

Universidade Federal do Rio de Janeiro – UFRJ
Centro de Ciências Da Saúde
Faculdade de Odontologia

**BRÁQUETES AUTOLIGADOS VERSUS CONVENCIONAIS:
ASPECTOS MICROBIOLÓGICOS E BIOMECÂNICOS**

Leonard Euler Andrade Gomes do Nascimento
CD, MO

Tese submetida ao corpo docente da Faculdade de Odontologia da Universidade Federal do Rio de Janeiro – UFRJ, como parte dos requisitos, para a obtenção de título de Doutor em Odontologia (Área de Concentração em Ortodontia).

Rio de Janeiro
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Orientadora: Profa. Dra. Margareth Maria Gomes de Souza, CD, MO, DO

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- 1. Bráquetes**
- 3. Biomecânica**

- 2. *Streptococcus mutans***
- 4. Tese**

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aos meus pais:

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a todos os meus sobrinhos:

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LISTA DE SIGLAS, ABREVIATURAS E SÍMBOLOS

* - rates of tooth movement followed by the same letters do not differ among themselves at a 5% significance level when compared using Tukey's test (p -value=5%) with Minimum Significant Difference=0.5407

§ - means followed by the same letters do not differ among themselves at a 5% significance level when compared using Tukey's test (P -value=5%)

AA - *Aggregatibacter actinomycetemcomitans*

AL – bráquetes autoligados

ANOVA - analysis of variance

AT - apical third

ATP - adenosine triphosphate

BFR - bone formation rate

BHI - brain heart infusion

C - compression side

C1 - control teeth 1

C2 - control teeth 2

CCT - ensaios clínicos controlados

CFU - colony forming unit

CIOMS/WHO - Council for International Organizations of Medical Sciences

CON - bráquetes convencionais

CrNi – fio de aço inoxidável

D - distal side

EW – conventional edgewise brackets

GT - gingival third

M - mesial side

Max - maximum

Min – minimum

MS - *Mitis Salivarius*

MSD - Minimum Significant Difference

MT - middle third

NDB - non-defined breed

P - prospectivo

PBS - Paraformaldehyde and Phosphate Buffer

PDL – Periodontal Ligament

PICO - Population Intervention Comparator Outcomes - População Intervenção
Comparação Resultados

RCT - Ensaio Clínicos Randomizados

SD – Standard Deviation

SEM - Scanning Electron Microscopy

SL - edgewise self-ligating brackets

SRD – sem raça definida

SS - stainless steel wire

T - traction side

T₀ - beginning of orthodontic mechanics, just before the activation with gray
chain elastics

T₁₅ - after 15 days of the beginning of orthodontic mechanics

UESPI - Universidade do Estado do Piauí

UFC - Unidades de Formação de Colônias

UFPI - Universidade Federal do Piauí

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RESUMO

NASCIMENTO, LEONARD EULER ANDRADE GOMES DO. Bráquetes autoligados versus convencionais: aspectos biomecânicos e microbiológicos. Orientadora: Profa. Dra. Margareth Maria Gomes de Souza. Rio de Janeiro: UFRJ/Faculdade de Odontologia, 2013, Tese (Doutorado em Odontologia – Ortodontia). 99f.

Introdução: Esta pesquisa teve como objetivo realizar comparações múltiplas de dois sistemas de bráquetes *edgewise*: convencionais e autoligados, verificando: a influência da configuração destes bráquetes na aderência e formação de colônias de *Streptococcus mutans*; a movimentação ortodôntica e as atividades de remodelação óssea. **Material e Método:** No primeiro estudo realizou-se uma revisão sistemática, onde avaliou-se a evidência científica de sistemas de bráquetes com o nível de microrganismos bucais. No segundo estudo, realizou-se estudo clínico em 10 humanos adultos masculinos, que serviram de hospedeiro para os bráquetes. Coletas de microrganismos foram realizadas de 20 bráquetes estéticos convencionais e 30 autoligados. Já no terceiro estudo, avaliou-se em 20 cães masculinos adultos, a movimentação dentária por deslize, promovida por sistemas convencionais e autoligados sob o ponto de vista clínico e celular. **Resultados/Conclusões:** No aspecto microbiológico, pesquisado por meio de revisão sistemática, seis artigos elegíveis, de elevada qualidade metodológica científica, não foram unânimes no relato da influência da configuração dos bráquetes (convencionais ou

autoligados) na adesão e a formação de colónias de *Streptococcus mutans*. No estudo clínico não houve diferença estatisticamente significativa na colonização de *S. mutans* quando comparados com os estéticos convencionais. As diferenças de colonização foram relacionadas com a composição do material que constitui o bráquete. Já quanto à movimentação ortodôntica, não teve significado estatístico semelhante, tendo os bráquetes autoligados as taxas mais elevadas: 3,1 milímetros (maxila) e 2,9 mm (mandíbula). No número de osteoblastos, os dentes com autoligados tiveram significado estatístico semelhante no lado de tensão no terço gengival da raiz e no lado da compressão no terço apical, da mesma forma na contagem de osteoclastos, no lado da compressão no terço gengival e lado de tensão no terço apical. Ao comparar a quantidade da movimentação ortodôntica e a avaliação histológica da atividade de remodelação, observou-se maior presença de movimento de translação dos dentes com o sistema convencional, e maior de inclinação nos dentes com os autoligados.

SUMMARY

NASCIMENTO, LEONARD EULER ANDRADE GOMES DO. Bráquetes autoligados versus convencionais: aspectos biomecânicos e microbiológicos. Orientadora: Profa. Dra. Margareth Maria Gomes de Souza. Rio de Janeiro: UFRJ/Faculdade de Odontologia, 2013, Tese (Doutorado em Odontologia – Ortodontia). 99f.

Introduction: This study aimed to perform multiple comparisons of two systems of edgewise brackets: conventional and self-ligating, assessing the influence of the configuration of these brackets on adherence and formation of colonies of *Streptococcus mutans*; orthodontic movement and activities in bone remodeling. **Material and Methods:** In the first study we carried out a systematic review, which evaluated the scientific evidence of brackets systems with the level of oral microorganisms. In the second study, clinical study was conducted in 10 adult human male, who served as host for the brackets. Collections of microorganisms were performed from 20 conventional aesthetic brackets and 30 self-ligating. In the third study with 20 adult male dogs, it was evaluated the tooth movement by sliding mechanics with conventional and self-ligating under clinical and cellular assessment. **Results/Conclusions:** In the microbiological aspect, searched through systematic review, six eligible articles, with high quality scientific methodology, were not unanimous in reporting the influence of the configuration of the brackets (conventional or self-ligating) on the adherence and the formation of colonies of *Streptococcus mutans*. In the

clinical study there was no statistically significant difference in the colonization of *S. mutans* when compared with the conventional cosmetic. The differences in colonization were related to the composition of the material constituting the bracket. As for the orthodontic movement, was not statistically significantly similar, with the brackets SL higher rates: 3.1 mm (maxilla) and 2.9 mm (lower jaw). The number of osteoblasts were statistically significant in the teeth with such self-ligating at the tension side of the gingival third of the root and the side of the compression in the apical third, likewise the count of osteoclasts on the compression side at the gingival third and on the tension side at the apical third. By comparing the amount of orthodontic movement and histological evaluation of remodeling activity, we observed a higher presence of translational movement of the teeth with the conventional system, and greater tipping in the teeth with self-ligating.

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1 INTRODUÇÃO

Os bráquetes convencionais (CON) estão associados com o uso de ligaduras elásticas ou de aço inoxidável para manter o fio ortodôntico inserido no interior da canaleta (Forsberg, Brattstrom *et al.*, 1991). O termo autoligados (AL), em Ortodontia, refere-se à bráquetes ortodônticos que possuem mecanismo próprio, de abertura e fechamento da canaleta, para a fixação do fio, não requerendo ligaduras metálica ou elástica para este fim (Berger, 1999; Cacciafesta, Sfondrini *et al.*, 2003). Os AL podem ser considerados passivos, com cliques rígidos (Damon, Smart Clip, Vision); ativos, com cliques flexíveis que sempre pressionam o arco independentemente da sua espessura (Quicklear, In-Ovation, Speed) e ainda híbridos, sendo ativos ou passivos (T3-American Orthodontics), dependendo do diâmetro e posição do arco ortodôntico (Agarwal, Valiathan *et al.*, 2008; Cacciafesta, Sfondrini *et al.*, 2006; Rinchuse e Miles, 2007).

A revisão sistemática realizada por Fleming e Johal (2010), investigou a influência do bráquete AL na eficiência do alinhamento, experiência subjetiva da dor, taxa da falha de colagem, mudanças dimensionais no arco, taxa de fechamento de espaço, resultados periodontais e reabsorção da raiz. Os resultados mostraram que há dúvidas para priorizar o uso de aparelhos ortodônticos fixos com AL em detrimento aos sistemas CON e se os aparelhos AL conferem vantagem especial em

relação à experiência de dor subjetiva e, se o tratamento ortodôntico é mais ou menos eficiente com os sistemas de bráquetes AL.

A diversidade dos dispositivos utilizados em aparelhos ortodônticos pode promover alterações específicas no ambiente bucal, tais como o pH ácido, maior aderência de microrganismos e desenvolvimento de biofilme (Balenseifen, Madonia *et al.*, 1970; Forsberg, Brattstrom *et al.*, 1991; Menzaghi, Saletta *et al.*, 1991; Rosenbloom e Tinanoff, 1991), aumentando o risco de descalcificação do esmalte (Saemundsson, Bergmann *et al.*, 1992; Sansone, Vanhoute *et al.*, 1993). As características clínicas e as propriedades físicas dos bráquetes variam consideravelmente (Anhoury, Nathanson *et al.*, 2002), o que pode influenciar diretamente a adesão da placa dental e, conseqüentemente, a indução de gengivite (Alexander, 1991; Loe, Theilade *et al.*, 1965; Shelley, 1981; Theilade, Wright *et al.*, 1966; Zachrisson e Zachrisson, 1972). Existe relação direta entre a placa bacteriana e inflamação gengival: quanto maior a colonização da placa bacteriana, maior a presença de gengivite (Quirynen, Dekeyser *et al.*, 1991; Quirynen, Dekeyser *et al.*, 1991; Ramberg, Axelsson *et al.*, 1995; Rowshani, Timmerman *et al.*, 2004; Socransky, Haffajee *et al.*, 1991). As características da superfície dos dentes, gengivas e secreção salivar podem influenciar a formação espontânea de biofilme em quantidade e qualidade (Bollen e Quirynen, 1994; Quirynen, Marechal *et al.*, 1988; Satou, Fukunaga *et al.*, 1988). No entanto, quando são usados aparelhos ortodônticos, o fato de que eles podem induzir a aderência de microrganismos e o desenvolvimento contínuo de biofilme deve ser considerado (Benson, Parkin *et al.*, 2004; Gorelick, Geiger *et al.*, 1982; Mattousch, van der Veen *et al.*, 2007; Mizrahi, 1982; Øgaard, 1989), pois os microrganismos encontram um nicho ecológico muito

favorável na superfície rebuscada que constitui os bráquetes (Batoni, Pardini *et al.*, 2001; Eliades, Gioka *et al.*, 2004; Maza, Elguezabal *et al.*, 2002).

A alteração da microbiota bucal pode estar relacionada a fatores como: variações no formato, material e tamanho dos bráquetes, o nível de higiene bucal individual, fluxo salivar, variáveis de tratamento, tipos de ligaduras, procedimentos de colagem e idade dos indivíduos (Brusca, Chara *et al.*, 2007; Faltermeier, Burgers *et al.*, 2008; Quirynen, Marechal *et al.*, 2008; Sukontapatipark, el-Agroudi *et al.*, 2001).

É questionável, no entanto, se a aderência de microorganismos e desenvolvimento do biofilme seriam reduzidos pela remoção de ligaduras dos bráquetes CON, com a utilização do mecanismo de abertura e fechamento dos AL. Mesmo com as mudanças nos modernos tipos de aparelhos fixos, o problema de acúmulo de placa em torno dos bráquetes ainda é cotidiano e persistente na Ortodontia (Türkkahraman, Sayin *et al.*, 2005; Yu e Qian, 2007).

Na biomecânica, o atrito é particularmente determinado pelo método de amarração utilizado, que pode ser por ligaduras elásticas, metálicas ou *clips* (Raveli, Goes *et al.*, 2008). Os sistemas de bráquetes AL foram desenvolvidos com a finalidade de reduzir a resistência de atrito entre o arco e acessórios (Pandis, Strigou *et al.*, 2006). Autores (Cacciafesta, Sfondrini *et al.*, 2003; Damon, 1998; Fernandes, Almeida *et al.*, 2008; Pandis, Strigou *et al.*, 2006; Yu e Qian, 2007) informam através de pesquisas que, quando os sistemas de bráquetes geram menor quantidade de atrito, eles reduzem significativamente o tempo de tratamento durante a mecânica de deslizamento (Berger, 1999; Cacciafesta, Sfondrini *et al.*, 2003; Damon, 1998; Fleming, DiBiase *et al.*, 2008; Fernandes, Miguel *et al.*, 2010; Fleming, 2007; Yu e Qian, 2007). Os sistemas de bráquetes *edgewise*, sejam os AL ou os CON, devem

promover a menor quantidade de atrito possível no sistema bráquete/fio ortodôntico (Berger, 1990; Fernandes, Miguel *et al.*, 2010; Pizonni, Ravnholt *et al.*, 1998; Scott, Scherriff *et al.*, 2008) sem prejudicar a qualidade do movimento planejado. Os bráquetes AL idealizados possuem diferentes formas, tamanhos e capacidade considerável para reduzir a fricção (Badawi, Toogood *et al.*, 2008; Henao e Kusy, 2004; Loftus, Artun *et al.*, 1999; Miles, 2007; Pizonni, Ravnholt *et al.*, 1998). Há relatos de que alguns sistemas de bráquetes SL apresentam menor atrito (Badawi, Toogood *et al.*, 2008; Berger, 1990; Henao e Kusy, 2004; Loftus, Artun *et al.*, 1999; Miles, 2007; Scott, Sherriff *et al.*, 2008; PizonniL, Ravnholt *et al.*, 1998), permitindo maior movimentação ortodôntica. No entanto, não existe qualquer informação sobre a atividade celular de remodelação óssea do movimento obtido com estes sistemas, os quais podem apresentar vantagens e desvantagens nos aspectos da biomecânica ortodôntica, assim como na aderência de microrganismos (*Streptococcus mutans*, Lactobacilos) e desenvolvimento de biofilme.

Portanto, é importante ainda verificar se a configuração dos bráquetes (CON ou AL) apresenta influência na aderência e formação de colônias de *Streptococcus mutans* e no comportamento biomecânico dos sistemas de bráquetes, observando clinicamente as respostas da movimentação ortodôntica, e também a atividade celular da remodelação óssea.

2 PROPOSIÇÃO

Comparar dois sistemas de bráquetes *edgewise*: convencionais e autoligados, quanto a:

- 2.1 Aderência e formação de colônias de *Streptococcus mutans* por meio de revisão sistemática.
- 2.2 Aderência e formação de colônias de *Streptococcus mutans* por meio de estudo *in vivo*.
- 2.3 Quantidade e tipo de movimento dentário e a remodelação óssea por meio de estudo em animais.

3 DELINEAMENTO DA PESQUISA

3.1 REVISÃO SISTEMÁTICA

3.1.1 Estratégia da Pesquisa

A estratégia para esta revisão sistemática foi baseada no Serviço Nacional de Saúde Centro de Análises e Disseminação (www.york.ac.uk/inst/crd/crdrep.htm). Quatro bases de dados (Cochrane Central Register of Controlled Trials; Ovid ALL EMB Reviews, PubMed e Bireme) foram selecionadas para pesquisar artigos relevantes, abrangendo o período de janeiro de 1965 a dezembro de 2012. Utilizou-se as palavras-chave *convencionais* e/ou *autoligados*, que também foram cruzados com as combinações dos termos: biofilme; e/ou *Streptococcus mutans* e/ou revisão sistemática. Os dois revisores (A.R.P.A. e L.E.A.G.N.) procuraram separadamente por publicações relevantes adicionais, que poderiam estar ausentes nas buscas do banco de dados, como busca manual de periódicos e contato com autor através de e-mail. Não houve restrições de idiomas. Os revisores selecionaram, em primeiro consenso, os artigos pela leitura do título e resumos. O texto integral foi obtido de publicações que cumpriram todos os critérios de inclusão.

3.1.2 Critérios de Inclusão e Exclusão

Os critérios de **inclusão** para os estudos foram:

- Estudos em humanos,
- Situação saudável periodontal assegurada antes do início de cada estudo,
- Idade maior ou igual que 11 anos,
- Ensaios clínicos randomizados e controlados,

- Bráquetes *edgewise*: convencionais e/ou autoligados e
- Relatos de casos, artigos de revisão, resumos e cartas ao Editor.

