental Materials **27**(1): 29-38.

Gao, H., B. J. Yang, et al. (2015). "Bisphenol A and hormone-associated cancers: current progress and perspectives." Medicine **94**(1): e211.

Geurtsen, W. and G. Leyhausen (2001). "Chemical-Biological Interactions of the resin monomer triethyleneglycol-dimethacrylate (TEGDMA)." Journal of dental research **80**(12): 2046-2050.

Goto, S., Y. Hasegawa, et al. (2019). "Effects of contact compressive force on bracket bond strength and adhesive thickness : Study using orthodontic resins with different viscosities." Journal of orofacial orthopedics = Fortschritte der Kieferorthopadie : Organ/official journal Deutsche Gesellschaft fur Kieferorthopadie.

Gupta, S. K., P. Saxena, et al. (2012). "Release and toxicity of dental resin composite." Toxicology international **19**(3): 225-234.

Howdeshell, K. L., A. K. Hotchkiss, et al. (1999). "Exposure to bisphenol A advances puberty." Nature **401**(6755): 763-764.

Issa, Y., D. C. Watts, et al. (2004). "Resin composite monomers alter MTT and LDH activity of human gingival fibroblasts in vitro." Dental materials : official publication of the Academy of Dental Materials **20**(1): 12-20.

Komurcuoglu, E., S. Olmez, et al. (2005). "Evaluation of residual monomer elimination methods in three different fissure sealants in vitro." Journal of oral rehabilitation **32**(2): 116-121.

Kotyk, M. W. and W. A. Wiltshire (2014). "An investigation into bisphenol-A leaching from orthodontic materials." The Angle orthodontist **84**(3): 516-520.

MacAulay, M., L. E. Tam, et al. (2017). "In Vivo Biodegradation of bisGMA and Urethane-Modified bisGMA-Based Resin Composite Materials." JDR clinical and translational research **2**(4): 397-405.

Maffini, M. V., B. S. Rubin, et al. (2006). "Endocrine disruptors and reproductive health: the case of bisphenol-A." Molecular and cellular endocrinology **254-255**: 179-186.

Malkiewicz, K., J. Turlo, et al. (2015). "Release of bisphenol A and its derivatives from orthodontic adhesive systems available on the European market as a

potential health risk factor." Annals of agricultural and environmental medicine : AAEM **22**(1): 172-177.

Michelsen, V. B., H. B. Kopperud, et al. (2012). "Detection and quantification of monomers in unstimulated whole saliva after treatment with resin-based composite fillings in vivo." European journal of oral sciences **120**(1): 89-95.

Moreira, M. R., L. G. Matos, et al. (2017). "Bisphenol A release from orthodontic adhesives measured in vitro and in vivo with gas chromatography." American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics **151**(3): 477-483.

Moritsuka, M., Y. Kitasako, et al. (2006). "Quantitative assessment for stimulated saliva flow rate and buffering capacity in relation to different ages." Journal of dentistry **34**(9): 716-720.

Murphy-Ullrich, J. E., V. A. Lightner, et al. (1991). "Focal adhesion integrity is downregulated by the alternatively spliced domain of human tenascin." The Journal of cell biology **115**(4): 1127-1136.

Nathanson, D., P. Lertpitayakun, et al. (1997). "In vitro elution of leachable components from dental sealants." Journal of the American Dental Association **128**(11): 1517-1523.

Polydorou, O., R. Trittler, et al. (2007). "Elution of monomers from two conventional dental composite materials." Dental materials : official publication of the Academy of Dental Materials **23**(12): 1535-1541.

Putzeys, E., S. M. Cokic, et al. (2017). "Simultaneous analysis of bisphenol A based compounds and other monomers leaching from resin-based dental materials by UHPLC-MS/MS." Journal of separation science **40**(5): 1063-1075.

Putzeys, E., S. Nys, et al. (2019). "Long-term elution of monomers from resin-based dental composites." Dental materials : official publication of the Academy of Dental Materials **35**(3): 477-485.

Sawai, C., K. Anderson, et al. (2003). "Effect of bisphenol A on murine immune function: modulation of interferon-gamma, IgG2a, and disease symptoms in NZB X NZW F1 mice." Environmental health perspectives **111**(16): 1883-1887.

Schwengberg, S., H. Bohlen, et al. (2005). "In vitro embryotoxicity assessment with dental restorative materials." Journal of dentistry **33**(1): 49-55.

Theilig, C., Y. Tegtmeier, et al. (2000). "Effects of BisGMA and TEGDMA on proliferation, migration, and tenascin expression of human fibroblasts and keratinocytes." Journal of biomedical materials research **53**(6): 632-639.

Thonemann, B., G. Schmalz, et al. (2002). "Responses of L929 mouse fibroblasts, primary and immortalized bovine dental papilla-derived cell lines to dental resin components." Dental materials : official publication of the Academy of Dental Materials **18**(4): 318-323.

Van Landuyt, K. L., T. Nawrot, et al. (2011). "How much do resin-based dental materials release? A meta-analytical approach." Dental materials : official publication of the Academy of Dental Materials **27**(8): 723-747.

Zimmerman-Downs, J. M., D. Shuman, et al. (2010). "Bisphenol A blood and saliva levels prior to and after dental sealant placement in adults." Journal of dental hygiene : JDH **84**(3): 145-150.

5 CONSIDERAÇÕES FINAIS

A colagem do esporão ao induzir a remoção da interposição lingual, permite que forças da musculatura peribucal sejam aplicadas sobre os dentes, o que proporcionou o aumento de biomarcadores no FCG. Estas citocinas desempenham papel importante na erupção dos dentes e na remodelação óssea, sendo relevante para o tratamento da mordida aberta anterior. Além disso, foi possível identificar a liberação de subprodutos resinosos na saliva das crianças em tratamento. Embora os mesmos possam interferir na migração celular, ainda não há estudos que quantifiquem a exposição mínima capaz de induzir alterações no indivíduo. Sendo assim, os benefícios da colagem do esporão com materiais resinosos se sobrepõem aos efeitos adversos dos subprodutos.

6 REFERÊNCIAS

ALHASHIMI, N.; FRITHLOF, L.; BRUDVIK, P.; BAKHIET, M. Orthodontic tooth movement and de novo synthesis of pro-inflammatory cytokines. *American Journal of Orthodontics and Dentofacial Orthopedics,* v. 119, n. 3, p. 307-312, 2001.

AL-HIYASAT, A. S.; DARMANI, H. In vivo effects of BISGMA-a component of dental composite-on male mouse reproduction and fertility. *Journal of Biomedical Materials Research Part A*, v. 78, n. 1, p. 66–72, 2006.

ARTESE, A.; DRUMMOND, S.; NASCIMENTO, J. M.; ARTESE, F. Criteria for diagnosing and treating anterior open bite with stability. *Dental Press J Orthod.* v. 16, p. 136-161, 2011.

AZUMA, Y.; KAJI, K.; KATOGI, R.; TAKESHITA, S.; KUDO, A Tumor necrosis factor-alpha induces differentiation of and bone resorption by osteoclasts. *J Biol Chem.* v. 275, n. 7, p. 4858-64, 2000.

BAŞARAN, G.; OZER, T.; KAYA, F.A.; HAMAMCI, O. Interleukins 2, 6, and 8 levels in human gingival sulcus during orthodontic treatment. *Am J Orthod Dentofacial Orthop.* v. 130, n. 1, p. 1-6, 2006.

BOLLING, A.K. *et al*. Dental monomers inhibit LPS - induced cytokine release from the macrophage cell line RAW264.7. *Toxicology Letters,* v.216, p.130-138, 2013.

BORONAT-CATALÁ, M.; CATALÁ-PIZARRO.; BAGÁN SEBASTIÁN, J.V. Salivary and crevicular fluid interleukins in gingivitis. *Journal of Clinical and Experimental Dentistry*, v.6, n.2, p.175-179, 2014.

BUONOCORE, M. G. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *J Dent Res*. V. 34, n. 6, p.849-53, 1955.

CARRANZA, J. R. A. F. e SZNAJDER, N. G. *Compendio de Periodoncia*. 5. ed. Argentina: Editora Médica Panamericana, 1996. 209 p.

CELIK, N., ASKIN, S. The effect of restorative materials on cytokines in gingival crevicular fluid. *Archives of oral biology* v. 84, p. 139-144, 2003.

COZZA, P.; MUCEDERO, M.; BACCETTI, T.; FRANCHI, L. Early orthodontic treatment of skeletal open bite malocclusion: a systematic review. *Angle Orthod*. v. 75, n. 5, p 707-13, 2005.

DAVIDOVITCH, Z. Tooth movement. *Crit Rev Oral Biol Med* v. 2, p. 411-50, 1991.

DAVIDOVITCH, Z.; NICOLAY, O.; NGAN, P.W.; SHANFELD, J.L. Neurotransmitters, cytokines and the control of alveolar bone remodeling in orthodontics. *Dent Clin North Am*. v. 32, p. 411-35, 1988.

DIAS, F.A.; ASSISURNAU, F.D.; PEDRON OLTRAMARI, P.V.; LUPION POLETI, M.; RODRIGUES DE ALMEIDA, M.; FREIRE FERNANDES, T.M. Stability of early

treatment of anterior open bite: clinical performance of bonded lingual spurs. *Journal of orthodontics.* v. 46, p. 68-73, 2019.

DUDIC, A.; KILIARIDIS, S.; MOMBELLI, A.; GIANNOPOULOU, C. Composition changes in gingival crevicular fluid during orthodontic tooth movement: comparisons between tension and compression sides. *European journal of oral sciences.* v. 114, p. 416-422, 2006.

FERRACANE, J. L. Elution of leachable components from composites. *Journal of Oral Rehabilitation*, v.21, p.441-452, 1994.

FERRACANE, J. L. Resin composite – state of the art. *Dental Materials*, v.27, n.1, p. 29-38, 2011.

GAO, H.; YANG, B. J.; LI, N.; FENG, L. M.; SHI, X. Y.; ZHAO, W. H.; LIU, S. J. Bisphenol A and hormone-associated cancers: current progress and perspectives. *Medicine (Baltimore)*. v. 94, n. 1, p. 211, 2015.

GARCIA-LOPEZ, S.; MEIKLE, M. C.; VILLANUEVA, R. E.; MONTANO, L.; MASSO, F.; RAMIREZ-AMADOR, V.; BOJALIL, R.; Mechanical deformation inhibits IL-10 and stimulates IL-12 production by mouse calvarial osteoblasts in vitro. *Arch Oral Biol*. v. 50, p. 449–452, 2005.

GONZALEZ, P.; MARTINEZ, M. B.; SIERRA, V.; RUEDA, Z.V.; BOTERO-MARIACA, P. Tongue position assessment during oral phase deglutition in children with anterior open bite and normal vertical overbite. *Journal of the Indian Society of Pedodontics and Preventive Dentistry*. v.37, p.167-171, 2019.

GOTO, S. Y. H., *et al*. Effects of contact compressive force on bracket bond strength and adhesive thickness : Study using orthodontic resins with different viscosities. *Journal of orofacial orthopedics = Fortschritte der Kieferorthopadie : Organ/official journal Deutsche Gesellschaft fur Kieferorthopadie*. 2019.

GRANT, M.; WILSON, J.; ROCK, P.; CHAPPLE, I. Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement. *European journal of orthodontics*. v. 35, p. 644-651, 2013.

GUPTA, S. K. *et al*. Release and toxicity of dental resin composite. *Toxicology International*, v. 19, n. 3, p. 225–234, 2012.

HOWDESHELL, K. L.; HOTCHKISS, A. K.; THAYER, K. A.; VANDENBERGH, J. G.; VOM SAAL, F. S. Exposure to bisphenol A advances puberty. *Nature.* v. 401, p. 763-4, 1999.

ISSA, Y.; WATTS, D. C.; BRUNTON, P. A.; WATERS, C. M.; DUXBURY, A. J. Resin composite monomers alter MTT *and* LDH activity *of* human gingival fibroblasts *in* vitro*. Dent Mater*. *v.* 20, p. 12-20, 2004.

JAYAPRAKASH, P. K.; BASAVANNA, J. M.; GREWAL, H.; MODI, P.; SAPAWAT, P.; BOHARA, P. D. Elevated levels of Interleukin (IL)-1β, IL-6, tumor necrosis factor-α, epidermal growth factor, and β2-microglobulin levels in gingival crevicular fluid during human Orthodontic tooth movement. *J Family Med Prim Care*. v. 8, n. 5, p.1602-1606, Mai, 2019.

KASPARAVICIENE, K.; SIDLAUSKAS, A.; ZASCIURINSKIENE, E.; VASILIAUSKAS, A.; JUODZBALYS, G.; SIDLAUSKAS, M.; MARMAITE, U. The prevalence of malocclusion and oral habits among 5-7-year-old children. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*. v. 20, p. 2036-2042, 2014.

KIMOTO, S.; MATSUZAWA, M.; MATSUBARA, S.; KOMATSU, T.; UCHIMURA, N.; KAWASE, T.; SAITO, S. Cytokine secretion of periodontal ligament fibroblasts derived from human deciduous teeth: effect of mechanical stress on the secretion of transforming growth factor-beta 1 and macrophage colony stimulating factor. *J Periodontal Res.* v. 34, p. 235–243, 1999.

KUAN, Y.H. *et al*. The up regulation of tumor necrosis factor-α and surface antigens expression on macrophages by bisphenol A - glycidyl- methacrylate. *International Endodontic Journal,* v.45, p.619-626, 2012.

LAMBRECHTS, H.; DE BAETS, E.; FIEUWS, S.; WILLEMS, G. Lip and tongue pressure in orthodontic patients. *European journal of orthodontics.* v. 32, p. 466-471, 2010.

LARA. T. S.*; SILVA FILHO*, O. G.; YATABE, M. S.; BERTOZ, F. A.. *Mordida aberta anterior dentoalveolar*: *diagnóstico morfológico* e *abordagens* terapêuticas. *Ortodontia* SPO, São Paulo, v.42, n.2, p.123-132, 2009.

LI, C.; CHUNG, C. J.; HWANG, C. J.; LEE, K. J. Local injection of RANKL facilitates tooth movement and alveolar bone remodelling. *Oral Dis.* v. 25, n. 2, p. 550-560, Mar, 2019.

LI, H.; LIN, X. Positive and negative signaling components involved in TNF-a-induced NF-kB activation. *Cytokine*. v. 41, p 1-8, 2008.

LI, S.; LI, Q.; ZHU, Y.; HU, W. GDF15 induced by compressive force contributes to osteoclast differentiation in human periodontal ligament cells. *Exp Cell Res.* v. 1387, n. 1, p.111745, Fev, 2020.

LINDHE, J.; LANG, N.P.; KARRING,T .*Tratado de Periodontia Clínica e Implantologia* Oral. 5 ed. Rio De Janeiro: Guanabara Koogan, 2014. 1304 p.

LING, H. T. B.; SUM, F.; ZHANG, L.; YEUNG, C. P. W.; LI, K. Y.; WONG, H. M. l. The association between nutritive, non-nutritive sucking habits and primary dental occlusion. *BMC oral health.* v.18, p.145, 2018.

MAFFINI, M. V.; RUBIN, B. S..; SONNENSCHEIN, C.; SOTO, A. M. Endocrine disruptors and reproductive health: the case of bisphenol-A. *Mol Cell Endocrinol.* v. 25, p .254-255:179-86, Jul, 2006.

MALKIEWICZ, K., J. T. Release of bisphenol A and its derivatives from orthodontic adhesive systems available on the European market as a potential health risk factor. *Annals of agricultural and environmental medicine : AAEM* v. 22, n. 1, p. 172-177, 2015.

MEAGER A. Cytokine regulation of cellular adhesion molecule expression in inflammation. *Cytokine Growth Factor Rev* v. 10, p. 27–39, 1999.

MEIKLE, M. C. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. *Eur J Orthod*. v. 28, p.221–40, 2006.

MEYER-MARCOTTY, P.; HARTMANN, J.; STELLZIG-EISENHAUER, A. Dentoalveolar open bite treatment with spur appliances. *J Orofac Orthop*. v.68, n. 6, p. 510-21, 2007.

MICHELSEN, V. B.; KOPPERUD, H. B.; LYGRE, G. B.; BJÖRKMAN, L.; JENSEN, E.; KLEVEN, I. S.; SVAHN, J.; LYGRE, H. Detection and quantification of monomers in unstimulated whole saliva after treatment with resin-based composite fillings in vivo. *Eur J Oral Sci.* v. 120, n. 1, p. 89-95, 2012.

MOURA, J. K. D. *et al.* Quantitative assessment of stimulated saliva flow rate in children and adolescents, *Journal of Dental Science*, v. 23, I.4, 2008.

MUNDY, G. R. Cytokines and local factors which affect osteoclast function. *Int J Cell Cloning* v. 10, p. 215–222, 1992.

NOGUEIRA, F. F.; MOTA, L. M.; NOUER, P. R. A.; NOUER, D. F. Esporão lingual colado *Nogueira*®: tratamento coadjuvante da deglutição atípica por pressionamento *lingual*. *Rev Dental Press Ortod Ortop Facial.* v. 10, n. 2, p. 129-56, 2005.