Os critérios de **exclusão** foram:

- Estudos com animais,
- Estudos *in vitro*,
- Planos de tratamentos que incluíram exodontias de pré-molares,
- Estudos com pacientes abaixo de 11 anos de idade,
- Presença de problemas periodontais,
- Usuários de solução anti-séptica, antibióticos, alcoólatras e fumantes e
- Artigos, em que os pacientes utilizassem dispositivos mecânicos de ancoragem, e aqueles com aparelhos Hyrax ou disjuntor tipo Haas.

Após a seleção dos artigos, a qualificação da relevância científica, dos artigos selecionados, foi realizada de forma independente pelos revisores, no caso de divergência, entre os mesmos, foi adotada a técnica do consenso. Esta avaliação utilizou o PICO (Population Intervention Comparator Outcomes - População Intervenção Comparação Resultados) (<http://www.efsa.europa.eu>) para a construção da pesquisa e bibliografia.

3.1.3 Avaliação da Relevância Científica dos Estudos Elegíveis

De cada artigo selecionado foram coletados os seguintes dados: autor/ano de publicação, revista, desenho do estudo, idade, dentes envolvidos, tipo de bráquete e da marca, tipo de ligadura, objetivo e método de análise, acompanhamento, análise estatística e resultados. Dos artigos incluídos, a avaliação da relevância científica (Jadad, Moore *et al.*, 1996) foi realizada de acordo com dez critérios:

1. Desenho do estudo (ensaios clínicos randomizados-RCT, prospectivo-P, ensaios clínicos controlados-CCT).....	...2 pontos
2. Descrição do estudo adequado.....1 ponto
3. Tamanho de amostra adequado.....1 ponto
4. Descrição adequada da seleção da amostra.....1 ponto
5. Descrição de “drop outs”.....1 ponto
6. Descrição adequada do método de medida do biofilme.....	..0,5 ponto
7. Estudo cego.....	..0,5 ponto
8. Estatísticas adequadas.....1 ponto
9. Considerado os fatores de confundimento.....1 ponto
10. Significado clínico.....1 ponto
	Total .10 pontos

Os dez critérios acima especificados foram avaliados para identificar a relevância científica da qualidade metodológica dos artigos revisados. O padrão de cada trabalho foi avaliado utilizando a seguinte classificação da relevância científica em “baixa” quando o número de pontos somados era menor ou igual a 4, “média” quando total de 5 a 8 pontos, ou “alta” quando obtido 9 ou 10 pontos.

3.2 AVALIAÇÃO MICROBIOLÓGICA

Este estudo teve aprovação do Comitê de Ética e Pesquisa da Universidade Estadual do Piauí (UESPI), sob protocolo número 28625 (Anexo 1, página 93).

3.2.1 Caracterização das amostras

Dez voluntários do sexo masculino (idades: 28-40 anos), selecionados aleatoriamente, e apresentando dentição permanente, com os terceiros molares ausentes. Excluiu-se da amostra pacientes em tratamento ortodôntico, com lesões de cárie, problemas periodontais e, em uso de antimicrobianos nos últimos três meses.

3.2.1.1 Bráquetes estéticos:

A amostra foi constituída de 50 bráquetes estéticos. 20 bráquetes CON, com ligaduras elásticas, utilizados como controle e 30 AL, como unidades experimentais. Uma semana após receber as instruções de higiene bucal foi realizada a colagem dos

bráquetes. Em cada voluntário foi instalado bráquetes CON: Mystique (Dentsply GAC, Bohemia, NY-USA) (Figura 1a) e Clarity (3M Unitek, Monrovia, Calif-USA) (Figura 1b). Os bráquetes AL avaliados são do tipo ativo: Quicklear (Forestadent, Pforzheim-Deutschland) (Figura 2a) e In-Ovation-C (Dentsply GAC) (Figura 2b) e do tipo passivo: Damon 3 (Ormco, Glendora, Calif-USA) (Figura 2c).

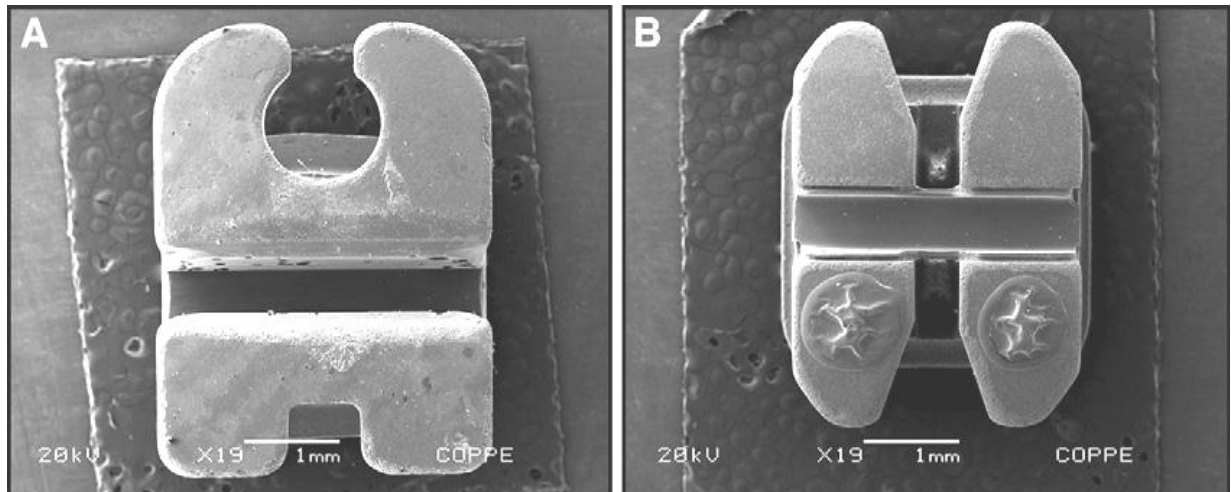


Figura 1. Bráquetes estéticos convencionais avaliados: a) Mystique (GAC-USA); b) Clarity (Unitek-USA).

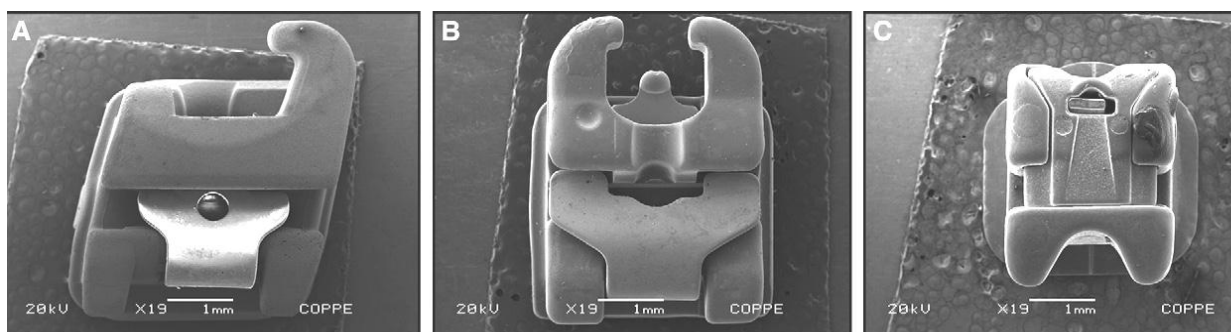


Figura 2. Bráquetes estéticos autoligados avaliados: a) Quicklear (Forestadent-BD); b) In-Ovation-C (GAC-USA); c) Damon 3 (Ormco-USA).

Os indivíduos receberam instruções de higiene bucal básica, com a intenção de padronizar escovações durante o estudo. Os voluntários receberam “kit” de higiene bucal, que incluiu escova de dentes (Procter & Gamble / Oral B, São Paulo, Brasil) e creme dental (Colgate-Palmolive, São Paulo, Brasil). A técnica de escovação utilizada foi a de Bass modificado,

3.2.2 Primeira coleta do biofilme

Previamente à primeira coleta do biofilme, os pacientes foram orientados a não ingerir alimentos e não escovar os dentes por período mínimo de 12 horas antes da coleta do biofilme dental. A primeira coleta foi realizada com o auxílio de 20 cones de papel absorventes estéreis (Dentsply Ind. e Com. Ltd., Petrópolis, RJ, Brasil), os quais serviram para coleta do biofilme das áreas supragengival das superfícies: vestibulo-cervical, méso- e disto-interproximal, obtidos a partir dos caninos, primeiros e segundos pré-molares e molares inferiores.

3.2.3 Colagem dos bráquetes estéticos

Os cinco diferentes tipos de bráquetes foram, aleatoriamente, colados nos dentes: caninos, primeiros e segundos pré-molares, primeiros e segundos molares no hemiarco inferior esquerdo. Todos os bráquetes foram colados com Transbond XT (3M Unitek, Monrovia, Calif, USA), de forma sistemática, de acordo com as instruções do fabricante. Apesar da ausência de fio ortodôntico, ligaduras elásticas foram colocadas nos bráquetes CON.

3.2.4 Segunda coleta dos microorganismos

A segunda coleta de biofilme foi realizada 21 dias após a colagem dos bráquetes, a partir das regiões das aletas, canaletas e cervicais destes.

Todo o material, obtido na primeira e segunda coleta, foi colocado em tubos Eppendorf 1,5 ml, estéreis, de plástico e de utilização única. Esses tubos foram identificados quanto ao paciente, dente e bráquete e pesados em balança eletrônica de precisão (modelo BG200, Ind e Com. Eletro-Eletrônica Gehaka Ltda, São Paulo, SP, Brasil). A quantidade de biofilme coletada foi diluída e homogeneizada com vibrador mecânico, utilizando 1 ml de solução salina constituída por cloreto de sódio 0,85% e 1% de tioglicolato de sódio, para cada 1 mg de placa coletada. Com o

auxílio de micropipeta, uma parte alíquota de 0,1 ml do meio de suspensão diluída foi retirada do tubo Eppendorf e colocado em tubo de ensaio contendo 0,9 ml da mesma solução de soro fisiológico e, seguido de homogeneização. Em seguida, alíquotas de 0,1 ml de cada diluição foram semeadas em placas de Petri contendo meio de cultura *Mitis salivarius* seletivo para o crescimento de *Streptococcus mutans*. As placas de Petri foram incubadas durante o período de contagem (72 horas) a 38° C, em condições anaeróbicas. As placas selecionadas tinham que mostrar colônias macroscopicamente visíveis, para realizar a leitura e contagem de colônias, que foi realizada por um único examinador (L.E.A.G.N.) previamente treinado e calibrado.

No dia 28 após a colagem, os bráquetes foram removidos com alicates ortodônticos próprios para este fim (Direct Bond Removing Angled Plier, Rocky Mountain Orthodontics, Denver, Colorado, USA), e colocados, imediatamente, em tubos de ensaio contendo 0,5 ml de solução salina estéril (cloreto de sódio a 0,9%), para evitar danos às colônias microbianas. Posteriormente, os bráquetes foram colocados em casulos numerados e identificados de acordo com o paciente e o dente ao qual o bráquete pertencia. Os mesmos foram submetidos à fixação por série gradual de álcoois a partir de concentrações de 50% a 70%, 75%, 90% e 100%, cada um com 10 minutos de intervalo. Após esta fase, todos os resíduos hídricos foram eliminados, utilizando dispositivo de Secador de Pontos Críticos (CPD 030, Bal-Tec AG, Balzers, Liechtenstein). Os bráquetes desidratados foram preparados com adesivo à base de prata e submetidos à metalização com cobertura a ouro (Union FL 9496; Bal-Tec AG). As peças foram então colocadas sobre placa de acrílico e examinadas no microscópio electrónico de varredura (JEOL Microscópio SM-5310, Tokyo, Japan)

3.3. AVALIAÇÃO DO MOVIMENTO DENTÁRIO

Este estudo obteve aprovação do Comitê de Ética e Pesquisa da Universidade Federal do Piauí (UFPI), sob protocolo número 004/08 (Anexo 2, página 95).

3.3.1 Caracterização das amostras

3.3.1.1 Animais:

Vinte cães adultos machos sem raça definida (SRD), três anos de idade (+ / - 0,5 anos) e peso médio de 12 kg (+ / - 1 kg) foram selecionados para participar do estudo e submetidos a quarentena. Para o procedimento de sedação dos animais, administrou-se por via intramuscular: 0,7 ml de acepromazina (0,1% Acepran--Univet), 0,8 ml de cloridrato de cetamina (Vetanarcol-König) e 0,8 mL de dihidrotiazine cloridrato (Rompum-Bayer).

Profilaxia foi realizada semanalmente em todos os dentes. Os incisivos laterais, que não foram movimentados ortodonticamente, serviram como unidades de controle em cada animal, para observação dos aspectos clínicos e histológicos em relação ao desenvolvimento biológico normal das estruturas ósseas, dentes e tecidos periodontais.

3.3.1.2 Biomecânica utilizada

Como parte dos requisitos da biomecânica ortodôntica, os incisivos centrais foram removidos da mandíbula e maxila em ambos os lados. O aparelho, inicialmente montado na região anterior inferior, constituiu-se de bandas com bráquetes CON soldados (Morelli, Sorocaba, São Paulo) de canaletas *standard* 0.022" x 0,028" cimentadas aos incisivos intermediários superiores e inferiores de todos os 20 animais, no lado direito, e bráquetes AL híbridos (T3-American

Orthodontics) de canaletas pré-ajustadas 0,022" x 0,028" aos dentes correspondentes no lado esquerdo, totalizando 40 bráquetes CON e 40 AL. Todas as bandas foram cimentadas com Transbond XT (3M Unitek). O mesmo tipo de compósito foi adicionado na porção incisal das bandas, a fim de proporcionar maior retenção mecânica. Segmento de fio 0,019" x 0,025" de aço inoxidável (CrNi) (Unitek) foi inserido nas canaletas de todos os bráquetes. Para amarrar o fio às canaletas dos bráquetes CON, ligaduras metálicas de 0,008" foram usadas. Após 30 dias do alinhamento inicial das canaletas dos bráquetes soldados às bandas, a ativação da mecânica de deslizamento foi realizada utilizando elásticos em cadeia cinza (Morelli, Sorocaba, São Paulo) com carga de 250gf aplicada dos bráquetes dos incisivos laterais intermediários no lado direito aos correspondentes do lado esquerdo nos arcos dentários mandibular e maxilar (Figura 3).



Figura 3. Mecânica ortodôntica aplicada para avaliar a movimentação dentária e a atividade de remodelação óssea entre os bráquetes *edgewise* convencionais e autoligados.

3.3.2 Avaliação da quantidade de movimento ortodôntico

Paquímetro digital eletrônico (0-100 mm) foi utilizado nos seguintes pontos de referência: duas perfurações com broca diamantada esférica n^o 1013 (KG-SORENSEN) feitas no terço gengival (GT) e incisal (IT), nas superfícies distais dos incisivos intermédios, e nas superfícies mesial das coroas dos caninos. As medidas de distância foram feitas com o mesmo operador, previamente calibrado. O valor médio das três medidas consecutivas de distância foi registrado, em milímetros, a partir dos pontos de referência das coroas dos incisivos intermédios aos pontos nas coroas dos caninos, no início da mecânica ortodôntica, imediatamente antes da ativação com elásticos em cadeia cor cinza (T₀) e após 15 dias (T₁₅). Para registrar a movimentação dentária no nível dos terços gengival e incisal, foi feita a diferença entre: (GT)T₁₅ - (GT)T₀ e (IT)T₁₅ - (IT)T₀. Após este período, todos os animais foram eutanasiados com dose letal de anestésico perfundido através da artéria carótida externa.

3.3.3 Avaliação da atividade celular de remodelação óssea

Os segmentos dentoalveolares de interesse foram identificados, dissecados, colocados em paraformaldeído a 4% e tampão fosfato (0,1 M PBS), e preparados para análise em microscopia de luz. Os segmentos foram cortados na direção vertical, seguindo o eixo longitudinal das raízes, no plano coronal, e submetidos à coloração pelo método de hematoxilina-eosina de Harris (Merck) e, em seguida, montados com Entellan (Merck). A leitura das estruturas histológicas foi realizada com o auxílio do microscópio HM-LUX Nikon E600 sob a seguinte resolução: 4NF x 0,10. Um sistema com análise de imagem computadorizada (Qwin Leica versão D-1000, 4,1) capturou 60 campos por dente, 30 distribuídos uniformemente sobre os

lados medial e distal, entre os terços gengival, médio e apical dos dentes controle, bem como aqueles movimentados com bráquetes CON e AL. A histomorfometria óssea (Compston, 2004) realizou a avaliação da atividade celular de remodelação óssea e estimou quantitativamente os osteoclastos e os osteoblastos nos 60 campos histológicos capturados.

3.4 ANÁLISE ESTATÍSTICA

3.4.1 Análise microbiológica

Os dados encontrados foram organizados em Tabelas, separados por tipos de bráquetes e por localização das coletas feitas a partir dos bráquetes. Os bráquetes CON com ligaduras elásticas foram utilizados como controle e os bráquetes AL como unidades experimentais. Os valores encontrados foram transformados, tomando a radiciação para obter a norma e aplicado ANOVA, e interações significativas entre os grupos foram analisadas separadamente e em conjunto, utilizando o método de Tukey, com um valor de $p=0,05$ de probabilidade.