PALMQUIST, P.; LUNDBERG, P.; LUNDBERG, I.; HANSTROM, L.; LERNER, U. H. 11-1β and TNF-a regulate IL-6-type cytokines in gingival fibroblasts. *Journal of Dental Research.* v. 87, p. 558-563, 2008.

PERINETTI, G.; SERRA, E.; PAOLANTONIO, M.; BRUE, C.; DI MEO, S.; FILIPPI, M. R. Lactate dehydrogenase activity in gingival crevicular fluid during orthodontic treatment: A controlled, short-term longitudinal study. *Journal of Periodontology.* v. 76, p. 411-417, 2005.

PHELAN, A.; FRANCHI, L.; BACCETTI, T.; DARENDELILER, M. A.; MCNAMARA, J. A. Longitudinal growth changes in subjects with open-bite tendency: a retrospective study. *Am J Orthod Dentofacial Orthop*. v. 145, n. 1, p. 28-35, 2004.

POLYDOROU, O. *et al*. Elution of monomers from two conventional dental composite materials. *Dental Materials,* v.2, n.3, p.1535-1541, 2007.

PUTZEYS, E. Simultaneous analysis of bisphenol A based compounds and other monomers leaching from resin-based dental materials by UHPLC–MS/MS. *Journal of Separation Science*, v. 40, n. 5, p. 1063–1075, 2017.

REN, Y.; MALTHA, J. C.; VAN'T HOF, M. A; VON DEN HOFF, J. W.; KUIJPERS-JAGTMAN, A. M.; ZHANG, D. Cytokine levels in crevicular fluid are less responsive to orthodontic force in adults than in juveniles. *J Clin Periodontol*. v. 29, n. 8, p. 757-62, 2002.

ROSA, M.; QUINZI, V.; MARZO, G. Paediatric Orthodontics Part 1: Anterior open bite in the mixed dentition. *European journal of paediatric dentistry* v. 20, p. 80-82, 2019.

RUAN, W. H.; CHEN, M. D.; GU, Z. Y.; LU, Y.; SU, J. M.; GUO, Q. Muscular forces exerted on the normal deciduous dentition. *The Angle orthodontist* v. 75, p. 785-790, 2005.

RUBIN, J.; ACKERT-BICKNELL, C. L.; ZHU, L.; FAN, X.; MURPHY, T. C.; NANES, M. S.; MARCUS, R.; HOLLOWAY, L.; BEAMER, W. G.; ROSEN, C. J. IGF-I regulates osteoprotegerin (OPG) and receptor activator of nuclear factor-kappaB ligand in vitro and OPG in vivo. *J Clin Endocrinol* Metab. v. 87, p. 4273–4279, 2002.

SAITO, S.; NGAN, P.; SAITO, M.; KIM, K.; LANESE, R.; SHANFELD, J.; DAVIDOVITCH, Z. Effects of cytokines on prostaglandin E and cAMP levels in human periodontal ligament fibroblasts *in vitro*. *Arch Oral Biol* v. 35, p. 387–395, 1990.

SAWAI, C.; ANDERSON, K.; WALSER-KUNTZ, D. Effect of bisphenol A on murine immune function: modulation of interferon-gamma, IgG2a, and disease symptoms in NZB × NZW F1 mice. *Environ Health Perspect*. v. 111, p. 1883–1887, 2003.

SCHRÖDER, A.; BAUER, K.; SPANIER, G.; PROFF, P.; WOLF, M.; KIRSCHNECK, C. Expression kinetics of human periodontal ligament fibroblasts in the early phases of orthodontic tooth movement. *J Orofac Orthop*. v. 79, n. 5, p.337-351, 2018.

SCHWENGBERG, S. *In vitro* embryotoxicity assessment with dental restorative materials. *Journal of Dentistry,* v. 33, n. 1, p. 49–55, 2005.

SILVA FILHO, O. G.; GOMES GLONCALVES, R. J.; MAIA, F. A. *Sucking habits*: clinical managementin dentistry. *J Clin Pediatr Dent*. v. 15, n. 3, p. 137-56, 1991.

SILVESTRINI-BIAVATI, A.; SALAMONE, S.; SILVESTRINI-BIAVATI, F.; AGOSTINO, P.; UGOLINI, A.; Anterior open-bite and sucking habits in Italian preschool children. *Eur J Paediatric Dent.* v.1, n. 1, p. 43-6, 2004.

SAMPAIO, I. B. M. Estatística aplicada à experimentação animal. 3ª Ed. Belo Horizonte. Fundação de Ensino e Pesquisa em Medicina Veterinária e Zootecnia, 2007.

SOCRANSKY, S. S.; HAFFAJEE, A. D. Periodontal microbial ecology. *Periodontol 2000.* v. 38, p.135-87, 2005.

SPAGNUOLO, G. *et al*. NF-KB Protection against apoptosis indeced by HEMA. *Journal of Dental Research,* v.83, n.11, p.837-842, 2004.

TAUSCHE, E.; LUCK, O.; HARZER, W. Prevalence of malocclusions in the early mixed dentition and orthodontic treatment need. *Eur J Orthod.* v.26, n. 3, p. 237-44, 2004.

TEIXEIRA, C. C.; KHOO, E.; TRAN, J.; CHARTRES, I.; LIU, Y.; THANT, L. M. Cytokine expression and accelerated tooth movement. *Journal of dental research* v. 89, p. 1135-1141, 2010.

THEILIG C.; TEGTMEIER, Y.; LEYHAUSEN, G.; GEURTSEN, W. Effects of BisGMA and TEGDMA on proliferation, migration, and tenascin expression of human fibroblasts and keratinocytes. *J Biomed Mater Res*. v. 53, n. 6, p. 632-9, 2000.

TUNCER, B. B.; OZMERIC, N.; TUNCER, C.; TEOMAN, I.; CAKILCI, B.; YUCEL, A. Levels of interleukin-8 during tooth movement. *The Angle orthodontist.* v. 75, p. 631-636, 2005.

UEMATSU, S.; MOGI, M.; DEGUCHI, T. Interleukin (lL)-1β, tumor necrosis factor-α epidermal growth factor, and β2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. *Journal of Dental Research*. v. 75, p. 562-567, 1996.

VAN LANDUYT, K.L. *et al*. How much do resin-based dental materials release? A meta-analytical approach. *Dental Materials*, v.27, p.723-747, 2011.

VANDEVSKA-RADUNOVIC, V. Neural modulation of inflammatory reactions in dental tissues incident to orthodontic tooth movement. A review of literature. *European Journal of Orthodontics*. v. 21, p. 231-247, 1999.

WEI, S.; KITAURA, H.; ZHOU, P.; ROSS, F. P.; TEITELBAUM, S. L. IL-1 mediates TNF-induced osteodastogenesis. *The Journal of Clinical Investigation.* v. 115, p. 282-290, 2005.

WISE, G. E.; KING, G. J. Mechanisms of tooth eruption and orthodontic tooth movement. *Journal of Dental Research*. v. 87, p. 414-434, 2008.

YAMAGUCHI, M.; KASAL, K. Inflammation in periodontal tissues in response to mechanical forces. *Archivum Immunologiae et Therapiae Experimentalis*. v. 53, p. 388-398, 2005.

YAMAGUCHI, M.; YOSHII, M.; KASAI, K. Relationship between substance P and interteukin-1β in gingival crevicular fluid during orthodontic tooth movement in adults. *European Journal of Orthodontics*. v. 28, p. 241-246, 2006.

ZIMMERMAN-DOWNS, J. M.; SHUMAN, D. Bisphenol A blood and saliva levels prior to and after dental sealant placement in adults. *Journal of dental hygiene : JDH* v. 84, n. 3, p. 145-150, 2010.

ANEXO 1 - Termo de Consentimento Livre e Esclarecido

(Destinados aos pacientes para avaliação periodontal, saliva e fluido gengival)

O menor de idade pelo qual o(a) senhor(a) é responsável está sendo convidado(a) a participar da pesquisa "**Liberação de bisfenol-A e biocompatibilidade de sistema adesivo ortodôntico utilizado na colagem de esporões em pacientes com mordida aberta anterior: estudos *in vivo***". O objetivo do presente estudo é verificar: (1) a presença de bisfenol A, que é um plastificante (presente em garrafas plásticas, reservatórios de alimento e resina odontológica usada para colagem de acessórios ortodônticos denominados esporões) na saliva; (2) presença de moléculas inflamatórias no fluido gengival; e, (3) avaliar a resposta periodontal. Sua participação no estudo consistirá em doar 1 mL de saliva (que será expelida num tubo de vidro), permitir a coleta do fluido gengival e análise periodontal, em quatro datas e horários a serem combinados (antes da colagem esporão, no dia da colagem do aparelho, 24 horas e 1 semana após a instalação). A partir da saliva será verificada a presença do bisfenol A. O fluido gengival será usado para verificar as moléculas inflamatórias. A pesquisa será realizada nas clínicas odontológicas da Faculdade de Odontologia da Universidade Federal de Minas Gerais (UFMG). Há o risco de um desconforto na língua durante o tratamento ocasionado pelos esporões e desconforto durante as coletas de saliva/ fluido e análise periodontal. Para minimizar estes acontecimentos a colagem dos esporões e as coletas serão feitas por pessoa capacitada e treinada, utilizando material estéril. Mas, caso o desconforto ocorra, você deverá entrar em contato com algum dos pesquisadores para que este seja averiguado e tratado. A resina é um material já disponível no mercado e muito utilizada por ortodontistas. Todas as medidas serão tomadas para minimizar as possibilidades de risco, como a padronização dos procedimentos e utilização de um material confiável. Com esta pesquisa seu filho (a) terá o benefício de corrigir a mordida aberta anterior além da melhora da estética. Em nenhum momento você ou o paciente terão o nome divulgado, e mesmo com a publicação dos resultados a sua identidade será preservada. Você não terá qualquer ônus ou ganho financeiro por participar da pesquisa, porém será beneficiado recebendo o tratamento (portanto, não está previsto nenhuma forma de ressarcimento). Seu filho (a) poderá recusar e/ou deixar de participar deste estudo a qualquer momento, sem nenhum constrangimento ou prejuízo na sua relação com os pesquisadores e a UFMG. Os pesquisadores responsáveis por este projeto podem decidir sobre a exclusão de seu filho (a) do estudo por razões científicas, a respeito das quais você deverá ser devidamente informado. Em caso de qualquer dúvida deverá e/ou poderá entrar em contato a qualquer hora com os pesquisadores responsáveis Soraia Macari ou Gabriel Antônio dos Anjos Tou (31) 34092426.

TERMO DE LIVRE CONSENTIMENTO

Declaro que li e entendi as informações fornecidas nesse termo. Tive a oportunidade de realizar perguntas e todas minhas dúvidas foram respondidas de forma satisfatória. Permito a utilização dos dados e resultados da pesquisa para divulgação e ensino, respeitando meu direito de não ser identificado. Este formulário está sendo assinado por mim em duas vias de igual teor e forma. Recebi uma via deste documento e outra via permaneceu com os pesquisadores.

Local:_____ Data___/___/_____

_____ _____

Nome do paciente Assinatura do responsável

Documento apresentado: _____ N°:_____

Pesquisadores: Soraia Macari / Gabriel Antônio dos Anjos Tou Tel.:(31) 3409-2426
E-mail: soraiamacari@gmail.com / gabrieltou@hotmail.com

_____ _____

Assinatura do pesquisador responsável Assinatura do pesquisador auxiliar

Endereço: Av. Antônio Carlos, 6627. Faculdade de Odontologia. Campus Pampulha. Sala 3204.
Em caso de dúvidas éticas o Comitê de Ética em Pesquisa (COEP – UFMG) poderá ser contactado. Av. Presidente Antonio Carlos, 6627 – Unidade Administrativa II – 2º andar – Sala 2005 – Telefax: 3409 4592 – Belo Horizonte – MG.

ANEXO 2- Termo de Assentimento Livre e Esclarecido (TALE)

ESTUDO: LIBERAÇÃO DE BISFENOL-A E BIOCOMPATIBILIDADE DE SISTEMA ADESIVO ORTODÔNTICO UTILIZADO NA COLAGEM DE ESPORÕES EM PACIENTES COM MORDIDA ABERTA ANTERIOR: Estudo *in vivo*

Prezado participante,

*Você está sendo convidado a participar da pesquisa "***Liberação de bisfenol-A e biocompatibilidade de sistema adesivo ortodôntico utilizado na colagem de esporões em pacientes com mordida aberta anterior: estudo *in vivo****". Seus pais permitiram que você participasse e para isso, gostaríamos de contar com sua ajuda. Sua colaboração neste estudo será muito importante para nós.*

Queremos saber quais os efeitos que o uso do aparelho esporão tem na liberação de uma substância chamada bisfenol-A em sua saliva. Participarão da pesquisa as crianças entre 6 e 9 anos de idade que estão em tratamento com aparelho (esporão) para mordida aberta anterior. Você não é obrigado a participar da pesquisa e não terá nenhum problema se desistir.

A pesquisa será feita nesta clínica que você está em tratamento, onde você vai ter o aparelho colado. Depois disso, um dentista vai examinar os seus dentes e gengiva e pedir para que você cuspa saliva em um pequeno frasco de vidro. Este exame ocorrerá em uma sala separada, onde só você vai ficar para que não se sinta envergonhado. Ninguém saberá que você está participando, não contaremos para outras pessoas e não daremos à estranhos as informações do seu exame e do questionário que você vai responder. Portanto, não precisa se envergonhar.

Se você tiver alguma dúvida, você pode me perguntar.

Eu _____, aceito participar da pesquisa (Liberação de bisfenol-A e biocompatibilidade de sistema adesivo ortodôntico utilizado na colagem de esporões em pacientes com mordida aberta anterior: estudo *in vivo*), que tem o objetivo de descobrir a influência que o uso do aparelho fixo causa na qualidade de vida da criança/adolescente. Esclareço que obtive todas as informações necessárias. Recebi uma via deste termo de assentimento, li e concordo em participar da pesquisa.

Local:_____ Data___/___/_____

_____ _____

Sua assinatura Assinatura do responsável

Documento apresentado: _____ N°:_____

Pesquisadores: Soraia Macari / Gabriel Antônio dos Anjos Tou Tel.:(31) 3409-2426
E-mail: soraiamacari@gmail.com / gabrieltou@hotmail.com

_____ _____

Assinatura do pesquisador responsável Assinatura do pesquisador auxiliar

Endereço: Av. Antônio Carlos, 6627. Faculdade de Odontologia. Campus Pampulha. Sala 3204.
Em caso de dúvidas éticas o Comitê de Ética em Pesquisa (COEP – UFMG) poderá ser contactado. Av. Presidente Antonio Carlos, 6627 – Unidade Administrativa II – 2º andar – Sala 2005 – Telefax: 3409 4592 – Belo Horizonte – MG.

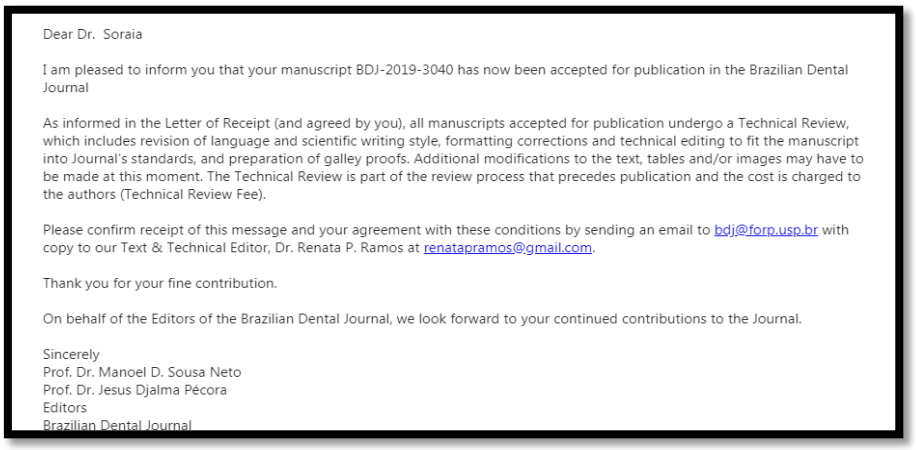
PRODUÇÃO CIENTÍFICA E ATIVIDADES DESENVOLVIDAS

- Apresentação de trabalho científico:

“AVALIAÇÃO DOS EFEITOS DE SUBPRODUTOS DE UM ADESIVO ORTODÔNTICO EM QUERATINÓCITOS HUMANOS” de autoria Tou GAA, Rinco LSO, Arruda JAA, Oliveira RF, Marquiore LF, Souza JVR, Macari S, Diniz IMA, apresentado na 36ª Reunião da Sociedade Brasileira de Pesquisa Odontológica.