Além disso, foi feita distinção entre os bráquetes formadores de maior e menor quantidade das colônias de *S. mutans*. As análises estatísticas foram repetidas entre os grupos de bráquetes em separado, para ver se existia diferença significativa entre eles, na colonização de *S. mutans*.

3.4.2 Análise da biomecânica

Os dados obtidos foram organizados em Tabelas. A análise estatística foi realizada para comparar movimento dos incisivos dos lados direito e esquerdo com bráquetes CON e AL, utilizando a análise de variância (ANOVA) em esquema de parcelas subdivididas, com observações obtidas nos T_0 e T_{15} quando as medidas

foram tomadas nos mesmos animais. Para o movimento dentário, a análise assumiu quatro locais: direito (CON) e esquerdo (AL) versus maxila e mandíbula (fatorial 2 x 2), que foram dependentes entre si. A análise do número de osteoblastos e osteoclastos foi realizada utilizando ANOVA de fatorial 3 x 3: 2 bráquetes (CON, AL) e os dentes controle contra três sítios (terços gengival, médio e apical). Em ambas as análises, comparações múltiplas de médias foi utilizado pelo teste de Tukey com *p-value*=5%.

4 DESENVOLVIMENTO DA PESQUISA

4.1 ARTIGO 1

Nascimento LEAG, Azevedo ARP, Souza MMG and Maia LC. Do self-ligating brackets have less influence on *Streptococcus mutans* colony formations? A Systematic Review. Aceito para publicação no periódico: **Dental Press Journal of Orthodontics**.

4.2 ARTIGO 2

Nascimento LEAG, Pithon MM, Santos RL, Farias AOA, Alviano DS, Nojima LI, Nojima MCG and Ruellas ACO. Colonization of *Streptococcus mutans* on esthetic brackets: self-ligating vs conventional. Publicado no periódico: **American Journal of Orthodontics and Dentofacial Orthopedics**. vol.143, n.4, April, p:S72-77. 2013.

4.3 ARTIGO 3

Nascimento LEAG, Souza MMG, Pithon MM, Filho ACG, Ruellas ACO, Franzotti ES, e Bolognese AM. Tooth movement and bone remodeling activity: Self-ligating versus conventional brackets. Submetido e na segunda revisão no periódico: **American Journal of Orthodontics and Dentofacial Orthopedics**.

4.1 ARTIGO 1

TÍTULO:

Are self-ligating brackets related to less formation of *Streptococcus mutans* colonies?

A Systematic Review

ABSTRACT

Objectives: To verify, from a systematic review, whether the design of brackets (conventionals or self-ligatings) has influence on adherence and formation of colonies of *Streptococcus mutans*. **Methodology:** Search Strategy: four databases (Cochrane Central Register of Controlled Trials, Ovid ALL EMB Reviews, PubMed and BIREME) were selected to search for relevant articles covering the period January 1965 to December 2012. Selection Criteria: in first consensus by reading the title and abstract. The full text was obtained from publications that met the inclusion criteria. Data collection and analysis: Two reviewers independently extracted data using the key words: conventional, self-ligating, biofilm, *Streptococcus mutans*, and systematic review, and evaluated independently, the quality of the studies (Jaddad 1996). In case of divergence was adopted the technique of consensus. **Results:** The search strategy resulted in 1,401 articles. The classification of scientific relevance revealed high quality of the 6 eligible articles, whose outcomes were not unanimous in reporting the influence of the design of the brackets (conventional or self-ligating) on the adhesion and the formation of colonies of *Streptococcus mutans*, and that other factors such as quality of types of brackets, the level of individual oral hygiene,

bonding and age, may have greater influence. **Conclusions:** There is no evidence for a possible influence of the design of the brackets (conventionals or self-ligatings) on colony formation and adherence of *Streptococcus mutans*. (Dental Press Journal of Orthodontics)

DESCRIPTORS: Biofilms, Orthodontic Brackets, *Streptococcus mutans*, Systematic Review

INTRODUCTION

Oral microbiota attachment of *Streptococcus mutans* and Lactobacillus is associated with the onset of tooth demineralization and periodontal disease, especially in orthodontic patients because they present risk of greater colonization by these microorganisms¹⁻⁴. It seems that the main factor for increasing the accumulation of dental plaque and inflammatory response is the new locations of retention around the components of fixed orthodontic appliance⁵. The devices used in orthodontic appliances, such as bands, wires, ligatures or brackets, can promote changes in the oral environment, such as pH, amount of *Streptococcus mutans*, the biofilm⁶⁻⁹ and enamel decalcification¹⁰⁻¹⁶. The clinical characteristics and physical properties of the bracket types are very different¹⁷, and thus can directly influence the biofilm adhesion amount and consequently gingivitis^{5,18-22}. The surface characteristics of the teeth and gingiva influence the spontaneous formation of plaque, both in quantity and quality^{18,23-30}. Saliva composition and secretion rate also influence plaque formation²⁷.

The conventional brackets (EW) are associated to the use of either elastic or stainless steel ligature to keep the orthodontic wire inside the slot⁸. The term self-

ligating (SL) in orthodontics refers to orthodontic brackets that have their own mechanism for opening and closing of the slot, not requiring any metallic or elastic ligature as method for the wire fixation^{31,32}. All these methods have advantages and disadvantages, but in relation to retaining biofilm, the literature^{8,33} suggests that elastic ligatures have greater retention of biofilm. Orthodontic treatment with EW usually presents as side effects some changes by the difficulty of periodontal hygiene and the greater accumulation and qualitative alteration of the plaque^{3,5,6,8,19,20}, so in order to improve this brackets conventional systems deficiencies, SL were developed, which according to the manufacturers and some studies^{8,34-38}, would allow better hygiene, because they say the SL brackets are less susceptible to bacterial colonization by its shape and the absence of elastic and metallic ligatures³³. It is questionable, however, if the microorganism adherence and biofilm development will be reduced by the removal of ligatures of conventional brackets with the use of the opening and closing mechanism in the SL systems. Even with the changes in modern brackets types, the problem of accumulation of plaque around the brackets is still persistent^{37,39}.

Over the years many publications^{6-11,33,34,38-41} reported different results concerning microorganism adherence and biofilm development for EW and SL brackets. The biofilm adherence on the brackets is measured by different systems and the varied scientific quality makes the evaluation of such results difficult. Therefore, it was proposed to verify, through a systematic review, whether the design of the brackets (conventionals or self-ligatings) has influence on adherence and formation of colonies of *Streptococcus mutans*. Additionally, an assessment of the quality of methodological soundness of the studies included in the review was undertaken.

METHODOLOGY

Search Strategy

The strategy of this review was based on the National Health Service Center for Reviews and Dissemination⁴². Four databases (Cochrane Central Register of Controlled Trials; Ovid ALL EMB Reviews, PubMed and Bireme) were screened to find relevant articles, covering the period from January 1965 to December 2012. The search used the keywords “conventional” and/or “self-ligating”; which were also crossed with combinations of the terms: “biofilm” and/or *Streptococcus mutans* and/or “systematic review” through the electronic databases. Two reviewers (L.E.A.G.N. and A.R.P.A.) sought separately for publications. As a first step the reviewers selected the articles by reading the titles and abstracts. The full text was obtained from publications that met the inclusion criteria. After the selection of articles for inclusion criteria, classification of scientific relevance of the selected articles was performed independently by the reviewers and in the case of divergence, we adopted the technique of consensus. This review used the PICO (Population Intervention Comparator Outcomes) strategy⁴³ for the construction of the research and bibliographic search (Table 1).

Table 1 – The PICO (Population Intervention Comparator Outcomes) strategy description for the construction of the research and bibliographical search.

Acronym	Description
Population	Patients with corrective orthodontic treatment appliances using conventional or self-ligating edgewise brackets
Intervention	Amount biofilm assessment and microbiota attachment on conventional or self-ligating brackets.
Comparator	Through the levels of biofilm amount on orthodontic brackets systems.
Outcomes	The measurement of colonies of <i>Streptococcus mutans</i> and / or their effects on periodontal tissues.

Inclusion and Exclusion Criteria

The inclusion criteria for the retrieved studies were concerned with:

- humans studies,
- 11 years old or more,
- healthy periodontal situation ensured before the study beginning,
- randomized and controlled clinical trials,
- studies with conventional edgewise and/or self-ligating brackets and
- case reports, review articles, abstracts and letters to the Editor.

The exclusion criteria were:

- studies with animals,
- *in vitro* studies,
- treatments plans that included extractions of premolars,
- patients under 11 years of age,
- periodontal problems, antibiotics and oral antiseptic solution users, alcoholics and smokers
- articles, in which patients used mechanical and anchoring devices, Hyrax and Hass.

Assessment of the Scientific Relevance of the Eligible Studies

The following data was collected from each paper retrieved: author/year of publication, study design, age, teeth involved, bracket type and brand, ligature type, aim and method of analysis, follow up, statistical analysis and outcome. A quality assessment⁴⁴ was performed on each article included, according to ten criteria:

1. study design (randomized clinical trials-RCT, prospective-P, or controlled clinical trials-CCT)..... ..2 points
2. adequate study description..... ..1 point
3. adequate sample size..... ..1 point

4. adequate selection description.....1 point
5. drop outs description.....1 point
6. adequate biofilme measurement method description.....	..0,5 point
7. blinding in measurement.....	..0,5 point
8. adequate statistics provided.....1 point
9. confounder factors considered.....1 point
10. clinical significance.....1 point
Total	..10 points

The ten criteria specified above were used to identify the scientific relevance of the reviewed papers. The rating was given as "low" when the points given were less than or equal to 4, "medium" for 5 to 8 points and "high" for 9 or 10 points.

RESULTS

Outcomes of the Search Strategy

The search strategy yielded 1,401 articles, having 195 repeats. 1,194 articles were not relevant to the review, leaving a total of twelve potentially relevant articles^{33,45-55}, that were chosen for retrieval and evaluation of the full text using a summarized data extraction sheet (Table 2).

Table 2. Search Dates, Search Strategies, and Number of Results for Each Database

Database	Search Strategy	Results	Selected Papers
Cochrane C.R.C. Trials	conventional OR Self-ligating	160	2
Ovid ALL EMB Reviews	exp Orthodontic Appliances/ or edgewise.mp. AND exp Orthodontic Appliance Design/ or exp Orthodontic Brackets/ or self-ligating.mp. or exp Orthodontic Appliances/ AND biofilm.mp. or exp Dental Biofilm Index/ AND streptococcus mutans.mp. or exp Streptococcus mutans/	53	4
PubMed (NLM)	conventional AND self-ligating, OR biofilm OR Streptococcus mutans	788	5
Bireme	conventional OR Self-ligating	400	1
TOTAL		1,401	12

Out of the twelve full text articles retrieved, 6 articles were excluded because: one article⁴⁵ the sample had premolar extractions, three^{47,49,51} were *in vitro* studies, and two others^{50,53} did not had the direct comparison between the two systems of EW and SL brackets. This left six articles^{33,46,48,52,54,55}, which were qualified for the final analysis as they evaluated periodontal and clinical variables due to bacterial adhesion in the patients with C and SL brackets (Figure 1).

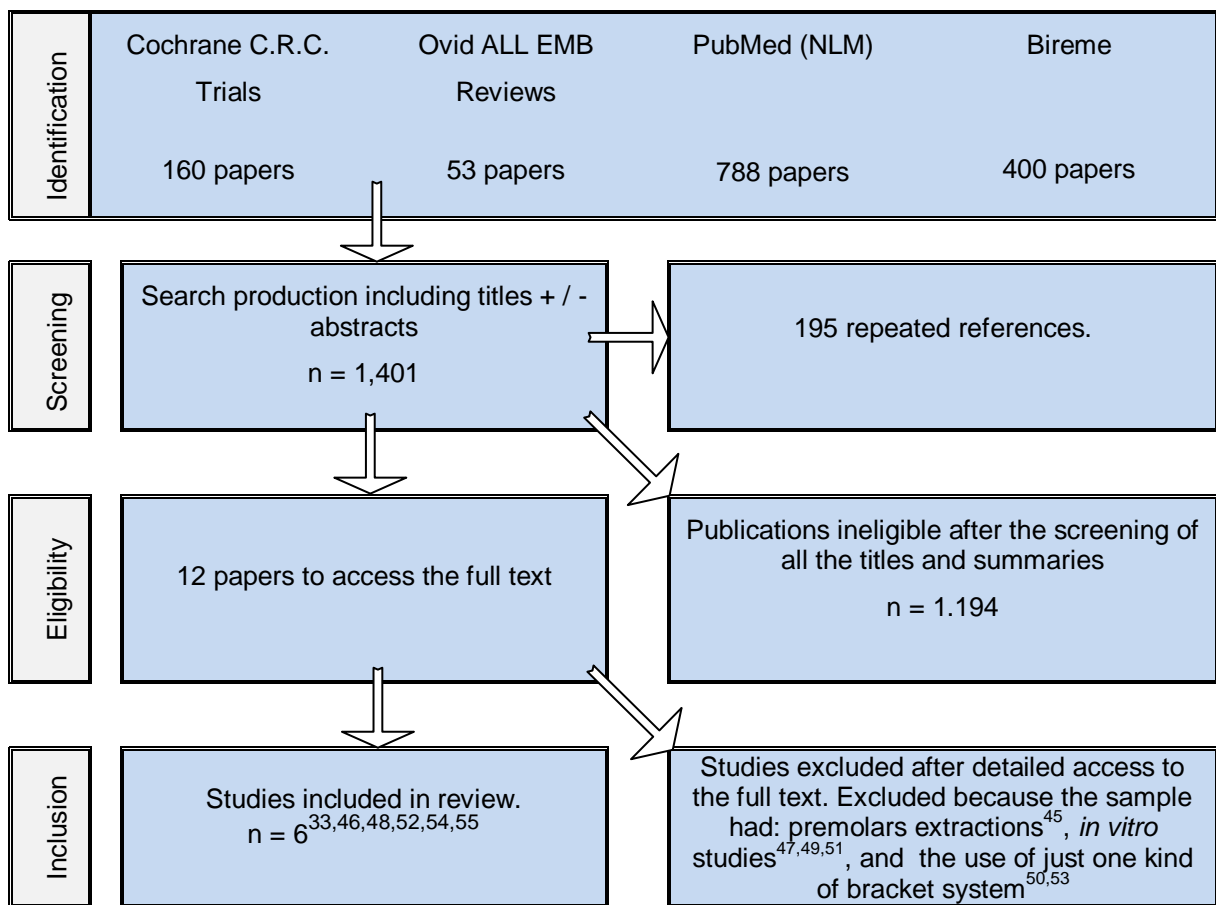


Figure 1: Flowchart summary of review.

Assessment of the Scientific Relevance of the Eligible Studies

The six articles^{33,46,48,52,54,55} included in this review (Table 3) met the inclusion criteria, although with differences among their ways of study, sampling, methods of analysis and follow up. All the eligible studies^{33,46,48,52,54,55} compared the two types of systems: conventional versus self-ligating edgewise brackets. Pandis⁴⁶ also gave

Table 3. Summarized data of the six studies included in the review

Author Year Paper	Pellegrini et al.³³ 2009 AJODO	Pandis et al.⁴⁶ 2008 Orthod Craniofac Res	Van Gastel et al.⁴⁸ 2007 Journal of Clinical Periodontology	Pithon et al.⁵² 2011 Braz J Oral Sci.	Pejda et al.⁵⁴ 2012 Angle Orthod	Pandis et al.⁵⁵ 2010 Eur J Orthod
Study	Randomized controlled trial	Prospective Cohort	Randomized controlled trial	Randomized controlled trial	Randomized controlled trial	Randomized controlled trial
Patients	18 p	100 p	16 p	5 p	38 p	32 p
Age	11-17 y	12-17 y	17-27 y	20-30 y	11-18 y	11-17 y
Teeth	Lateral incisors	maxilla and mandible	1 st and 2 nd premolars	canines; 1 st and 2 nd premolars and molars (lowers)	maxilla and mandible	maxilla and mandible
Bracket type/brand	14p: EW - Mini-Ovation 14 p: SL - Innovation-R GAC [®]	50 p: EW - GAC [®] 50 p: SL - In-Ovation-R - GAC [®]	16 EW - GAC [®] 16 SL - Speed [®]	10 EW - Morelli 40 SL: GAC [®] ; Aditek; Ormco; 3M Unitek.	19 p: EW - Sprint Forestadent 19 p: SL - Damon 3MX, Ormco	16 p: EW - GAC [®] 16 p: SL - In-Ovation-R - GAC [®]
Ligature type	Elastic ligatures to the EW brackets	Elastic ligatures to the EW brackets	Elastic ligatures to the EW brackets	Elastic ligatures to the EW brackets	Elastic ligatures to the EW brackets	Elastic ligatures to the EW brackets
Objective of analysis	Plaque amount. To determine if ATP by bioluminescence may be useful in assessing the plaque index	Index gingival plaque and calculus of the pocket depth	Crevicular fluid and pocket depth. Colonies (an) aerobic	<i>S. mutans</i> attachment and others microorganisms on EW and SL.	Amount of 6 kinds of microorganisms on EW and SL.	Effect of the brackets (EW or SL) at the levels of <i>S. mutans</i> in saliva
Method of analysis	MSB specific for <i>S. mutans</i> and determination by bioluminescence	Clinical periodontal parameters	Clinical and microbiological periodontal parameters	MSB specific for <i>S. mutans</i> and BHI, not specific for bacteria and fungus	Clinical periodontal parameters and PCR	MSB specific for <i>S. mutans</i>
Follow up	5 w	18 m	7 d	21 d	18 w	2-3 m
Statistical analysis	t tests (1-tailed, with P <0.05). Chi-squared	Chi-squared. Wilcoxon rank sum. STATA [®] 10	ANOVA Tukey-Kramer	SPSS 13.0 program. Wilcoxon (p-value <0.05)	Teste <i>t</i> Sidak post hoc Testes Fischer e χ^2	ANCOVA Minitab 14.20 Chi-squared
Outcome	SL favor reduced accumulation of <i>S. Mutans</i> and ATP by bioluminescence is useful in assessing plaque index	No advantages of SL over EW with respect to the periodontal status of the mandibular anterior teeth	Bracket design can have a significant impact on bacterial load and on periodontal parameters	The hypothesis that SL favor greater aggregation of microorganisms was proved	The bracket design does not seem to have a strong influence on clinical parameters and periodontal pathogens in subgingival plaque	The levels of <i>S. mutans</i> in whole saliva do not seem to be significantly different between EW and SL