- Artigo Aceito

Artigo aceito pelo periódico “Brazilian Dental Journal” (Qualis A2) em participação no Projeto de atendimento Ortodôntico à pacientes portadores de Fissura labiopalatina UFMG. Texto se encontra no formato da revista



Dear Dr. Soraia

I am pleased to inform you that your manuscript BDJ-2019-3040 has now been accepted for publication in the Brazilian Dental Journal

As informed in the Letter of Receipt (and agreed by you), all manuscripts accepted for publication undergo a Technical Review, which includes revision of language and scientific writing style, formatting corrections and technical editing to fit the manuscript into Journal's standards, and preparation of galley proofs. Additional modifications to the text, tables and/or images may have to be made at this moment. The Technical Review is part of the review process that precedes publication and the cost is charged to the authors (Technical Review Fee).

Please confirm receipt of this message and your agreement with these conditions by sending an email to bdj@forp.usp.br with copy to our Text & Technical Editor, Dr. Renata P. Ramos at renatapramos@gmail.com.

Thank you for your fine contribution.

On behalf of the Editors of the Brazilian Dental Journal, we look forward to your continued contributions to the Journal.

Sincerely
Prof. Dr. Manoel D. Sousa Neto
Prof. Dr. Jesus Djalma Pécora
Editors
Brazilian Dental Journal

Early management of cleft lip and palate treated with nasoalveolar molding (NAM): a case report

Nathália Viegas de Oliveira[1], Gabriel Antônio Anjos Tou[2], Raquel Souto Silva[3], Elizabeth Maria Bastos Lages[4], Sérgio Edriane Rezende[5], Henrique Pretti[4], Soraia Macari[6]

1 Resident student, Department of Pediatric Dentistry and Orthodontics, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

2 Master student, Department of Pediatric Dentistry and Orthodontics, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

3 Undergraduate student. Departamento de Odontopediatria e Ortodontia, Faculdade de Odontologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

4 Associated professor, Ph.D., Department of Pediatric Dentistry and Orthodontics, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

5 Associated professor, Ph.D., Department of Head and Neck Surgeon andSkull-Maxillofacial Surgeon, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

6 Assistant professor, Ph.D., Department of Pediatric Dentistry and Orthodontics, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Corresponding author: Reprint requests to: Soraia Macari. Mailing address: Departamento de Odontopediatria e Ortodontia, Faculdade de Odontologia, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos 6627, CEP 31.270-901, Belo Horizonte, Minas Gerais, Brazil. E-mail: soraiamacari@gmail.com

The first-year follow-up of a cleft lip and palate patient treated with nasoalveolar molding (NAM)

A cleft lip and palate patient treated with NAM

ABSTRACT

The objectives of pre-surgical orthopedics are to allow surgical repair with minimal tension of the involved tissues and less restriction to the craniofacial growth. The aim of this study was to evaluate the benefits of nasoalveolar model (NAM) as a pre-operative therapy in a patient with bilateral cleft lip and palate followed by labioplasty and palatoplasty. A 15-day-old patient underwent orthopedic treatment with NAM. After pre-operative treatment, retraction of the pre-maxilla was observed with reduction of the fissure. Due to the successful effects of NAM treatment the patient had a one-step surgery for lip correction. Six months later, due to lip pressure the fissure was further decreased. After six months, the patient underwent palatoplasty. Both surgeries contributed to the remaining closure of the fissure, which were reduced by half compared to the end of pre-operative treatment. The uses of NAM as a pre-operative treatment approached the alveolar segments, centralized the pre-maxilla, decreased the cleft palate resulting in a marked improvement of the arch and provide superior surgical results. In addition, it allows the primary repair of the patient's lip with asymmetric bilateral fissure in only one-step surgery; in consequence, it will reduce treatment morbidity and decrease cost of treatment.

Key words: nasoalveolar molding, cleft lip and palate, labioplasty, palatoplasty.

INTRODUCTION

The protocol for the treatment of cleft lip and palate is considered a challenge in dentistry (1), the approach consists of the use of the nasoalveolar molding (NAM) proposed by Grayson et al. (2). The treatment with NAM preceding the surgical intervention is used to nasal cartilages reposition, to approach the alveolar processes, centralize the pre-maxilla, and to elongate the deficient columella (3, 4). Cleft lip and palate may be unilateral or bilateral (5). In the bilateral cleft deformity the lower lateral cartilages fails to migrate up into the nasal tip to stretch the columella. The prolabium is positioned directly on the end of the shortened columella. The alar cartilages are positioned along the alar margins and pre-maxilla is suspended from the tip of the nasal septum, whereas the lateral alveolar segments remains behind it (6).

The surgical repair of bilateral cleft lip and palate deformity presents additional challenges for satisfactory results (7), especially when it is accompanied by asymmetry of the alveolar and palate segments with the displaced and projected pre-maxilla (8). During treatment, bilateral cleft lip repair can be performed in one or two stages (8-10); and in cases of asymmetric or incomplete bilateral cleft with displaced or severely projected pre-maxilla, short or absent columella, two-stage lip closure is more feasible (7, 10, 11).The purpose of pre-surgical orthopedics is to reduce the width of cleft, to obtain the alignment of the segments before the labioplasty allowing the surgical repair with minimum tension, to improve the shape of the arch and to normalize the swallowing pattern avoiding the dorsal positioning of the tongue in the cleft (3, 7, 12).

Considering that the NAM technique has been shown to significantly improve the surgical outcome of the primary repair in cleft lip and palate patients compared to other techniques of pre-surgical orthopaedics (7), the objective of this article is to show the progress of the pre-operative treatment with the use of NAM and its contribution for the accomplishment of labioplasty in only one-stage surgical time followed by the palatoplasty in a patient with complete bilateral lip and palate cleft using a clinical case report.

Diagnosis and Etiology

A male child, 15-old-day patient was referred to an orthodontic evaluation to facilitate the surgical repair of the lip and cleft palate. The patient had a complete bilateral incisive trans-foramen lip and cleft palate with pre-maxilla shifted to left (Figure 1).According to the mother's report, the complete cleft palate was confirmed at the end of the pregnancy.

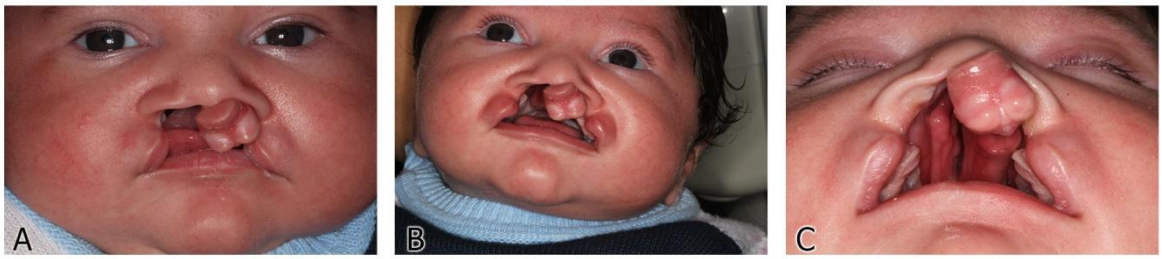


Figure 1: A male child, 15-old-day had a complete bilateral incisive trans-foramen lip and cleft palate with premaxilla shifted to left.

The initial impression of the maxilla was made to fabricate the NAM conventional molding plate (Figure 2). The casts obtained during the follow-up treatment were used to measure the width of the cleft, the length of the arch and the distance between the alveolar segments (Figure 3). The outermost portion of each segment was identified by letters as shown in Figure 3. The cleft was measured by the distance from the mesial segment of the maxillary arch to the distal part of the pre-maxilla segment bilaterally. The width of the alveolar ach was analyzed what was equivalent to the canine (anterior arch width A-D), first (middle palatal arch width E-F) and second molars distance (posterior palatal arch width G-H). Additionally the length of the arch was represented by the I-J line (Figure 3). The values of the measurements are showed in Table 1. From these measurements it was verified that the pre-maxilla was protruded and decentralized compared to the alveolar segments (Figure 1).