Legend: p=patients; y=years; m=months; w=weeks; d=days; h=hours; C=conventional brackets; SL=self-ligating brackets; *S.*=*Streptococcus*; SEM=scan electronic microscopy ; ATP= adenosine triphosphate; MSB=Mitis Salivarius agar; BHI=brain heart infusion; PCR=polymerase chain reaction

references to the plaque, gingival and calculus index, whereas the article by Van Gastel⁴⁸ examined the amount of crevicular fluid and anaerobic and aerobic colonies. Another study Pandis⁵⁴ collected saliva after 2-3 months of the orthodontic appliances bonding and *Mitis Salivarius* culture medium (MS), specific for *S. mutans* was used for counting of colony forming units (CFU). Pithon⁵² made the collection of plaque samples, directly from SL and EW brackets of different brands, and after 3 weeks of bonding, the CFU held in: MS, specific for *S. mutans* and BHI (Brain Heart Infusion), not specific to bacteria and fungi. In this estudo⁵², CFU was performed visually after 24, 48 and 72 h of incubation. Pejda et al.⁵⁴ collected the plaque samples of subgingival sulcus after 18 weeks of treatment, performing the count of 5 periodontal pathogens by PCR, while Pellegrini et al.³³ collected from tooth surfaces surrounding the brackets, after 5 weeks of bonding, and the CFU was analyzed by MS agar and bioluminescence of ATP (adenosine triphosphate).

When evaluating the scientific relevance of the six eligible articles^{33,46,48,52,54,55}, we found that the description of the sample selection was appropriate, however the number of dropouts was declared in studies of Pellegrini³³, Pandis⁴⁶, van Gastel⁴⁸ and Pejda⁵⁴. The studies^{33,48,52,54} provided the approval of the ethics committee, which was not clearly seen in the both articles of Pandis^{46, 55}, where was asked only the consent of patients/parents before starting the study. Considering the confounding factors, similar oral routine and hygiene instructions were given to subjects in these six studies^{33,46,48,52,54,55}. In the papers^{46, 54}, full alignment of the mandibular arch was necessary to eliminate the crowding as a confounding factor, but the clinical variables were evaluated by the same periodontist, and the examiner of Pandis⁴⁶ was not blinded, which could influence the outcome of the research,

Outcomes Assessment of the Eligible Studies

Among the selected studies, four^{46,48,54,55} had their outcomes consistent in reporting that a) there is no advantage of SL over EW in periodontal condition of anterior lower teeth⁴⁶, b) the design of the brackets can have significant impact on bacterial load and periodontal parameters periodontais⁴⁸ c) sub-gingival plaque and whole saliva, do not seem to be significantly different in total levels of *S. Mutans* and periodontal pathogens between EW and SL^{54,55}. However, a study⁵² confirmed the hypothesis that the SL favor the accumulation of micro-organisms, while another study³³ reported that SL promote lower retention of *S. mutans* when compared to EW (Table 3). The outcomes of the eligible studies^{33,46,48,52,54,55}, were not unanimous in reporting that there is evidence of a possible influence of the design of the brackets (conventionals or self-ligatings) on the adhesion and the formation of colonies of *Streptococcus mutans*.

DISCUSSION

A systematic review is a tool that can confirm the quality of research and methodological soundness of works selected from the literature and present the outcomes for consideration of the clinical and scientific communities. Evidence based practice requires the construction of a research question and literature review.

Conventionally, to attach the wire to the brackets, three methods are used: metallic ligature, elastic ligatures, and the open-close devices of the SL. All these methods have advantages and disadvantages, but with regard to the accumulation of biofilm, the literature^{8,33} suggests that the elastic ligatures favor the retention of biofilm in comparison with the two other methods of ligatures. Questioning was prepared for this revision to verify the hypothesis that the SL brackets are related to

less formation of *Streptococcus mutans* colonies due to elimination of the ligatures (elastic or metallic) which are considered part of the conventional brackets, due of their function to retain the orthodontic wire within the conventional slot. The microorganisms exhibit significant adherence to brackets because there are favorable ecological niches in the porous, rough and irregular surfaces of these brackets^{39,47,49,51,56}. So bracket surface characteristics can be considered as harboring favorable sites for the adhesion of biofilm.

Outcomes of the Search Strategy

This research was highly sensitive, addressed the evidence of minimum bias. The study of Jordan & LeBlanc⁵⁰ was excluded due to: a) rated only one bracket system, b) not blinded examiner and c) statistical analyzes unspecified. *In vitro* studies excluded^{47,49,51}, do not account the inherent characteristics which contribute to the development of the intraorally biofilm and may provide bias results for clinical periodontal condition²². The differences observed between the results of some papers^{33,46,48-50, 52}, may be related to factors that include: variations in the shape, material and size between the SL and EW brackets, the individual level of oral hygiene, salivary flow, treatment variables, types of ligatures, bonding procedures and age of individuals^{24,45,49,51,55}. Thus, the bracket type itself would not be the deciding factor for the biofilm development, but its composition and material type should be included as factors of *Streptococcus mutans* colonies formation⁵⁶.

Assessment of the Scientific Relevance of the Eligible Studies

Statistical analysis of our results was not feasible because methodological designs, of the eligible articles, were heterogeneous, however the quality assessment

revealed high-quality research and methodological soundness of the six studies^{33,46,48,52,54,55}, as shown in their final notes according to the scale of Jadad (1996)⁴⁴.

Although the SL brackets not require ligatures, their opening and closing mechanism may provide sites for biofilm amount in the same way as the conventional bracket⁴⁶. This mechanism of the SL brackets is not renewed, as with elastomeric modules in conventional brackets. The plaque calcification in SL leads to a malfunction of the opening and closing mechanisms. Thus, the SL theoretical advantages over the conventional can be eliminated how confirms others studies^{46,52}. When using conventional brackets neither the elastomeric rings nor the metallic ligatures appear to affect the distribution of bacterial morphotypes in brackets or on the enamel surface³. The aged elastic surface can apparently favor the plaque retention in relation to the polished stainless steel ligatures, but no difference between periodontal conditions of patients treated with these two types of ligatures^{8,57}. However, studies^{41,58} report that brackets with elastomeric rings favored damage to gingival conditions, with significant accumulation of biofilm, while the metallic ligature had lower retention of biofilms in comparison with the other brackets. There are reports^{59,60} that the brackets EW are directly related to the retention of biofilme, however, the study⁵² suggests the cross-infection due to exchange of the elastomeric rings, is controllable with the use of brackets EW, because the EW favor lower formation of *S. Mutans* colonies, agreeing with the study⁴⁸ that showed no difference between EW and SL in gingival bleeding.

Outcomes Assessment of the Retrieved Studies

Oral microbiota attachment of *Streptococcus mutans* and Lactobacillus in patients is associated with use of orthodontic appliances^{6,8,9,33,45}, being either with brackets EW or SL. This increase leads to: high cariogenic plaque, pH low enough to change the clinical periodontal parameters^{46,48,54} and increased risk of enamel demineralization^{6,47}.

Some eligible studies^{52,54} evaluated not only the presence of *S. mutans*, but also of other microorganisms related to periodontal disease in patients with brackets EW or SL. The study of Pejda et al.⁵⁴ found 23.8 times more chance of the presence of *Aggregatibacter actinomycetemcomitans* (AA) in subgingival plaque of patients with EW brackets, but the increase of AA does not represent a risk factor for local periodontitis as studies of Paolantonio et al.^{61, 62} confirm. The differences found between the results at one study⁵² with others eligible assessed studies^{33,46,48,54,55}, may have been because of methodological difference of some others studies^{46,48,54,55}, where the CFU were counted from material collected from saliva and another study³³, collected from tooth surfaces surrounding the bracket, while the study of Pithon⁵² it was directly performed from the brackets, in the cervical-distal regions of the winglets, slot and in the cervical region of the bracket base, which is why this latest study should have found statistically significant differences, revealing greater accumulation of biofilm on brackets SL.

Clinical Implications

Some studies^{8,33-39} report that SL brackets are less susceptible to bacterial colonization by its shape and lack of metallic or elastomeric ligatures. However, adequate control of biofilm is strongly influenced by the correct orientation and

cooperation of the patients^{24,55} than simply choosing the selection of a system of brackets or other. The outcomes of eligible studies^{33,46,48,52,54,55} were not unanimous in reporting a possible influence of the design of the brackets (conventionals or self-ligatings) on the adhesion and the formation of colonies of *Streptococcus mutans*.

The decisions of orthodontists to use SL in their clinical routine, instead of EW, aimed at improving the hygiene / plaque accumulation, cannot yet be applied due to lack of scientific evidence, considering what the literature says^{46,48,52,54,55}. After this review we presume that there is not enough evidence to support the use of the appliances with SL brackets in place of systems with EW or vice versa, according to the study by Fleming et al.⁶³.

Based on the limitations of some works^{64 66}, further studies with other types of brackets, as for example, aesthetic self-ligatings must be performed to see periodontal complications arising from different shapes, sizes and material types of brackets, and with that guide the development of new system designs brackets to reduce the formation of colonies of *Streptococcus mutans*.

CONCLUSIONS

There is no evidence for a possible influence of the design of the brackets (conventionals or self-ligatings) on colony formation and adherence of *Streptococcus mutans*.

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4.1 ARTIGO 2

Nascimento LEAG, Pithon MM, Santos RL, Farias AOA, Alviano DS, Nojima LI, Nojima MCG and Ruellas ACO. Colonization of *Streptococcus mutans* on esthetic brackets: self-ligating vs conventional. Publicado no periódico: **American Journal of Orthodontics and Dentofacial Orthopedics**. vol.143, n.4, April, p:S72-77. 2013.

Colonization of *Streptococcus mutans* on esthetic brackets: Self-ligating vs conventional

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Introduction: Self-ligating orthodontic brackets rely on clips, rather than ligatures, to hold the archwire in place. It is unknown whether replacing ligatures with clips affects the adherence of *Streptococcus mutans*. The aim of this research was to evaluate whether self-ligating brackets have an advantage over conventional brackets as determined by the adherence of *S mutans*. **Methods:** The sample consisted of 50 esthetic brackets, divided into 3 experimental groups and 2 control groups of 10 brackets each. Two experimental groups were active self-ligating brackets (QuickClear; Forestadent, Pforzheim, Germany; and In-Ovation C; Dentsply GAC, Bohemia, NY); the other was a passive self-ligating bracket (Damon 3; Ormco, Glendora, Calif). The 2 control groups were conventional brackets (Mystique; Dentsply GAC; and Clarity; 3M Unitek, Monrovia, Calif). The brackets were randomly bonded to the canines, first and second premolars, and first and second molars in the mandibular left hemiarch of 10 male participants. Biofilm was collected from the tooth surfaces before bonding and from the brackets on day 21 and placed in Petri dishes containing *Mitis salivarius* agar. The brackets were removed on day 28 and examined by using scanning electron microscopy. Statistical analysis, analysis of variance, and the Tukey correction with a *P* value of 0.05 were used. **Results:** The greatest numbers of colonies were found in an active self-ligating bracket group (In-Ovation C), and the fewest colonies were in a conventional bracket group (Clarity). The largest colonies formed on active self-ligating brackets. In the slot, the greatest formation was in a control group (Mystique). **Conclusions:** Self-ligating esthetic brackets do not promote greater or lesser *S mutans* colonization when compared with conventional brackets. Differences were found to be related to the material composition of the bracket. (Am J Orthod Dentofacial Orthop 2013;143:S72-7)

The diversity of devices used in orthodontic appliances can promote specific alterations in the oral environment, such as acidic pH, greater adherence of microorganisms (*Streptococcus mutans*), and the development of biofilm.¹⁻⁴ These alterations increase the

risk of enamel decalcification.^{5,6} Clinical characteristics and physical properties of brackets vary considerably,⁷ and these can directly influence the adherence of dental plaque and consequently cause gingivitis.⁸⁻¹² A direct relationship exists between gingival inflammation and dental plaque: with more gingivitis, there is greater colonization of bacterial plaque.¹³⁻¹⁸ The surface characteristics of the teeth and gingivae, and salivary secretions can influence the quantity and quality of biofilm formation.^{13,15-21} The porous structure of the material of the brackets provides a highly favorable ecologic niche for the adherence of microorganisms and the continuous development of biofilm.²²⁻²⁹

Self-ligating brackets have a mechanical mobile device to close the slot, converting it into a tube. Some systems are considered passive (Damon 3, Smart Clip, Vision, Oyster), and others are active (Speed, In-Ovation, QuickClear). Some advantages are attributed to the self-ligating bracket systems: eg, more rapid orthodontic movement and consequent reduction in treatment time for the mechanical closing of spaces.³⁰⁻³⁴ However, it is not known whether adherence of

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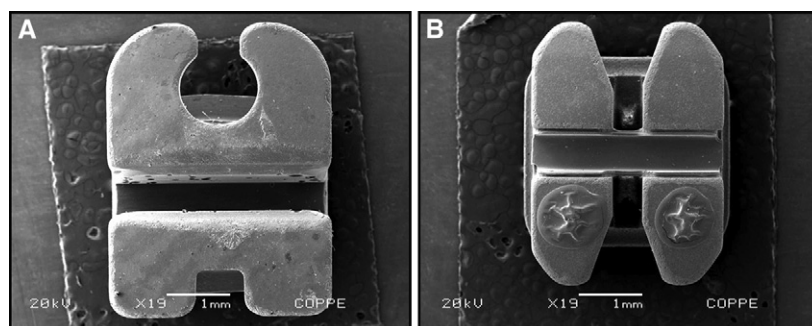


Fig 1. Conventional esthetic brackets evaluated: **A**, Mystique; **B**, Clarity.

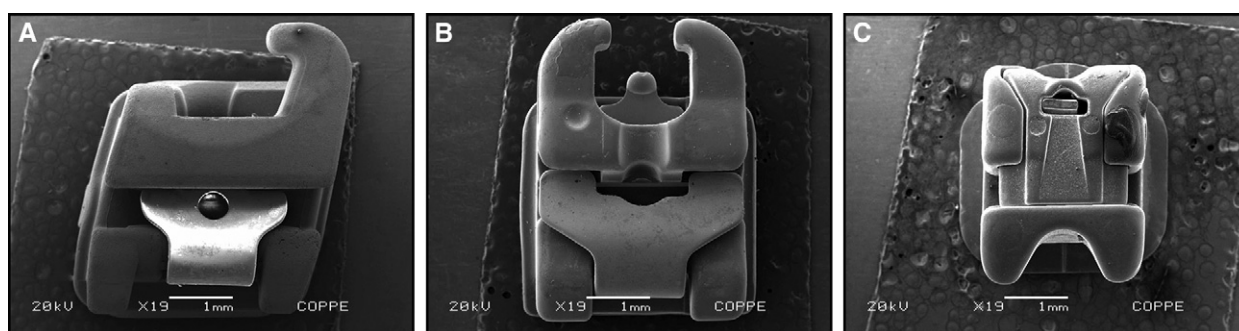


Fig 2. Self-ligating esthetic brackets evaluated: **A**, Quicklear; **B**, In-Ovation C; **C**, Damon 3.

microorganisms and the development of biofilm are diminished when self-ligating brackets are used, because the ligatures (metallic or elastic) necessary for maintaining the orthodontic wire in place with conventional brackets are replaced by the opening and closing mechanism (clip) of the self-ligating brackets. The alteration of microbial adherence depends also on factors such as variations in design, size, and composition of the self-ligating and conventional esthetic brackets, methods of bonding and of tying the wire to the slot, level of oral hygiene, and age of the patient.^{19,35-37}

As the numbers of adult patients grow, so does the demand for esthetic self-ligating bracket systems. Thus, the aim of this study was to evaluate whether self-ligating esthetic brackets have advantages over conventional esthetic brackets with respect to surface retention of *S mutans* colonies.

MATERIAL AND METHODS

The sample consisted of 50 esthetic brackets—20 conventional and 30 self-ligating—bonded in 10 male volunteers (ages, 28-40 years). One week after receiving oral hygiene instructions, 1 bracket of each type was placed in each subject. The conventional brackets were Mystique (Dentsply GAC, Bohemia, NY) (Fig 1, A) and

Clarity (3M Unitek, Monrovia, Calif) (Fig 1, B). Two of the self-ligating brackets were active types: active Quicklear (Forestadent, Pforzheim, Germany) (Fig 2, A) and In-Ovation C (Dentsply GAC) (Fig 2, B); the other self-ligating bracket was passive: Damon 3 (Ormco, Glendora, Calif) (Fig 2, C). The conventional brackets, with elastic ties, were used as controls, and the self-ligating brackets were the experimental units. The 10 volunteers were randomly selected and had complete permanent dentitions. Exclusion criteria included orthodontic treatment, carious lesions, periodontal problems, and antimicrobial use in the last 3 months. This study was approved by the ethics committee of Universidade do Estado do Piauí (UESPI) (protocol number 28625). The subjects received basic oral hygiene instructions, with the intent to standardize brushing during the study. They were taught the modified Bass technique and were given an oral hygiene kit that included a toothbrush (Procter & Gamble/Oral B, São Paulo, Brazil) and toothpaste (Colgate-Palmolive, São Paulo, Brazil).

The first biofilm samples were collected before bonding. The patients were instructed not to eat food and not to brush their teeth for a minimum of 12 hours before the collection of the dental biofilm. Plaque was collected with size 20 absorbent paper cones (Dentsply Indústria e

Comércio, Petrópolis, Rio de Janeiro, Brazil), obtained from the mandibular canines, first and second premolars, and first and second molars from the supragingival areas: cervical-buccal, mesial-interproximal, and distal-interproximal surfaces.

The experiment then proceeded with the bonding of 5 different brackets in each patient. The brackets were randomly assigned to selected teeth: canines, first and second premolars, and first and second molars of the mandibular left hemiarch. All brackets were bonded with Transbond XT (3M Unitek), in a systematic manner and according to the manufacturer's instructions. No orthodontic wires were placed on any bracket; the conventional esthetic brackets received elastic ties, which are considered part of conventional brackets because of their function to retain the wire.

The second collection of biofilm was done 21 days after bonding the brackets and was obtained from the hooks, slots, and cervical regions. The material collected was placed in 1.5-mL sterile, plastic, single-use Eppendorf tubes (Axygen, Union City, Calif). These tubes were identified for each patient, tooth, and bracket, and weighed by precise electronic balance (model BG200; Indústria e Comércio Electro-eletrônica Gehaka, São Paulo, São Paulo, Brazil). The quantity of biofilm collected was diluted and homogenized with a mechanical vibrator, by using 1 mL of saline solution composed of 0.85% sodium chloride and 1% of sodium thioglycolate for each 1 mg of plaque collected. With a micropipette, an aliquot of 0.1 mL of the suspended diluted medium was taken from the Eppendorf tube and placed in a test tube containing 0.9 mL of the same saline solution, and then the mixture was homogenized. Then 0.1-mL aliquots of each dilution were plated on Petri dishes containing *Mitis salivarius* culture medium selective for *S mutans* growth. The Petri dishes were incubated during the counting period (72 hours) at 38°C in anaerobic conditions. The selected plates had to show macroscopically visible colonies to accomplish the reading and counting of the colonies, which were performed by 1 previously trained and calibrated examiner (L.E.A.G.N.).

On day 28, the brackets were removed with orthodontic pliers (Rocky Mountain Orthodontics, Denver, Colo) and placed immediately in test tubes containing 0.5 mL of sterile saline solution (0.9% sodium chloride) to avoid harming the bacterial colonies. Later, the brackets were placed in wells that were numbered and identified by the patient and tooth to which the bracket belonged. They were submitted to fixation by a gradual series of alcohol concentrations of 50% to 70%, 75%, 90%, and 100%, each at a 10-minute interval. After this phase, all hydrous residues were eliminated by using a critical point dryer device (CPD 030; Bal-Tec AG,

Table I. Mean numbers of colony forming units of *S mutans* before bonding the brackets

Area	Mean CFU
Cervical-buccal	5.40
Mesial-interproximal	3.60
Distal-interproximal	3.40

Table II. Mean total numbers of *S mutans* colonies by bracket type, without considering the collection location

Bracket	Mean total CFU	
Clarity	508.47	cd
Mystique	679.60	bc
Damon 3	705.07	bc
QuicKlear	909.87	ab
In-Ovation C	1043.80	a

Minimum significant difference, 287.66. Means with the same letters do not differ statistically. The Tukey test was applied ($P = 0.05$).

Balzers, Liechtenstein). The dehydrated brackets were prepared with a silver-based adhesive and underwent metallization with gold covering (Union FL 9496; Bal-Tec AG). The pieces were then placed on an acrylic plate and examined with a scanning electron microscope (SM 5310; JEOL, Tokyo, Japan).

Statistical analysis

The data were organized in tables, separated by types of brackets and by location of the collections from the brackets. The conventional brackets with elastic ties were used as controls, and the self-ligating brackets were the experimental units. The values were transformed by taking the root to obtain the normal and applying analysis of variance, and significant interactions between the groups were analyzed separately and together by using the Tukey method, with $P = 0.05$.

A distinction was made between brackets with the most and the least amounts *S mutans* colonies. The statistical analyses were repeated between the groups of brackets separately to determine whether there was a significant difference in the colonization of *S mutans* among them.

RESULTS

Table I shows the mean values of bacterial plaque collected from dental surfaces before bonding the brackets.

Tables II through IV compile the data from the collection of microorganisms taken directly from the brackets (hook, slot, and cervical regions) used in the experiment.

Table III. Mean total numbers of colonies of *S mutans* on the locations of the brackets, without considering type of bracket

Location	Mean total CFU	
Hook	51.67	c
Slot	1152.50	a
Cervical	908.87	b

Minimum significant difference, 166.35. Means with the same letters do not differ statistically. The Tukey test was applied ($P = 0.05$).

Table IV. Mean numbers of colonies of *S mutans* (CFU) counted and the interactions between brackets, with their varying types and the locations on the bracket where the microorganisms were collected

Bracket	Location					
	Hook		Slot		Cervical	
Clarity	7.20	a	208.60	c	1309.60	ab
		B		B		A
Mystique	6.00	a	1427.20	a	605.60	cd
		C		A		B
Damon 3	84.60	a	1190.00	a	840.60	bc
		B		A		A
QuickClear	110.60	a	1620.40	a	998.60	abc
		C		A		B
In-Ovation C	87.00	a	1661.60	a	1382.80	a
		B		A		A

Minimum significant difference columns = 498.24 (lowercase letters indicate vertical evaluations). Minimum significant difference rows = 407.47 (uppercase letters indicate horizontal evaluations). Means with the same letters do not differ statistically. The Tukey test was applied ($P = 0.05$).

Table II gives the results of the evaluation of each bracket as a whole and shows that In-Ovation C had the highest numbers of colony forming units, and Clarity had the least. The largest colonies were formed on the active brackets. In Table III, it can be verified that the location on the bracket with the greatest formation of *S mutans* was in the slot, followed by the cervical region of the bracket, and then the hook. No statistically significant differences were observed between the conventional Mystique brackets and the self-ligating Damon 3, QuickClear, and In-Ovation C brackets with respect to the numbers of colonies formed in the slots (Table IV). There was statistical similarity among the self-ligating brackets and the conventional Clarity brackets.

Figure 3 shows, by scanning electron microscope, the In-Ovation C self-ligating bracket; its opening and closing mechanism (clip) shows additional local plaque retention. The Damon 3 self-ligating bracket slot is shown in Figure 4.

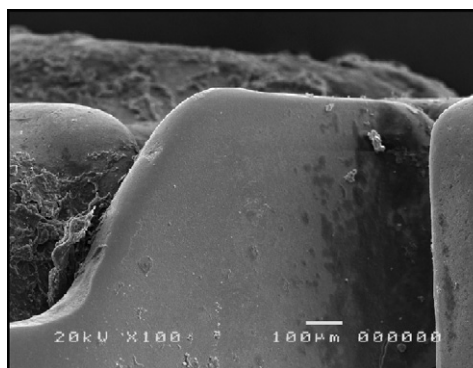


Fig 3. The In-Ovation C self-ligating bracket clip provided additional opportunities for local plaque retention.

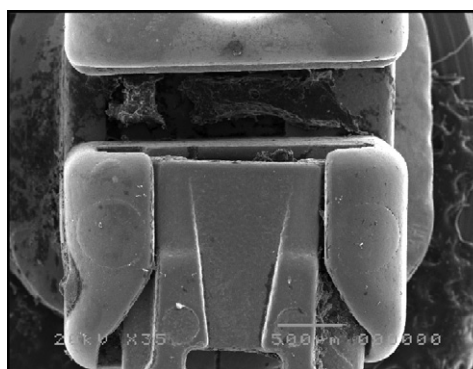


Fig 4. The slot of the Damon 3 self-ligating bracket was a biofilm harbor.

DISCUSSION

In this research, we evaluated differences in microbial adhesion between conventional bracket systems that include ligatures and self-ligating systems that do not use ligatures.

Because in-vitro studies lack some fundamental properties that modulate intraoral microbial colonization and plaque retention in patients, these studies ought to be considered to obtain quality in clinical evidence.^{38,39} To ensure that the study was blind, the samples were placed in Petri dishes, and the identification system was unknown to the examiner.³⁶

The variability of the number of colony forming units of *S mutans* among the brackets of this study is shown in Table II. This variation concurs with other studies that reported that the characteristics of the bracket, tooth, and gingival surfaces influence the spontaneous formation of biofilm, both in quantity and in quality.^{7,13,15,19,21,40} In the same manner, the composition and amount of salivary secretions can vary with age and influence bacterial adherence.^{17,41,42}

Even though self-ligating brackets eliminate the ligatures, they incorporate intrinsic opening and closing mechanisms that can provide additional opportunities for local plaque retention, where calcification of the plaque can lead to obstacles in the functioning of the opening and closing mechanism (Fig 3).³⁵ The elastic ligatures in the oral environment allow for the adsorption of potassium and sodium in the initial phase, followed by calcium and potassium precipitations, which stabilize the formed integument¹⁹; this could favor the accumulation of plaque, and also it did not have a statistical difference in relation to the results of this study when comparing the self-ligating brackets with the conventional Mystique. In the same manner, no difference was found in the periodontal conditions of the treated patients with either elastic or metallic ligatures.³

In the distinction between the brackets with greater or lesser formation of *S mutans* colonies, it was observed that there was little colonization on the hooks of the conventional esthetic and the self-ligating brackets (Table III), and the greatest formation of colonies occurred in the slots (Fig 4), followed by the cervical region of the brackets. When evaluating the brackets without considering the specific areas of collection (Table II), the conventional Clarity brackets had the least amount of colony formation, whereas the self-ligating In-Ovation C and Quicklear had the most. Some self-ligating brackets contain metallic and ceramic parts. Tronchin et al⁴³ demonstrated the capacity of forming a thin layer of biofilm on the surface of synthetic devices. Yet, there is a conflict with in-vitro studies, whose findings seem to indicate that the adherence of *S mutans* is weaker in metallic brackets than in plastic or ceramic ones.^{36,40} It is believed that this study shows reliability, since it was performed in vivo with microbiologic parameters.

This study also showed that the greatest *S mutans* colonization in the slots of the self-ligating brackets was with In-Ovation C (Table IV). The conventional Mystique bracket had the same statistical significance as did the self-ligating brackets (Table III). The lowest colonization was verified with the Clarity brackets. Ceramic brackets are more inclined to bacterial colonization than metallic ones, and this can be observed when comparing the conventional brackets of this study, Mystique and Clarity.⁴⁴ The slot of the Mystique bracket is ceramic, which is porous with rough areas; it had greater potential for accumulating microorganisms compared with the smoother, less porous metallic slot of the Clarity brackets (Fig 1).

Some authors compared edge-wise conventional and self-ligating metallic systems.^{45,46} They observed that greater or lesser microbial adherence and biofilm development is not determined by the system of brackets used. Since the characteristics of the materials

of the brackets vary considerably, the colonization of *S mutans* can be different among the types of brackets currently used, as shown in Table IV. More studies are needed to visualize periodontal complications of the different bracket systems used with fixed appliances so that the brackets can be projected to reduce the adherence of dental plaque.

CONCLUSIONS

Self-ligating esthetic brackets do not show greater or lesser amounts of colonization of *S mutans* when compared with conventional esthetic brackets. The differences were related to the type of material of the brackets.

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4.3 ARTIGO 3

TÍTULO

Tooth movement and bone remodeling activity: Self-ligating versus conventional brackets

ABSTRACT

Introduction: The aim of this study was to compare the tooth movement with bone remodeling after orthodontic movement with conventional and self-ligating brackets.

Methods: The experiments were conducted in 20 male dogs. Bands were cemented in all intermediate incisors, with conventional brackets (Morelli) on the right side and hybrid self-ligating ones (T3-American Orthodontics) on the left side. A 0.019" x 0.025" stainless steel wire with chain elastics (250 gf) performed the sliding mechanics. To the rates of tooth movement assessment, distances from intermediate incisors to canines were recorded at the beginning of the tooth movement and after 15 days. To the bone remodeling were evaluated the numbers of osteoclasts and osteoblasts by histomorphometry. Statistical analysis of variance and the Tukey correction with a *p*-value at 5% were used. **Results:** The rates of tooth movement at the incisal third crown level with self-ligating brackets were higher than the conventional ones. The number of osteoblasts in the teeth with self-ligating had equal statistical significance on the side of tension at the root gingival third versus side of compression at the root apical third; and also did in the osteoclasts count, in the compression side at the gingival third versus the tension side at the apical third. **Conclusion:** The comparison with tooth movement and

bone remodeling after tooth movement indicated more translation movement of teeth with the conventional brackets, and more tipping movement with self-ligating brackets.

DESCRIPTORS: bone; histomorphometry; orthodontic movement; self-ligating; brackets.

INTRODUCTION

Friction is particularly determined by the ligation method used, which can be elastomeric ligatures, wire ligatures or ligating clips.¹ Self-ligating bracket systems have been developed with the purpose of reducing frictional resistance between the archwire and accessories. Some are considered: passive, with rigid clips (Damon, Smart Clip, Vision); active, with flexible clips that press against the archwire constantly irrespective of thickness (Speed, In-Ovation); and hybrid (passive and active) depending on the diameter and position of the orthodontic archwire (T3).²⁻⁴

The literature^{3,5-8} reports that when the bracket systems cause less amount of friction, they significantly reduce treatment time during sliding mechanics. Bracket systems, whether edgewise self-ligating (SL) or conventional edgewise (EW), should promote the lowest amount of friction possible for the bracket/orthodontic wire system^{7,9-11} without impairing the quality of movement planned. The idealized SL brackets, which have different shapes, sizes, mechanics, and a considerable ability to reduce friction¹²⁻¹⁵ are widely used in the clinical routine.

A systematic review article¹⁶ investigated the influence of SL bracket type on alignment efficiency, subjective pain experience, bond failure rate, arch dimensional

changes, rate of orthodontic space closure, periodontal outcomes, and root reabsorption. The outcomes of the review¹⁶ showed: a) insufficient evidence to support the use of SL fixed orthodontic appliances over EW appliance systems or vice versa, b) SL appliances do not confer a particular advantage with regards to subjective pain experience and, c) insufficient evidence suggesting that orthodontic treatment is more or less efficient with SL systems. There are reports that some SL bracket systems present less friction,⁶⁻¹⁵ allowing greater orthodontic movement. However, there is no information about the cellular bone remodeling activity of the movement achieved with these systems. Therefore, the aim of this study was to assess the rates of orthodontic movement observed through clinical evaluation, and cellular bone remodeling of SL and EW brackets, in the periodontal ligament (PDL) after the application of sliding mechanics.

MATERIALS E METHODS

Twenty 3-year-old (+/- 0.5 years) adult male dogs of non-defined breed (NDB) and mean weight of 12 kg (+/- 1 kg) were subjected to quarantine and recruited to participate in this study. For the sedation procedure of the animals, the following drugs were administered intramuscularly: 0.7ml of acepromazine (Acepran-0.1%-Univet), 0.8ml of ketamine hydrochloride (Vetanarcol-König) and 0.8ml of dihydro-tiazine hydrochloride (Rompum-Bayer). The research was approved by the Ethics Committee for Animal Experimentation under report number 01/09 (Universidade Federal do Piauí). Throughout the entire experiment, the experimental procedures on the animals fulfilled the Proposed International Ethical Guidelines for Biomedical

Research involving animals (Council for International Organizations of Medical Sciences – CIOMS/WHO, 1985).