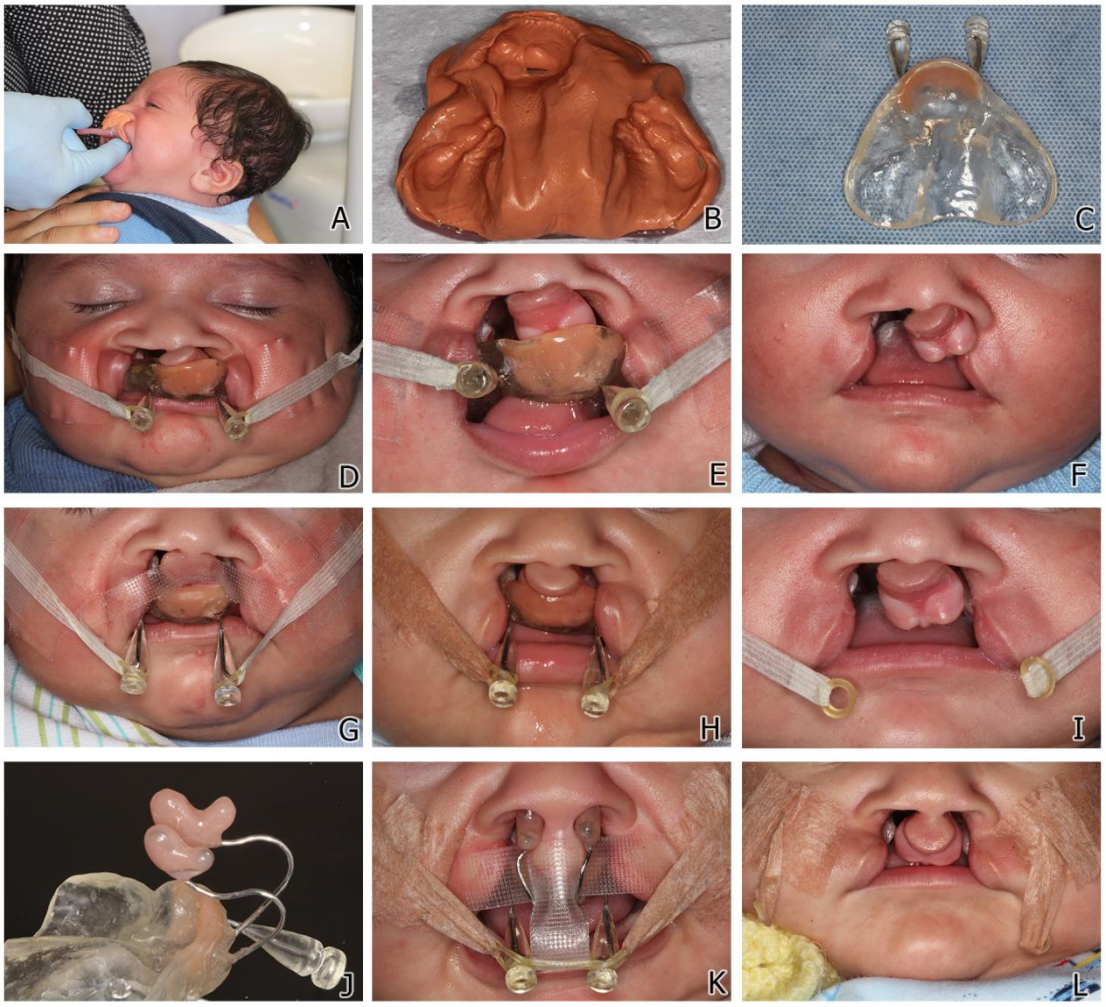


Figure 2: Sequence of treatment with the use of NAM.

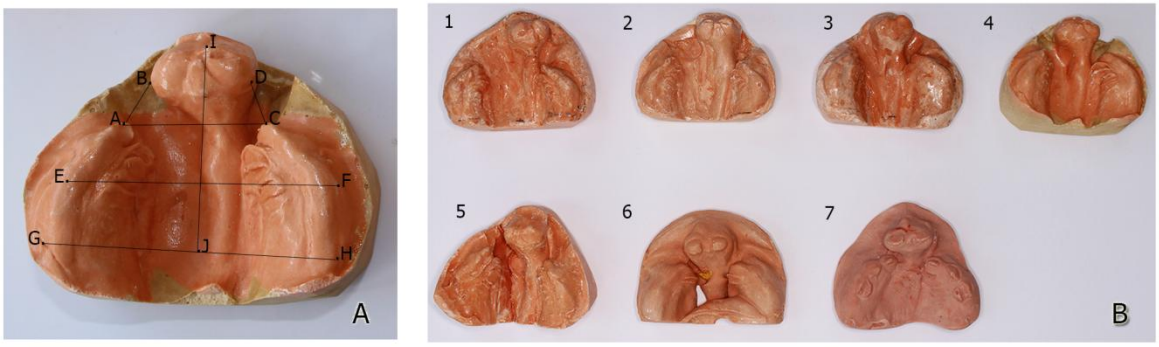


Figure 3: Marking of the outermost points in each anterior, middle and posterior segment of the arch and width of the clefts and registered patient models throughout preoperative and post-operative treatment

Treatment Objectives

The treatment objectives were to redirect the pre-maxilla to the center of the arch using an orthopedic device nasoalveolar molding (NAM) and to align and approximate the maxillary alveolar segments, and also to elongate the columella pre-operatively and therefore facilitate labioplasty repair within not in two, but in only one-stage surgical time. In addition, this treatment also aimed to reduce the tension in the soft tissues and muscle sutures involved to favor the prognosis of palatoplasty.

Treatment Progress

The treatment was performed in several stages: (A) pre-operative orthopedics using the NAM fifteen days post-birth, (B) labioplasty surgery three months post-birth, and (C) palatoplasty surgery sixteen months post-birth.

Intraoral maxillary impressions were made using heavy bodied impression material (Express XT ESPE, Unitek/3M, CA, USA) and casts were made from dental stone to prepare the diagnosis and working cast (Figure 2). The conventional custom molding plate was provided using acrylic resin (JET Clássico, Paulista, SP, Brazil) (3) (Figure 2). External forces were applied to the plate to retract and centralize the pre-maxilla (9) by an elastic band (tape Elastic Intraoral Latex 3/16 – Morelli, Sorocaba, SP, Brazil) attached to a custom-fitted head cap (Figure 2). Furthermore, to facilitate the lip repair and balanced orofacial growth hypoallergenic adhesive tapes (Micropore surgical tape; 3M, St Paul, MN) were placed by parents on the lip segments to adduct the lip segments and also to narrow the alar base of the cleft segment (7, 13) (Figure 2). Parents were advised to use NAM 24 hours a day, 7 days a week, in addition to a brief period of cleaning the device twice a day.

During each return visit, selective internal wear on the appliance was made as necessary to move the alveolar segments to the desired area to reduce the distance between the segments and to produce better contour to the alveolar arch. After the alveolar gap was reduced, the nasal stent was bent at the end of a 0.032-in stainless steel wire that was embedded into the anterior portion of the alveolar molding plate (5). An acrylic extension that pushes the nostrils upward against a counterforce of soft material across the nasolabial junction and stretches the diminutive columella (2, 3, 14) was added (Figure 2). The follow-up treatment was made by new impressions that were made during the appointments (Figure 3).

The active participation of parents and their ability to follow the instructions of home care was fundamental to the therapy. Appointments were scheduled at intervals of one week initially and later there was a two week (5) space.

Subsequently, after three months of intensive use of NAM, the patient underwent the labioplasty. Firstly, the distance between the outermost region of the pre-maxilla and the bilateral alveolar segments was measured before the incision to determine the extent of detachment of the involved tissues (Figure 4). The Mulliken technique (9) was used by the surgeon, as a one-step technique, and the cutaneous incisions were then performed to simulate the lip filter to reconstruct the fundus of the gingivolabial sulcus, interrupting or reducing nasal buccal communication in the anterior region (Figure 4). The orbicularis muscle strap was made in the pre-maxilla by a pressure favoring its retro position (Figure 4).

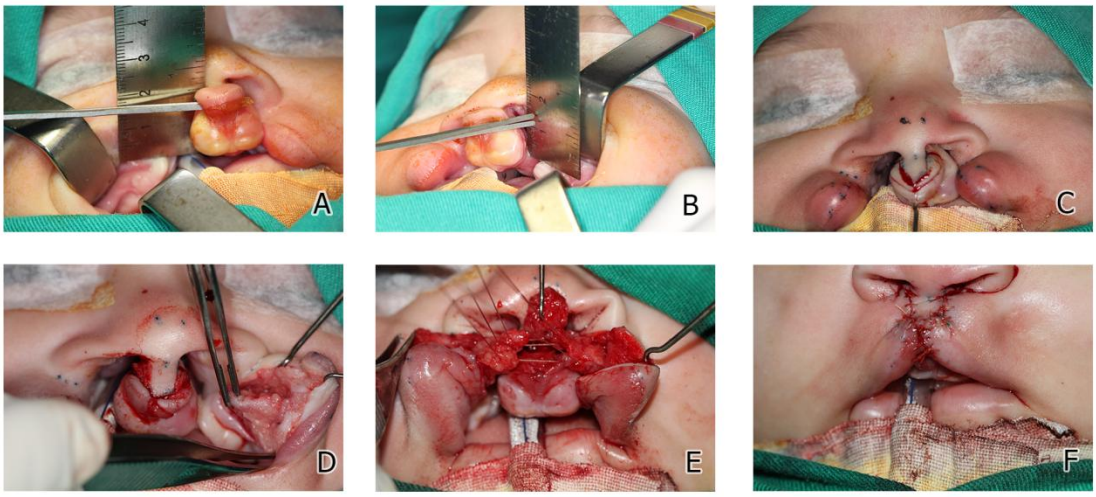


Figure 4: Primary surgery labioplasty.