Prophylaxis was performed weekly on the teeth. The lateral incisors were used as controls units in each animal for the observation of the clinical and histological aspects with regard to the normal biological development of the bone structures, teeth, and periodontal tissue and they were not treated orthodontically. As part of the required orthodontic mechanics, the central incisors were extracted from the mandible and maxilla on both sides, and after seven days bands with EW brackets (Morelli, Sorocaba, São Paulo) with a 0.022" x 0.028" slot, not preadjustable, were cemented to the maxillary and mandibular intermediate incisors on the right side, and hybrid SL brackets (T3-American Orthodontics) with a 0.022" x 0.028" and Roth prescription slot, to the corresponding teeth on the left side. All brackets were cemented with Transbond XT (3M Unitek). The same type of resin was added in the incisal portion of the bands in order to improve retention. A 0.019" x 0.025" Unitek stainless steel wires (CrNi) were passively inserted in the slots of all brackets. To tie the wire to the EW bracket slots, a 0.008" ligature wire was used.

After 30 days of the initial alignment of the slots, the sliding mechanics activation was performed using gray chain elastics (Morelli, Sorocaba, São Paulo) with a load of 250gf from the brackets of the intermediate lateral incisors on the right side to the ones on the left side on the mandibular and maxillary dental arches (Fig 1).



Fig 1. The orthodontics mechanics applied to assess the tooth movement and the bone remodeling between *edgewise* conventional and self-ligating brackets.

Assessment of the rates of orthodontic movement

An electronic digital caliper (0-100 mm) was used in the following references points: two perforations with a spherical diamond bur No 1013 (KG) done at the gingival (GT) and incisal third (IT), in the distal surfaces of the intermediate incisors, and in the mesial surfaces of the canines' crowns. The distance measurements were done with the same operator, who was previously calibrated. The mean value of the three consecutive distance measurements was recorded, in millimeters, from intermediate incisors to canines' crowns at the GT and IT, at the beginning of orthodontic mechanics, just before the activation with gray chain elastics (T_0), and after 15 days (T_{15}). To record the rates of tooth movement at the gingival and the incisal thirds, it was made the difference between: $(GT)T_{15}-(GT)T_0$ and $(IT)T_{15}-(IT)T_0$. After this period, all the animals were euthanized with a lethal dose of anesthetic infused through the external carotid artery.

Assessment of the cellular bone remodeling activity

The dentoalveolar segments of interest were identified, dissected, placed in 4% paraformaldehyde and phosphate buffer (0.1 M PBS), and prepared for light microscopy analysis. The slices were cut in the vertical direction following the longitudinal axis of the roots, in the coronal plane. The slices were submitted to staining by the Harris hematoxylin-eosin method (Merck) and then mounted with Entellan (Merck). The reading of the histological structures was performed with the aid of the HM-LUX Nikon E600 microscope under the following resolution: 4NF x 0.10. A system with computerized image analysis (Qwin Leica D-1000, version 4.1) captured 60 fields per tooth, 30 evenly distributed on the medial and distal sides, among the gingival, middle and apical thirds of the control teeth as well as those moved with EW and SL brackets. The bone histomorphometry¹⁷ performed the assessment of the bone remodeling activity and estimated quantitatively the osteoclasts and osteoblasts in the 60 histological captured fields.

Statistical Analysis

Statistical analysis was performed to compare the right side and left side incisor tooth movements with EW and SL brackets, using the analysis of variance (ANOVA) in a split plot design, with subplots observations obtained in the times T_0 and T_{15} when measurements were taken in the same animals. For the tooth movement, the analysis assumed 4 sites: right (EW) and left (SL) versus maxilla and mandible (2 x 2 factorial design), which were dependent with each other. The analysis of the number of osteoblasts and osteoclasts was performed using ANOVA in 3 x 3 factorial design: 2 brackets (EW,SL) and the control teeth versus 3 sites (gingival, medial and apical thirds). In both analyses, multiple comparisons of means was used by the Tukey test with p-value = 5%.

RESULTS

Assessment of the rates of orthodontic movement

Table I shows that the rates of tooth movement had equal clinical significance between the SL and EW brackets at the GT level. However, when assessing the rates of tooth movement at the IT level, no equal clinical significance between the EW and SL brackets systems were observed, which rates of movement with SL at the IT level was 3.1 mm (maxilla) and 2.9 mm (mandible) and at the GT level was 1.5 mm (maxilla) and 1.1 mm (mandible), which indicates that the crown axis after the orthodontic movement was more tipped with the SL brackets.

Table I. Mean of distance measurements from the references points from canines to intermediate incisors crowns at the incisal and gingival thirds to record the rates of tooth movement ($T_{15}-T_0$), caused by the use of self-ligating brackets compared quantitatively to that caused by conventional brackets, after application of sliding mechanics.

Bracket type, Bone and Third of the Crown			T_0 (mm)				T_{15} (mm)				$T_{15}-T_0$ (mm)*
			Mean	Max	Min	SD	Mean	Max	Min	SD	
EW	Maxilla (n=20)	IT	14.1	18.1	10.5	4.4	15.3	18.7	11.9	4.1	1.2 A
		GT	13.3	17.8	9.2	3.3	14.4	17.4	10.3	3.1	1.1 A
	Mandible (n=20)	IT	6.2	7.9	4.7	4.1	7.6	8.6	4.9	3.7	1.4 A
		GT	5.5	8.2	4.1	3.2	6.8	8.2	4.8	3.1	1.3 A
SL	Maxilla (n=20)	IT	14.1	17.5	10.7	4.1	17.2	18.9	11.5	4.4	3.1 B
		GT	13.4	16.3	9.3	3.1	14.9	17.9	10.9	3.4	1.5 A
	Mandible (n=20)	IT	5.9	7.3	4.9	3.3	8.8	9.1	5.8	3.7	2.9 B
		GT	5.6	8.3	4.6	3.1	6.7	8.2	4.9	3.1	1.1 A

Legend: EW=teeth with conventional brackets, SL= teeth with self-ligating brackets, n=sample numbers, Mean of distance measurements at the crowns from canines to intermediate incisors at the incisal third level (IT) and at the gingival third level (GT), T_0 =mean measurements before beginning sliding mechanics, T_{15} =mean measurements after 15 days of sliding mechanics, $T_{15}-T_0$ =the difference between T_{15} and T_0 , resulting the rates of tooth movement, Max=maximum, Min=minimum, SD=standard deviation, mm=millimeters. *=rates followed by the same letters do not differ among themselves at a 5% significance level when compared using Tukey's test (p -value=5%) with Minimum Significant Difference=0.5407.

Assessment of the cellular bone remodeling activity

When evaluating the control teeth, the periodontal ligament (PDL) showed regular and uniform thickness throughout entire root (Fig 2). In general, the collagen fibers remained parallel among them and they were perpendicularly inserted into the bone and cementum surfaces. Most fibroblasts presented a fusiform shape and were arranged in fascicles. Inflammatory cells were rarely found. A uniform distribution of blood vessels and nerves of various sizes were found throughout the periodontal ligament. The cementum surface was uniform and continuous. The bone crest was slightly irregular with the presence of few osteoclasts arranged in Howship's lacunae or juxtaposed to the bone surface. The osteoblasts were juxtaposed in the control teeth, but the osteoblasts were arranged with no organization in the teeth with both EW and SL brackets on the side of tension along the bone surfaces.

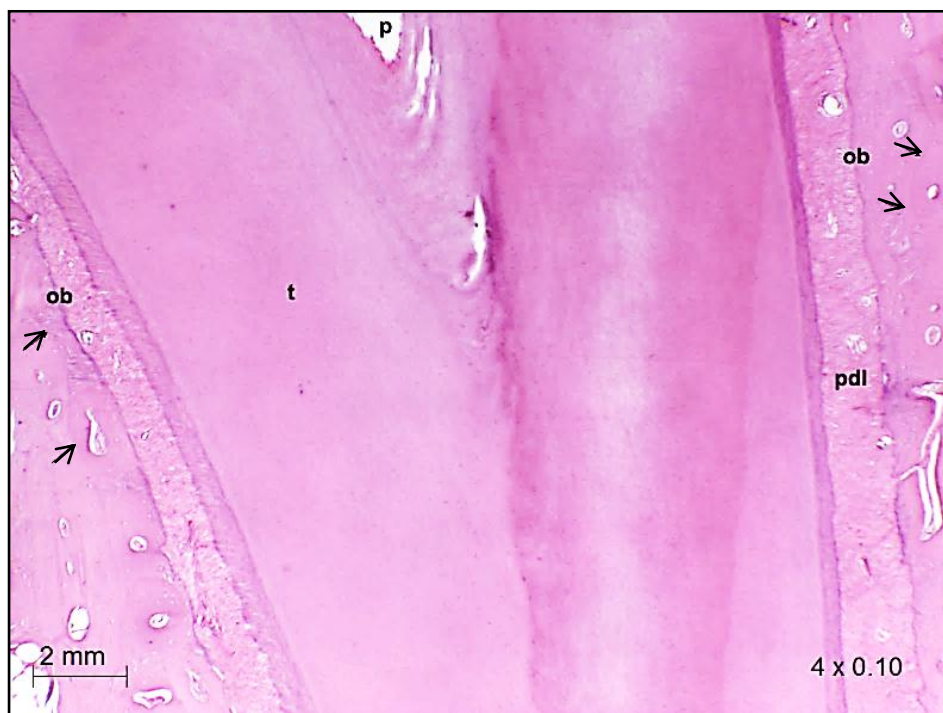


Fig 2. Photomicrograph of the control lower left lateral incisor, at magnification of 4x0.10, shows aspects of normality for the PDL. p=pulp; pdl=periodontal ligament; t=root tooth; b=bone; ob=osteoblasts; arrows=incremental growth lines.

On the tension side in the periodontium of the teeth with EW brackets, intense osteoblast activity arranged from the cervical to the apical thirds of the bone edges was observed, as well as the formation of cementum, indicating the presence of incremental growth lines (Fig 3). Adjacent to the bone surface of the periodontium of these teeth, at the gingival, middle and apical thirds, irregular bone with active osteoclasts, located within Howship's lacunae, was frequently found on the mesial surface, which corresponded to the side that received compression. Loss of organization and individuality of Sharpey's fibers, showing areas of frontal and undermining bone absorption, were observed (Fig 3).

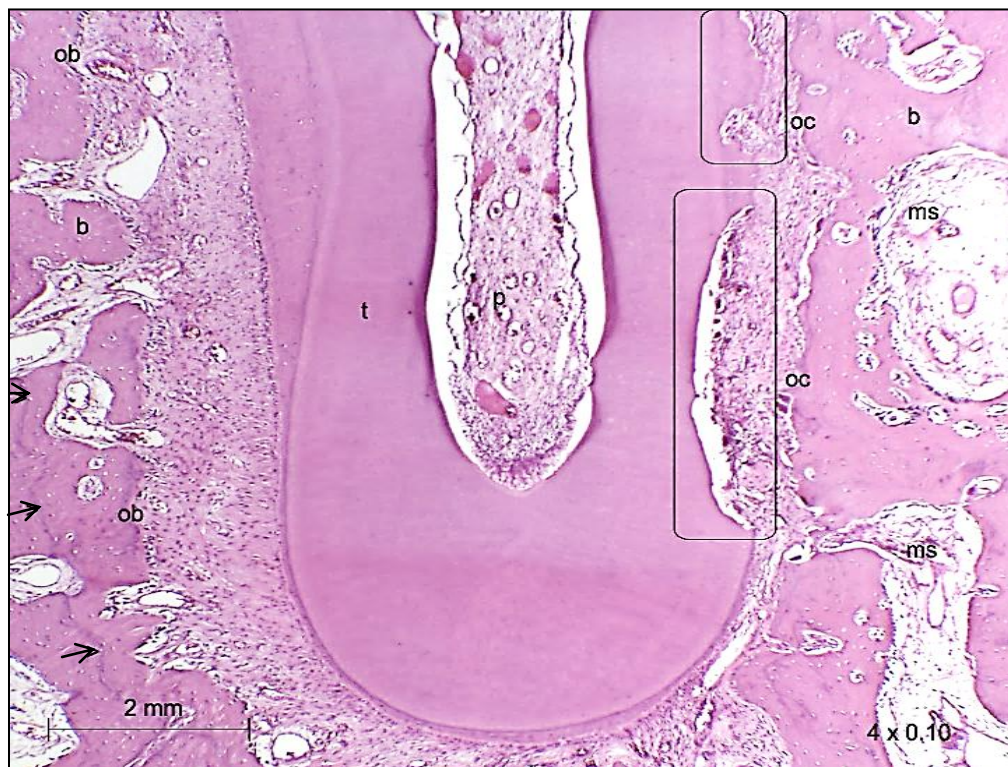


Fig 3. Photomicrograph (Magnification of 4x0.10) of the intermediate lower right incisor root, which had a conventional bracket, shows, on the side of compression, osteoclasts (oc), root (squares) and bone absorption (frontal and undermining), and on the side of tension, osteoblasts arranged on the bone edge (ob), apposition of cementum and incremental lines growth (>); p=pulp; ms=marrow space; t=root tooth; b= bone.

The reactions of apposition (on the tension side) and absorption (on the compression side) were frequent along the roots of teeth with the EW brackets, suggesting translational movement of the teeth because in the osteoclasts and

osteoblasts count, the amount of these cells was uniformly distributed along the sides with compression and tension, respectively, which can be seen in Tables II

Table II. Osteoclasts cell count performed on the tension and compression sides, at gingival, middle and apical thirds (teeth with SL and EW systems), and mesial and distal sides of the control group teeth.

Bracket & side	GT				MT				AT			
	Mean§	Max	Min	SD	Mean§	Max	Min	SD	Mean§	Max	Min	SD
EW T	0.9 A d	2	0	1.6	0.9 A d	2	0	2.2	1.1 A b	2	0	2.2
EW C	4.3 B c	7	2	2.7	5.7 A a	8	3	2.4	4.6 B a	9	2	4.1
SL T	0.4 C c	1	0	2.1	2.6 B c	6	1	2.4	5.3 A a	9	3	4.2
SL C	10.1 A a	13	6	4.1	4.8 B b	8	2	4.1	0.7 C b	3	0	2.2
C1 D	0.9 A c	2	0	2.0	0.8 A d	2	0	2.2	0.9 A b	2	0	2.2
C2 M	0.9 A c	2	0	1.9	0.8 A d	2	0	2.2	0.9 A b	3	0	2.3

Legend: EW=conventional edgewise brackets, SL= self-ligating edgewise brackets, T=traction side, C=compression side, C1 and C2=control teeth, D=distal side, M=mesial side, GT= gingival third, MT=middle third, AT=apical third, §=means followed by the same letters do not differ among themselves at a 5% significance level when compared using Tukey's test (P -value=5%), Max=maximum, Min=minimum, SD=standard deviation. Lowercase letters – vertical evaluation (tension/distal and compression/mesial sides), MSD=Minimum Significant Difference=0.7468. Uppercase letters – horizontal evaluation (thirds). MSD=Minimum Significant Difference=0.6151.

and III, showing the same statistical significance at the assessed root thirds.

Table III. Osteoblasts cell count performed on the tension and compression sides, at gingival, middle and apical thirds (teeth with SL and EW systems), and mesial and distal sides of the control group teeth.

Bracket & side	GT				MT				AT			
	Mean*	Max	Min	SD	Mean*	Max	Min	SD	Mean*	Max	Min	SD
EW T	12.2 A b	19	5	5.1	12.3 A a	19	7	3,7	13.1 A a	18	7	3.7
EW C	3.4 A d	7	1	4.23	3.9 A d	8	2	4.4	3.8 A c	9	1	4.2
SL T	15.5 A a	22	9	6.2	9.5 B b	13	5	5.4	5.1 C c	9	2	4.1
SL C	4.8 C d	10	1	4.1	8.1 B b	13	4	4.1	13.4 A a	19	8	5.6
C1 D	8.1 A c	11	7	2.9	7.7 A c	10	5	2.9	8.7 A b	10	5	2.9
C2 M	8.1 A c	10	6	2.9	7.7 A c	11	6	2.9	8.8 A b	11	6	2.9

Legend: EW=conventional edgewise brackets, SL= self-ligating edgewise brackets, T=traction side, C=compression side, C1 and C2=control teeth, D=distal side, M=mesial side, GT= gingival third, MT=middle third, AT=apical third, *=means followed by the same letters do not differ among themselves at a 5% significance level when compared using Tukey's test (P -value=5%), Max=maximum, Min=minimum, SD=standard deviation. Lowercase letters – vertical evaluation (tension/distal and compression/mesial sides) with Minimum Significant Difference=1.5133. Uppercase letters – horizontal evaluation (thirds) with Minimum Significant Difference=1.2463.

The teeth with SL brackets, areas of frequent reabsorption were found with irregular bone, significant number of active osteoclasts in gingival thirds on the compression side (Fig 4A) and in the apical region of the roots on the tension side (Fig 4B). On the tension side at the apical third areas of root reabsorption were also found, as well as frontal and undermining bone reabsorption, with predominance of the latter.