Primary palatoplasty determines the prognosis of a patient, and an optimal surgical design should functionally restore functions, including speech, chewing, breathing and aesthetics, while at the same time preserving the normal growth potential in the involved area (15). The patient underwent palatoplasty using the Veau-Wardil-Kikner technique when he completed one year and four months of age (Figure 5) by the same team of surgeons who performed the labioplasty.

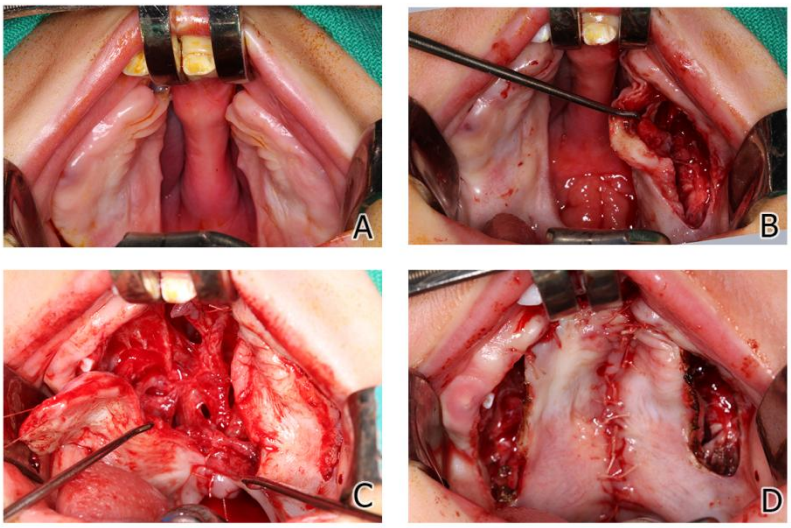


Figure 5: Primary surgery palatoplasty.

Treatment Results

At the end of the pre-operative treatment a reduction of 3 mm in the length of the arch was observed with the retraction of the pre-maxilla. There was a reduction of 8 mm from the right side fissure and 4 mm from the left side fissure due to the centralization of the pre-maxilla (Figure 3 model 5).The decrease in arch width was 3 mm in anterior part of palate and 2mm in posterior part (Table 1, model 5). The pre-maxilla was retracted by using the molding plate in conjunction with external tape and elastics (Figure 2).

Table 1. Alveolar cleft and alveolar arch measurements in millimeters (mm) within the different treatment phases

Stone plaster casts	Alveolar cleft in right side	Alveolar cleft in left side	Anterior arch width	Mid palatal arch width	Posterior arch width	Arch length
	A-B	C-D	A-D	E-F	G-H	I-J
	Mm					
1. Initial	14	10	24	50	48	29
2. Fifiteen days after NAM´s use	11	9	23	50	47	29
3. One month afer NAM´s use	10	7	21	50	46	28
4. Two month after NAM´s use	6	7	21	50	46	27
5. Pre-surgery	5	6	21	50	46	26
6. After labioplasty	3	4	16	50	49	22
7. After palatoplasty	2	3	10	48	51	22

Two impressions were made for post-surgical measurements (Figure 3 models 6 and 7). After three months of intensive treatment with NAM, the patient underwent labioplasty. Six months later, a significant reduction of cleft palate was measured in the plaster model and was compared with the initial model. As shown in Table 1, model 6, the pre-maxilla was retracted 7 mm and shifted to the right side by 11 mm after labioplasty. In this same period, the only decrease in arch width was 2mm in mid palatal part.

After six months of palatoplasty, a new cast was analyzed (Figure 3 model 7). Comparing these results and the model performed six months after the labioplasty (Figure 3, model 7 compared to model 6), any changes where seen in arch length. In despite of this, a significant decrease of 6 mm was observed in the anterior part of

the arch resulting from the closure of the palate and 2 mm in the middle part by traction of the tissues after surgery. There was a 2 mm increase in the width in the posterior part of the arch by the growth of skeletal structures. In addition, there was a reduction of 1 mm on both sides of clefts (Table 1). This shows that both labioplasty and palatoplasty surgeries contributed to closure of clefts (Figure 5 and Table 1). Measurements after the palatoplasty show a reduction on the cleft in half when comparing to the model after the pre-operative treatment (Table 1 and Figure 3 model 7 compared to model 5).

DISCUSSION

Treatment of patients with cleft lip and palate may combine surgery and orthopedics. The orthopedic treatment of patient described in our study started 15 days post birth. The patient presented a complete bilateral trans-forame lip and palate cleft and pre-maxilla displaced to the left. Alveolar gap bilateral were 14 mm and 10 mm successively. In view of these conditions, the nasal alveolar molding was used until the period of labioplasty. After orthopedic treatment, there was a centralization of the pre-maxilla and reduction of the clefts due to the approximation of the alveolar segments obtained by the use of NAM. Because of the good results (Figure 6), the surgical correction of the lip was made in only one surgery, unlike what is proposed by some authors regarding the severity of the initial case (8, 10).

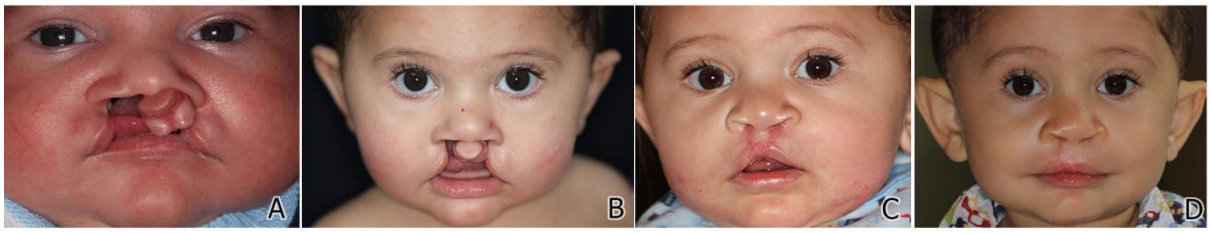


Figure 6: Pretreatment, post-treatment pre-operatory, one month after labioplasty and one month after palatoplasty, respectively.

The practice of NAM offers some significant benefits to the patient. The objectives of NAM, as described by many authors (1-4, 7, 12, 14, 16) are to provide symmetry to severely deformed nasal cartilages, to achieve projection of the flattened nasal tip, to provide nonsurgical elongation of the columella, to improve

alignment of the alveolar ridges, and to reduce the distance between the cleft lip segments. NAM can be applied to the entire range of cleft deformities, including complete clefts without an intact nasal floor (5) in corroboration with our case.

Several pre-surgical infant orthopedics techniques have been described, such as lip taping. This technique is a nonsurgical method of mobilizing and graft on the soft tissues of the lip, nose, and maxilla before primary cleft lip repair (17). It is a widely used method, but is a complementary technique to NAM therapy. Another alternative to the treatment is pre-directional appliance (16) which is the modification of Grayson's pre-surgical nasoalveolar molding. This technique has presented excellent results in a case report that the pre-maxilla was shifted to the left side by 5.5 mm in one patient with bilateral fissure and projected pre-maxilla asymmetry (16). This finding is compatible with our study, which also showed success in the centralization of the maxilla through the approximation of the segments, with a 7 mm retraction of the pre-maxilla and was displaced to the right side in 11 mm.

As shown in the present case report, excellent results were obtained in the approximation of the alveolar segments and in the centralization of pre-maxilla. This can be explained by the wearing of the acrylic in the direction of the desired movement and the addition of acrylic resin in the space of the pre-maxilla similarly proposed by Grayson (2). However, there was little improvement in the elongation of the columella. This may be justified by delayed activation of nasal stents.

The correction of the cleft nasal deformity is a great esthetic challenge to the orthodontist. The patient has used a conventional stent incorporated in the appliance when the alveolar cleft gap was reduced. Subramanian et al (4) modified the NAM device by making the nasal stent with titanium molybdenum alloy (TMA) wire. The advantage of the TMA wire is that it is more resilient, and hence, activation can be done once in 2 weeks. But this technique is appropriate only for unilateral cleft palate. Matsuo et al. (18) reported that cartilage alar is more malleable to the orthopedic maneuver soon after birth, a result of elevated levels of maternal estrogen circulating in the child's bloodstream and sialuronic acid levels in the nasal cartilage. We must take advantage of the plasticity of the infant cartilage, whereas at three months of age a reduction of this plasticity occurs. Through this, the authors declare pre-operative non-surgical correction should therefore begin as soon as possible (19, 20) to promote permanent correction in its form (3).

Lip taping or surgical lip adhesion as alone procedures can be a disadvantage for bilateral cleft lip and palate patients. If the control of the alveolar segments is not achieved, the pre-maxilla can descend vertically, and the anterior aspect of the posterior alveolar segments can collapse palatally (5). The lip taping is a simple inexpensively technique. It is applied on the lip along the cleft just after birth to reshape and approach the alveolar arch (17) and in our study it was used as auxiliary therapy to NAM (3). Also, the purpose of the use of facial adhesive tape associated with elastic was to assist in the fixation of NAM on the face of the baby and to press lightly against the pre-designed maxilla. Although head traction has been used for a long time, Graysonet al. (3) reported that wearing on the acrylic results in a more controlled positioning of the pre-maxilla.

Considering the results obtained from early cleft care with pre-operative orthopedics, the patient was submitted to labioplasty after three months of intensive use of NAM. Primary surgical correction protocol can be done in one or two-stages. For this, alveolar gap asymmetry, length of prolabium and pre-maxilla's projection should be evaluated. In cases of large asymmetry and severely projection which tension created during the surgery to join tissues is excessive, a two-stage procedure is recommended (10). In this case, first, closure on one side of the lip (usually the more severely involved) and then, the other side (9). However, getting the symmetric lip in two-stages is more difficult than in the simultaneous closure of both sides, it may require addition revisions (11), increasing patient morbidity(8) and a more expensive treatment (21). Therefore, our objective was to approximate alveolar segments to reduce tension in the sutures during primary surgery and to enable the procedure in one-stage. Labioplasty was successful. Furthermore simultaneous lip repair closure facilitated creation of a symmetric and balanced lip (10), thus palatoplasty was performed nine months later.

There is a direct relationship between increased size of cleft lip, increased tension of the tissue and severity of scars; thus, main post-surgical complications include unpleasant suture line scar and unresolved nasal deformities (20). In this way the pre-operative treatment with the NAM seems to be fundamental for better post - surgical results (21-24). That was shown not only in the present case report but also in a comparative study where patients who were treated with NAM obtained better aesthetic and functional results (20).

As previously reported, early cleft care using NAM are clearly described on the literature (13). There is no doubt about the gains made by pre-operative orthopedics (13). However, parental collaboration was the main factor that contributed to the success of the treatment. The birth of a child with a fissure can be traumatic and challenging for the family due to severe distortion of the nose, lip and maxillary arch (5). Therefore, the orthodontist should encourage parents during each visit to use NAM in so that the gains are compatible with the expected result.

CONCLUSION

The use of NAM as a pre-operative treatment approached the alveolar segments, centralized the pre-maxilla, and decreased the cleft palate resulting in a marked improvement of the arch. In addition, it allows the primary repair of the patient's lip with asymmetric bilateral fissure in only one-step surgery in consequence it will reduce treatment morbidity, decrease cost and provide better results during primary repair.

REFERENCES

(1) M. Eruz, A. Yagci, I. Ozyazgan. A New Technique for Retracting the Protrusive Premaxilla With Fewer Patient Visits. Cleft Palate Craniofac J 2019;4:543-547.

(2) B.H. Grayson, C. Cutting, R. Wood. Preoperative columella lengthening in bilateral cleft lip and palate. Plast Reconstr Surg 1993;92:1422-1423.

(3) B.H. Grayson, P.E. Santiago, L.E. Brecht, C.B. Cutting. Presurgical nasoalveolar molding in infants with cleft lip and palate. Cleft Palate Craniofac J 1999;36:486-498.

(4) C.S. Subramanian, N.K. Prasad, A.B. Chitharanjan, E.J. Liou. A modified presurgical orthopedic (nasoalveolar molding) device in the treatment of unilateral cleft lip and palate. Eur J Dent 2016;10:435-438.

(5) B.H. Grayson, J.S. Garfinkle. Early cleft management: the case for nasoalveolar molding. Am J Orthod Dentofacial Orthop 2014;145:134-142.

(6) D.R. Millard, Jr. Embryonic rationale for the primary correction of classical congenital clefts of the lip and palate. Ann R Coll Surg Engl 1994;76:150-160.

(7) B.H. Grayson, C.B. Cutting. Presurgical nasoalveolar orthopedic molding in primary correction of the nose, lip, and alveolus of infants born with unilateral and bilateral clefts. Cleft Palate Craniofac J 2001;38:193-198.

(8) H. Xu, K.E. Salyer, E.R. Genecov. Primary bilateral two-stage cleft lip/nose repair: part II, J Craniofac Surg 2009;20:1927-1933.

(9) J.B. Mulliken. Repair of bilateral cleft lip and its variants. Indian J Plast Surg 2009;42:S79-90.

(10) H. Xu, K.E. Salyer, E.R. Genecov. Primary bilateral one-stage cleft lip/nose repair: 40-year Dallas experience: part I. J Craniofac Surg 2009;20:S1913-1926.

(11) K. Chung, L.J. Lo. One-Stage vs. Two-Stage Repair of Asymmetric Bilateral Cleft Lip: A 20-Year Retrospective Study of Clinical Outcome. Plast Reconstr Surg 2018;141:1215-1224.

(12) D.J. Maull, B.H. Grayson, C.B. Cutting, L.L. Brecht, F.L. Bookstein, D. Khorrambadi, J.A. Webb, D.J. Hurwitz. Long-term effects of nasoalveolar molding on three-dimensional nasal shape in unilateral clefts. Cleft Palate Craniofac J 1999;36:391-397.

(13) B. Isik Aslan, A. Gulsen, K. Findikcioglu, D. Uzuner, N. Ucuncu. Effects of Nasoalveolar Molding Therapy on Alveolar and Palatal Cleft Deformities in Unilateral and Bilateral Cleft Lip and Palate. J Craniofac Surg 2018;29:e179-e184.

(14) R. Punga, S.M. Sharma. Presurgical orthopaedic nasoalveolar molding in cleft lip and palate infants: a comparative evaluation of cases done with and without nasal stents. J Maxillofac Oral Surg 2013;12:273-288.

(15) B. Shi, J.E. Losee. The impact of cleft lip and palate repair on maxillofacial growth. Int J Oral Sci 2015;7:14-17.

(16) P.S. Daigavane, P. Hazarey, R. Vasant, R. Thombare. Pre-directional appliance: a new approach to correct shifted premaxilla in bilateral cleft cases. J Indian Soc Pedod Prev Dent 2011;29:S39-43.

(17) R. Pool, T.K. Farnworth. Preoperative lip taping in the cleft lip. Ann Plast Surg 1994;32:243-249.

(18) K. Matsuo, T. Hirose, T. Tomono, M. Iwasawa, S. Katohda, N. Takahashi, B. Koh. Nonsurgical correction of congenital auricular deformities in the early neonate: a preliminary report. Plast Reconstr Surg 1984;73:38-51.

(19) K. Matsuo, T. Hirose. Preoperative non-surgical over-correction of cleft lip nasal deformity. Br J Plast Surg 1991;44:5-11.

(20) A. Kalantar-Hormozi, A. Ghalambor, M. Tolouei. Investigating the surgical outcomes of Nasoalveolar Molding (NAM) approach in cleft lip and palate repairing operations. International Journal of Pharmaceutical Research and Allied Sciences 2016;5:412-416.

(21) P.A. Patel, M.S. Rubin, S. Clouston, F. Lalezaradeh, L.E. Brecht, C.B. Cutting, P.R. Shetye, S.M. Warren, B.H. Grayson. Comparative Study of Early Secondary Nasal Revisions and Costs in Patients With Clefts Treated With and Without Nasoalveolar Molding. J Craniofac Surg 2015;26:1229-1233.

(22) Q. Yu, X. Gong, G. Shen. CAD presurgical nasoalveolar molding effects on the maxillary morphology in infants with UCLP. Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:418-426.

(23) V. Shetty, H.J. Vyas, S.M. Sharma, H.F. Sailer. A comparison of results using nasoalveolar moulding in cleft infants treated within 1 month of life versus those treated after this period: development of a new protocol. Int J Oral Maxillofac Surg 2012;41:28-36.

(24) Z. Liang, J. Yao, P.K.T. Chen, C. Zheng, J. Yang. Effect of Presurgical Nasoalveolar Molding on Nasal Symmetry in Unilateral Complete Cleft Lip/Palate

Patients after Primary Cheiloplasty without Concomitant Nasal Cartilage Dissection: Early Childhood Evaluation. Cleft Palate Craniofac J 2018;12:2612-2617.