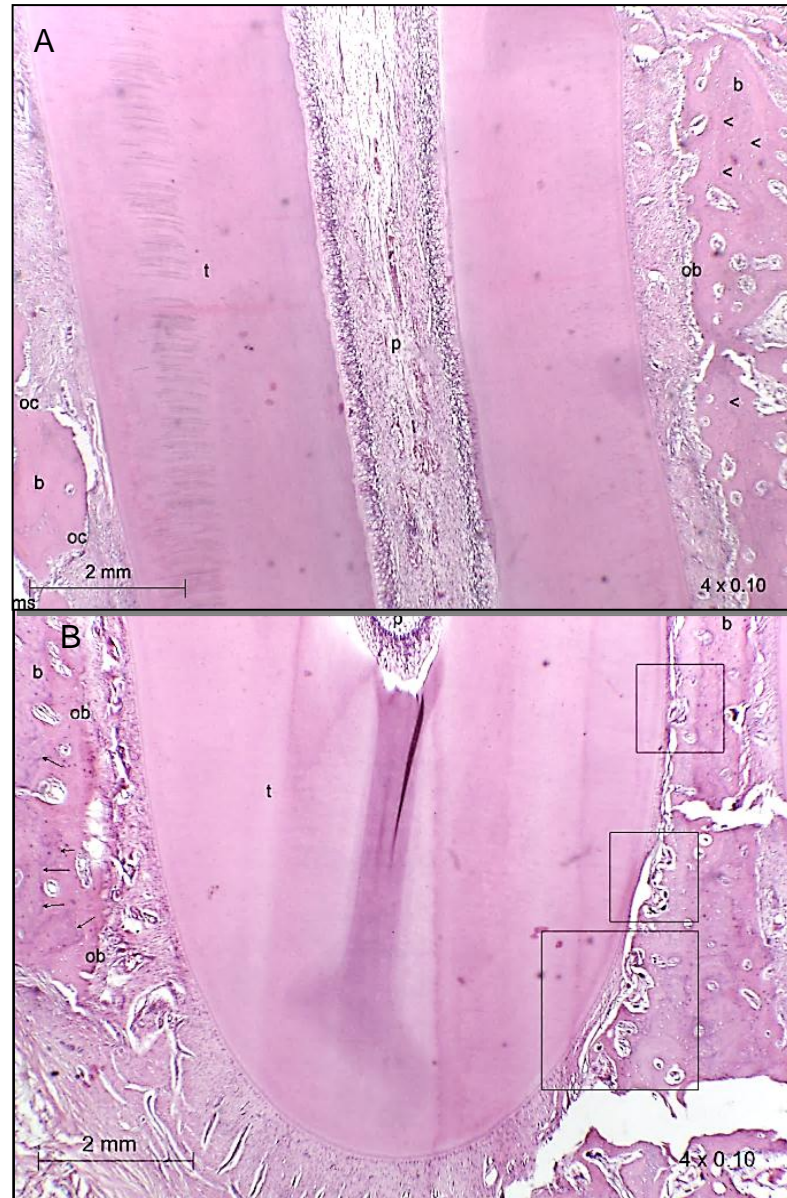


Fig 4. Photomicrographs (4x0.10) of intermediate lower left incisor, which had bracket SL. A) At the gingival third level: active osteoclasts with frontal and undermining absorption on the side of compression and the marginal bone crest with incremental lines growth and osteoblasts in the bone edge on tension side. B) At the root apical third level: irregular bone, significant number of active osteoclasts (squares) with frontal and undermining reabsorption on the side of tension and incremental lines growth and osteoblasts in the bone edge at the compression side. b=bone; t=root tooth; ob=osteoblasts; oc=osteoclasts; ms=marrow space; p=pulp; arrows=incremental lines growth.

The osteoblasts count on the side of tension showed equal statistical significance at the gingival third in comparison with the side with compression at the apical third (Table III). The same statistical significance was also observed in the osteoclasts count, when observing both the compression side at the gingival third and the tension side at the apical third (Table II). This indicates similar tissue reactions in these two areas, despite being on different sides and thirds. These findings suggest tipping movement of the teeth that received the SL brackets.

DISCUSSION

In the present study, we investigated the rates of orthodontic tooth movement and cellular bone remodeling activity after 15 days, using conventional brackets that required ligatures and self-ligating brackets that did not use ligatures. The rates of the orthodontic movement with sliding mechanics were observed through clinical evaluation, and its cellular bone remodeling activity, due to the responses of periodontal ligament cells (osteoclasts and osteoblasts). A verification of slot alignment and efforts to eliminate the friction or resistance generated due to differences in initial alignment of brackets slots was made to enhance the validity of our findings. Thus, a 0.019" x 0.025" CrNi wire was passively inserted in the slots of all brackets, and after 30 days of the initial alignment of band slots, the sliding mechanics activation begun.

Morphological changes in the periodontal support tissue visualized by light microscopy in our experiment were similar to findings reported in classical studies of Reitan¹⁸ and Rygh.¹⁹ Many previous studies on experimental tooth movement have been performed in rats.^{20,21} However, the rat as an experimental model has disadvantages. Rodents have continuous eruption of the incisors and physiological

distal drift of the molars. Continuous eruption of the incisors may affect the direction of the applied force because incisors are often used as the anchorage unit in these experiments. Distal drift might camouflage the amount of real tooth movement. Furthermore, a rat molar is about 60 times smaller than a human molar; thus, even a force in the range of 5 gf has to be considered a high one. By using the dog model, we could overcome these problems with good reasons to support the use of dogs as experimental animals in induced tooth movement. The use of dogs to study orthodontic tooth movement is not new, there are studies using this experimental model.^{22,23} Although the alveolar bone of dogs is generally denser than in human, differences between the anatomy of the periodontal ligament and alveolar bone of dogs and human are rather small and in this respect, dogs are generally accepted as a good model for comparison with human beings.^{24,25}

We performed the present study to investigate the rates of tooth movement and bone remodeling activity considering a short follow up time with animals models, because it is unequivocally comparable with activity observed in humans.^{23,26} To avoid lower number of replicates per treatment, as well as for security and availability of resources for our work, we adopted a high number of repetitions considered (n = 20) to perform the ANOVA, following the information from the literature regarding the number of repetitions²⁷.

Our research used bone histomorphometry because it enables the quantitative assessment of bone turnover and remodeling in histological sections of bone¹⁷. It provides unique information about mechanisms of bone loss and gain in therapeutic interventions¹⁷. Many of the measurements made in bone histomorphometry have a subjective element, and this, along with differences in staining techniques,

magnification, and measurement methods, contributes to significant inter- and intra-observer measurement variability²⁸⁻³⁰.

The application of force of 250 gf within 15 days was sufficient to observe clinical movement and significant histological reactions which is in agreement also with studies³¹⁻³⁶ that reported that approximately two days after the application of orthodontic force, local modifications allow the osteoblasts and osteoclasts to begin the process of bone remodeling.

These histological reactions were also observed in our study, but with greater presence of reabsorption areas than of apposition areas. In our research, the performance of clinical movement in the time interval of fifteen days did not show statistical significance between the EW and SL brackets at the crown GT level. This result is in agreement with the study¹⁵ conducted with the passive Smart Clip SL brackets and EW brackets in the same way as in studies^{37,38} with Damon 2 and EW brackets.

However, when we assessed the rates of tooth movement at the crown IT level, no equal statistical significance between the EW and SL was observed. At this level the movement was higher than at the GT level, indicating a tipping movement of the SL crowns. In the periodontal ligament of the teeth with EW brackets, an agglomeration of osteoblasts were uniform along the entire root of the teeth of these animals, while for the SL brackets, the prevalence of these cells was more at the gingival or apical thirds.

On tension side in the teeth with SL brackets, major tension was found at the gingival third. Instead, on the side of compression, tension was found in the apical third, suggesting that the tipping movement was present in the teeth with SL brackets. In the groups of teeth with EW brackets, tension was arranged along the

root thirds of the teeth only on the corresponding sides, suggesting translational movement for this group.

The degree of success achieved with orthodontic mechanics can be influenced by many variables such as: age, endocrine factors, systemic diseases, medication, bone density, type of material, size, shape and angle of the wire interface/slot and bond strength and ligation form.³⁹⁻⁴³ In our study, when evaluating the orthodontic behavior in the mandible and maxilla arches using the same system (either EW or SL), a statistically significant difference was not found for the brackets between the two bone arches, even though the values at the mandible arch had showed the lowest rate of tooth movement.

One study showed that clinically, the bone responds differently to orthodontic force resulting in a significant difference among the rates of tooth movement in maxilla and mandible.⁴⁴ Another study say that in the skeletally mature dogs (1- to 2-year-old), the bone formation rate (BFR), which is a measure of the bone turnover or remodeling in bone supporting permanent teeth, is substantially higher in the alveolar process surrounding erupted permanent teeth (mandible approximately 37%/y, maxilla approximately 19%/y) than the BFR found at other sites such as the femur (6.4%/y)⁴⁵.

Our results showed that the SL brackets had the same clinical movement behavior on both bone arches, however, they suffered greater tipping movement than translational movement, which is in agreement with some authors⁴⁶⁻⁴⁸ affirming that there is insufficient evidence that large rectangular wires, in the presence of tipping, the SL brackets can cause less friction in comparison with the EW brackets, and that the performance of orthodontic mechanics of EW and SL brackets may be related to the size of the orthodontic wire and not dependent merely on the system used.

However, since others studies⁴⁹ reported the application of methods in modern molecular biology focused on the role played by the periodontal ligament cells, further researches can explain which system could anticipate cellular stress even before starting orthodontic movement.

CONCLUSIONS

The comparisons of tooth movement and bone remodeling after tooth movement indicated more translation movement of teeth with the conventional brackets, and more tipping movement of teeth with self-ligating brackets.

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5 DISCUSSÃO

A revisão sistemática é ferramenta capaz de confirmar a qualidade da investigação e solidez metodológica de trabalhos selecionados a partir da literatura e apresentar os resultados para apreciação das comunidades clínica e científica. Prática baseada em evidências exige a construção de uma pergunta de pesquisa e revisão da literatura.

No primeiro artigo, o questionamento elaborado para a revisão sistemática foi verificar se a configuração dos bráquetes (convencionais ou autoligados) apresenta influência na formação de colônias de *Streptococcus mutans*. Os microorganismos apresentam adesão significativa para bráquetes porque há nichos ecológicos favoráveis nas superfícies porosas, ásperas e irregulares destes dispositivos (Brusca, Chara, *et al.*, 2007; Carneiro, 2008; Faltermeier e Bürgers, 2008; Türkkahraman, Sayin, *et al.*, 2005).

Esta pesquisa foi altamente sensível, abordando as evidências de mínimo viés. O estudo de Jordan & LeBlanc (Jordan e LeBlanc, 2002) foi excluído por ter avaliado apenas um sistema de bráquete, examinador não cego e as análises estatísticas não tem sido especificadas. Os estudos *in vitro* dos autores Brusca, Chara, *et al.*, 2007; Faltermeier, Bürgers, *et al.*, 2008 e Fournier, Payant, *et al.*, 1998, também foram excluídos por não terem as características inerentes para o desenvolvimento do biofilme. As diferenças observadas, entre os resultados de

alguns artigos (Brusca, Chara, *et al.*, 2007; Pandis, Vlachopoulos, *et al.*, 2008; Pellegrini, Sauerwein, *et al.*, 2009; Pithon, dos Santos, *et al.*, 2011; van Gastel, Quirynen *et al.*, 2007), podem estar relacionadas com fatores que incluem as variações no formato, o material e tamanho entre os bráquetes CON e AL, o nível de higiene bucal individual, o fluxo salivar, as variáveis de tratamento, os tipos de ligaduras, os procedimentos de colagem e a idade dos indivíduos (Fournier, Payant, *et al.*, 1998; Pandis, Papaioannou, *et al.*, 2010; Quirynen, Dekeyser, *et al.*, 1991; Sukontapatipark, el-Agroudi, *et al.*, 2001). Assim, o tipo do bráquete propriamente dito não seria o fator decisivo para o desenvolvimento do biofilme, mas sim a composição e o tipo de material destes devem ser incluídos como fatores de formação de colônias de *Streptococcus mutans* (Carneiro, 2008).

Na revisão sistemática, o tratamento estatístico dos resultados foi inviável em virtude dos desenhos metodológicos dos artigos elegíveis terem sido heterogêneos, entretanto, a avaliação da relevância científica revelou pesquisa de alta qualidade e solidez metodológica dos seis estudos selecionados (Pandis, Vlachopoulos, *et al.*, 2008; Pandis, Papaioannou, *et al.*, 2010; Pejda, Varga, *et al.*, 2012; Pellegrini, Sauerwein, *et al.*, 2009; Pithon, dos Santos, *et al.*, 2011; van Gastel, Quirynen *et al.*, 2007), como mostrado em suas notas finais, de acordo com a escala de Jadad (Jadad, Moore, *et al.*, 1996).

Há relatos de que os bráquetes CON estão diretamente relacionados com o acúmulo de biofilme (Batoni, Pardini, *et al.*, 2001; Eliades, Eliades, *et al.*, 1995), entretanto, o estudo de Pithon *et al.*, 2011 sugere que, devido a troca dos anéis elastoméricos, uma provável infecção é controlável com a utilização de bráquetes CON, porque estes bráquetes favorecem menor formação de colônias de

Streptococcus mutans, concordando com estudo de van Gastel, Quirynen *et al.*, 2007 que não mostrou diferença entre CON e AL no sangramento gengival.

O aumento da microbiota bucal de *Streptococcus mutans* e Lactobacilos está associado em pacientes com uso de aparelhos ortodônticos (Balenseifen e Madonia, 1970; Forsberg, Brattström, *et al.*, 1991; Pellegrini, Sauerwein, *et al.*, 2009; Rosenbloom e Tinanoff, 1991; Sukontapatipark, el-Agroudi, *et al.*, 2001), sejam com bráquetes CON ou AL. Este aumento leva a maior cariogenicidade da placa, ao baixo pH a ponto de alterar os parâmetros periodontais clínicos (Pandis, Vlachopoulos, *et al.*, 2008; Pejda, Varga, *et al.*, 2012; van Gastel, Quirynen *et al.*, 2007) e ao aumento do risco de desmineralização do esmalte (Faltermeier, Bürgers, *et al.*, 2008). Alguns estudos elegíveis (Pejda, Varga, *et al.*, 2012; Pithon, dos Santos, *et al.*, 2011) avaliaram não só a presença de *Streptococcus mutans*, como também a de outros microrganismos relacionados a doença periodontal em pacientes com bráquetes CON ou AL. O estudo de Pejda *et al.*(2012), encontrou 23,8 vezes mais chance da presença de *Aggregatibacter actinomycetemcomitans* (AA) na placa subgengival de pacientes com os bráquetes CON, mas o aumento destes microrganismos (AA) não representou fator de risco para periodontite localizada, conforme os estudos de Paolantonio *et al.* (1997,1999). As discordâncias de resultados encontradas entre o estudo de Pithon *et al.*(2011) com os demais estudos elegíveis analisados (Pandis, Vlachopoulos, *et al.*, 2008; Pandis, Papaioannou, *et al.*, 2010; Pejda, Varga, *et al.*, 2012; Pellegrini, Sauerwein, *et al.*, 2009; van Gastel, Quirynen *et al.*, 2007), podem ser explicadas pela diferença de metodologia. Em alguns estudos (Pandis, Vlachopoulos, *et al.*, 2008; Pandis, Papaioannou, *et al.*, 2010; Pejda, Varga, *et al.*, 2012; van Gastel, Quirynen *et al.*, 2007), as unidades das colônias formadas foram contadas a partir da saliva, os

estudos de Pellegrini, Sauerwein, *et al.* (2009), a partir das superfícies dentárias ao redor do bráquete. No estudo de Pithon (2011) as colônias foram contadas diretamente das superfícies dos bráquetes (aleta, canaleta e cervical), motivo pelo qual este último trabalho deve ter encontrado diferenças estatísticas significantes, revelando maior acúmulo de biofilme sobre os bráquetes AL.

Algumas pesquisas (Damon, 1998; Forsberg, Brattström, *et al.*, 1991; Paduano, Cioffi, *et al.*, 2008; Shivapuja. e Berger, 1994; Türkkahraman, Sayin, *et al.*, 2005; Yu e Qian, 2007) relatam que os bráquetes AL são menos susceptíveis a colonização bacteriana pelo seu formato e pela ausência de ligaduras elásticas ou metálicas. No entanto, o adequado controle de biofilme está fortemente influenciado pela correta orientação e colaboração dos pacientes quanto à higiene bucal (Pandis, Papaioannou, *et al.*, 2010; Quirynen, Dekeyser, *et al.*, 1991), do que simplesmente optar pela seleção de um sistema ou outro de bráquetes.

Os desfechos dos estudos elegíveis (Pandis, Vlachopoulos, *et al.*, 2008; Pandis, Papaioannou, *et al.*, 2010; Pejda, Varga, *et al.*, 2012; Pellegrini, Sauerwein, *et al.*, 2009; Pithon, dos Santos, *et al.*, 2011; van Gastel, Quirynen *et al.*, 2007) não foram unânimes ao relatar a possível influência da configuração dos bráquetes (convencionais ou autoligados) sobre a aderência e a formação de colônias de *Streptococcus mutans*.

As decisões de ortodontistas em utilizar os bráquetes AL na sua rotina clínica, em detrimento dos CON, com objetivo de melhorar a higienização/acúmulo de placa, não podem ser confirmadas por falta de evidência científica, considerando-se o que afirma a literatura (Pandis, Vlachopoulos, *et al.*, 2008; Pandis, Papaioannou, *et al.*, 2010; Pejda, Varga, *et al.*, 2012; Pithon, dos Santos, *et al.*, 2011; van Gastel, Quirynen *et al.*, 2007). Após esta revisão pode-se presumir que não há evidência

científica suficiente para apoiar o uso dos aparelhos ortodônticos fixos com bráquetes AL em substituição aos sistemas de aparelhos CON ou vice-versa, em conformidade com o estudo de Fleming e Johal, 2010.

Na presente pesquisa, o segundo artigo estudou comparações entre os sistemas de bráquetes CON e AL estéticos, avaliando a adesão microbiana *in vivo*, o que deve ser considerado para a obtenção de qualidade em evidência clínica. Para assegurar estudo cego, as amostras foram colocadas em placas de Petri, e o sistema de identificação era desconhecido do examinador (Jadad, Moore, *et al.*, 1996).

A variabilidade, do número de unidades formadoras de colônias de *S. mutans* entre os bráquetes estéticos, no presente estudo, coincide com a variação de outros estudos (Anhoury, Nathanson, *et al.*, 2002; Fournier, Payant, *et al.*, 1998; Menzaghi, Saletta, *et al.*, 1991; Quirynen, Dekeyser, *et al.*, 1991; Quirynen, Marechal, *et al.*, 1988; Satou, Fukunaga, *et al.*, 1988; van Pelt, Weerkamp, *et al.*, 1985). As características das superfícies anatômicas da gengiva influenciam a formação espontânea de biofilme, tanto em quantidade como em qualidade e da mesma forma, a composição e quantidade de secreção salivar podem variar com a idade e influenciar a aderência bacteriana (Addy Shaw, *et al.*, 1982; Ramberg, Axelsson, *et al.*, 1995; Ristic, Vlahovic, *et al.*, 2007).

Mesmo que os bráquetes AL eliminem as ligaduras, incorporando mecanismos intrínsecos de abertura e fechamento, estes podem proporcionar oportunidades adicionais para a retenção da placa local, que pode se calcificar e tornar-se obstáculo ao funcionamento do mecanismo de abertura e de fechamento (Faltermeier, Bürgers, *et al.*, 2008). As ligaduras elásticas no ambiente bucal permitem a adsorção de potássio e de sódio na fase inicial, seguida por precipitação

do cálcio e de potássio, estabilizando o tegumento formado (Quiryneen, Marechal, *et al.*, 1988), o que poderia favorecer o acúmulo de placa, mas também não se observou diferença estatística em relação aos resultados deste estudo ao comparar os AL com o Mystique convencional. Do mesmo modo, não foi encontrada diferença nas condições periodontais de pacientes tratados com ligaduras elásticas ou metálicas (Forsberg, Brattström, *et al.*, 1991).

Na diferenciação entre os bráquetes com maior ou menor formação de colônias de *S. mutans*, observou-se que houve pouca colonização sobre as aletas dos bráquetes estéticos CON e dos AL, e a maior formação de colônias ocorreu nas canaletas, seguida pela região cervical dos bráquetes. Ao avaliar os bráquetes sem considerar as áreas específicas da coleta, os CON Clarity tiveram a menor formação de colônias, enquanto os bráquetes AL In-Ovation C e Quicklear tiveram a maior. Tronchin *et al.* (1988) demonstraram a capacidade de formação de fina camada de biofilme sobre a superfície dos dispositivos sintéticos. No entanto, existe conflito com estudos in-vitro, cujos resultados parecem indicar que a aderência de *S. mutans* é mais fraca em bráquetes metálicos que nos de plástico ou cerâmica (Brusca, Chara, *et al.*, 2007; Fournier, Payant, *et al.*, 1998). O presente estudo também mostrou que a maior colonização de *S. mutans* localizou-se nas canaletas dos bráquetes dos AL do In-Ovation C. O bráquete convencional Mystique teve o mesmo significado estatístico dos AL. A canaleta do Mystique é de cerâmica, que é porosa com áreas rugosas, tinha, portanto, maior potencial para acúmulo de microrganismos em comparação com a metálica mais lisa e menos porosa canaleta do Clarity. Os bráquetes de cerâmica são mais propensos à colonização bacteriana do que os metálicos (Carneiro, 2008), e isso pode ser observado quando se compara os

convencionais Mystique e Clarity deste estudo. A menor colonização foi verificada com os bráquetes Clarity.

Alguns autores compararam os sistemas metálicos “edgewise” convencionais e autoligados (Pandis, Vlachopoulos, et al., 2008; van Gastel, Quirynen et al., 2007). Observaram que a maior ou menor aderência microbiana e de desenvolvimento de biofilme não é determinada pelo sistema de bráquetes utilizados. Uma vez que as características dos materiais dos bráquetes podem variar consideravelmente, a colonização de *S. mutans* pode ser diferente entre os tipos de bráquetes utilizados atualmente, como também foi evidenciado nesta presente pesquisa.

Observando as limitações de alguns trabalhos (Addy, Shaw, et al., 1982; Maza, Elguezabal, et al., 2002; Park, Gakunga, et al., 2007), pesquisas adicionais, com outros tipos de bráquetes, devem ser realizadas para visualizar as complicações periodontais decorrentes das diferentes formas, tipos de materiais e tamanhos dos bráquetes, e com isso nortear o desenvolvimento de novos projetos de sistemas de bráquetes com material afim de reduzir a formação das colônias de *Streptococcus mutans*.

Com o objetivo de estudar os aspectos biomecânicos envolvendo os sistemas de bráquetes CON e AL, os autores, no terceiro artigo, investigaram a correlação da quantidade de movimentação ortodôntica com a atividade de remodelação óssea celular após 15 dias, usando os bráquetes CON que necessitam de ligaduras e bráquetes AL que não as usam.

A quantidade do movimento ortodôntico com mecânica de deslizamento foi observada pela avaliação clínica, e a atividade de remodelação óssea celular, devido às respostas das células do ligamento periodontal (osteoclastos e osteoblastos).

O alinhamento das canaletas dos bráquetes e os esforços para eliminar o atrito ou resistência devido às diferenças no alinhamento inicial das canaletas foi feito para aumentar a validade deste estudo. Assim, um fio 0,019" x 0,025" de CrNi foi inserido nas canaletas de todos os bráquetes da forma mais passiva possível e, depois de 30 dias após o alinhamento destas, iniciou-se a ativação da mecânica de deslizamento.

Alterações morfológicas do suporte periodontal visualizadas por microscopia de luz neste experimento foram semelhantes aos encontrados em estudos clássicos (Reitan, 1974; Reitan e Kvam, 1971; Rygh, 1976). Muitos estudos anteriores sobre a movimentação dentária experimental foram realizados em ratos (Katona, Paydar, *et al.*, 1995; Silveira, Franco, *et al.*, 2006). No entanto, o rato como modelo experimental tem desvantagens. Roedores têm erupção contínua dos incisivos e ocorre migração distal fisiológica dos molares. A erupção contínua dos incisivos pode afetar a direção da força aplicada, porque os incisivos são frequentemente usados como a unidade de ancoragem nestas experiências. A migração distal pode camuflar a quantidade real do movimento dentário. Usando o cão, como modelo experimental, pode-se superar esses problemas com boas razões para apoiar o uso destes animais em pesquisas envolvendo movimentação dentária induzida (Jónsdóttir, Giesen, *et al.*, 2006; Lou, Fang, *et al.*, 2011). O estudo de Reitan (1974) propôs a força de 150 gf como força ótima para movimentação do canino humano. Neste estudo, a força adotada para movimentar os dentes foi maior (250 gf), considerando o osso alveolar de cães como variável da mecânica ortodôntica, pois geralmente este é mais denso do que em seres humanos (Reitan e Kvam, 1971). Embora com as diferenças entre a anatomia do ligamento periodontal e osso alveolar de cães e de seres humanos, os cães são geralmente aceitos como bom

modelo para comparação com os seres humanos (Pilon, Kuijpers-Jagtman, *et al.*, 1996; Reitan e Kvam, 1971) e a investigação da quantidade de movimentação dentária e atividade remodelação óssea considerando em curto período de acompanhamento com modelos animais, pode ser inequivocamente comparável com a atividade observada em humanos (Graber, 1996; Jónsdóttir, Giesen, *et al.*, 2006). No presente estudo, teve-se dificuldade no preparo de amostra suficiente para período de movimentação ortodôntica de 21 dias. O estudo de Midgett *et al.* (1981) também relata a dificuldade em trabalhos com animais experimentais. Portanto, os autores, para o período de 15 dias de movimentação ortodôntica, adotaram elevado número de repetições considerado ($n = 20$) para realizar o teste ANOVA (Sampaio, 1998).

A histomorfometria óssea permite a avaliação quantitativa de células e do remodelamento ósseo em cortes histológicos (Compston, 2004), fornecendo informações únicas sobre mecanismos de perda e ganho ósseo de intervenções terapêuticas (Compston, 2004). Muitas das medidas feitas na histomorfometria óssea têm um elemento subjetivo, e isso, juntamente com as diferenças de técnicas de coloração, de ampliação e métodos de medição, contribui para a variabilidade significativa nas medições inter e intra-observador (Chavassieux, Arlot, *et al.*, 1985; de Vernejoul, Kuntz, *et al.*, 1981).

No presente trabalho a aplicação da força de 250 gf no prazo de 15 dias foi suficiente para observar o movimento clínico e significativas reações histológicas, o que está de acordo também com estudos (Consolaro, 2002; Mackie, 2003; Melsen, 1999) que relataram que cerca de dois dias após a aplicação da força ortodôntica, modificações locais permitem que os osteoblastos e osteoclastos possam iniciar o processo de remodelação óssea. Essas reações histológicas

também foram observadas neste estudo, mas com maior presença de áreas de absorção do que de áreas de aposição. Nesta pesquisa, o desempenho do movimento clínico no intervalo de tempo de 15 dias não houve diferença na significância estatística entre os bráquetes CON e AL ao nível do terço gengival da coroa. Este resultado está de acordo com o estudo de Miles (2007) realizado com os bráquetes AL passivo Smart-Clip e CON, da mesma maneira como os estudos de Miles, Weyant, *et al.* (2006); Pandis, Polychronopoulou, *et al.* (2007) com bráquetes Damon 2 e CON. No entanto, diferenças na significância estatística foram observadas entre os CON e AL ao nível do terço incisal da coroa. Neste nível, o movimento ortodôntico dos AL foi maior que no terço gengival, indicando movimento de inclinação para os AL. Diferentes desempenhos das reações iniciais histológicas de absorção e aposição óssea foram encontrados entre CON e AL. No ligamento periodontal dos dentes com bráquetes CON, aglomeração de osteoblastos e osteoclastos foram uniformes ao longo de toda a raiz dos dentes destes animais, enquanto para os bráquetes AL, a prevalência destas células era maior nos terços gengivais ou apicais. No lado de tensão nos dentes com AL, foi encontrada compressão no terço apical e tensão no terço gengival. No lado da compressão, ocorreu o oposto, o que sugere que o movimento de inclinação está presente nos dentes com os bráquetes AL. O movimento de inclinação histologicamente identificado pode ter influenciado o resultado da quantificação do movimento clínico, que foi ligeiramente maior nos dentes superiores com os bráquetes AL, porém sem significância estatística. Nos grupos de dentes com bráquetes CON, tensão e compressão foram observadas ao longo dos terços radiculares dos dentes apenas nos lados correspondentes, o que sugere movimento de translação para este grupo.

O grau de sucesso alcançado com a mecânica ortodôntica pode ser influenciado por muitas variáveis, tais como: idade, fatores endócrinos, doenças sistêmicas, medicamentos, densidade óssea, tipo de material, tamanho, forma e ângulo da interface fio / canaleta, colagem e tipo de amarração (Henaó e Kusy, 2005; Zachrisson, 2006). Neste estudo, ao avaliar o comportamento do movimento clínico ortodôntico nas arcadas da mandíbula e maxila, quando utilizado o mesmo sistema de bráquetes, diferença estatisticamente significativa não foi encontrada entre os CON, o que não foi observado com os AL quando comparadas as medidas dos terços gengival e incisal avaliadas. Um estudo de Deguchi, Takano-Yamamoto, *et al.*(2008) mostrou que clinicamente, o osso responde de forma diferente a força ortodôntica, resultando em diferença significativa entre as taxas de movimentação dentária na maxila e mandíbula. Entretanto, outro estudo (Huja, Fernandez, *et al.*, 2006) relatou que, nos ossos de cães adultos, a taxa de formação óssea (BFR), que é uma medida do volume de osso ou de remodelação no osso de suporte dos dentes permanentes, é substancialmente maior no processo alveolar de dentes permanentes erupcionados (mandíbula aproximadamente 37% / ano, maxila aproximadamente 19% / ano) que o BFR encontrado em outros locais, como o fêmur (6,4% / ano).

No comportamento clínico de movimento dentário dos bráquetes AL, os autores observaram que, mesmo com maior movimento de inclinação do que o de translação, há evidência insuficiente de que fios retangulares, na presença de inclinações, os AL podem causar menor atrito, em comparação com os bráquetes CON, o que está de acordo com alguns autores (Ehsani, Mandichb, *et al.*, 2009; Harradine, 2001; Morina, E., T. Eliades, *et al.*, 2008), afirmando que tal acontecimento pode estar relacionado com o tamanho do fio ortodôntico e não

depende apenas do sistema utilizado. No entanto, uma vez que outro estudo (Kumamoto, Yamauchi, *et al.*, 2003) relatou a aplicação de métodos de biologia molecular moderna, focada no papel desempenhado pelas células do ligamento periodontal, novas pesquisas podem explicar qual sistema poderia antecipar o estresse celular, mesmo antes de iniciar a movimentação ortodôntica.

6 CONCLUSÃO

Após a comparação dos dois sistemas de bráquetes *edgewise*: convencionais e os autoligados, pode-se concluir que:

6.1 Não houve evidencia na literatura, por meio de revisão sistemática, da possível influência da configuração dos dois sistemas de bráquetes quanto a aderência e formação de colônias de *Streptococcus mutans*;

6.2 Por meio de estudo *in vivo*, os bráquetes autoligados estéticos não apresentam maior ou menor aderência e formação de colônias de *Streptococcus mutans*. As diferenças foram relacionadas com a composição do material que constitui o bráquete e

6.3 Por meio de estudo em animais, a quantidade e tipo de movimento dentário e a remodelação óssea indicaram maior presença de movimento de translação dos dentes com o sistema convencional, e maior de inclinação nos dentes com os autoligados.

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8 ANEXOS

8.1 PARECER DE COMITÉ DE ÉTICA

PROJETO DE PESQUISA

Título: A COLONIZAÇÃO DE "STREPTOCOCCUS MUTANS" NOS BRÁQUETES CONVENCIONAIS "EDGEWISE" E PRÉ-AJUSTADOS
Área Temática:

Pesquisador: LEONARD EULER ANDRADE GOMES DO
Instituição: NASCIMENTO

Versão: 2
CAAE: 02798412.6.0000.5209

PARECER CONSUBSTANCIADO DO CEP

Número do Parecer: 28625

Data da Relatoria: 26/05/2009

Apresentação do Projeto:

O Projeto de Pesquisa intitulado A COLONIZAÇÃO DE "STREPTOCOCCUS MUTANS" NOS BRÁQUETES CONVENCIONAIS "EDGEWISE" E PRÉ-AJUSTADOS terá como amostra 10 indivíduos do gênero masculino, com idades de 20 a 30 anos que receberão tratamento na clínica de Ortodontia do curso de especialização em Ortodontia da UFPI. O grupo 1 (controle) será constituído de 5 pacientes com aparelhos com a prescrição da técnica EW e o grupo 2 (experimental) de 5 pacientes usando bráquetes SL. A coleta inicial será feita, previamente à montagem da aparelhagem ortodôntica, nos dentes 11, 13 e 15, para se ter o perfil microbiológico de cada paciente. A segunda coleta do biofilme será feita, da canaleta e cervical dos bráquetes colados, nos dentes envolvidos, 21 dias após terem sido colados. A quantidade de biofilme colhida em cada dente de cada paciente será semeada em alíquotas de 0,1 ml de cada diluição em placas de Petri, contendo meio de cultura Mitis Salivarius específico para pesquisa de Streptococcus mutans. As placas de Petri serão incubadas durante o período da contagem (72h), a 38°C, em ambiente anaeróbico. As placas selecionadas deverão apresentar colônias, visíveis macroscopicamente, para se realizar a leitura e contagem do número de colônias, a qual será realizada por um único examinador, previamente calibrado e treinado. O material colhido será acondicionado em tubos plásticos Eppendorf, e semeados em placas de Petri, contendo meio de cultura Mitis Salivarius específico para Streptococcus mutans. Para a estatística utilizar-se-á teste de ANOVA e a correção de Tukey, ao nível de 5% de probabilidade.

Objetivo da Pesquisa:

GERAL: Comparar o acúmulo de biofilme sobre os bráquetes "edgewise" convencionais versus pré-ajustados.
ESPECÍFICO: Identificar a vantagem dos sistemas quanto ao acúmulo de biofilme.

Avaliação dos Riscos e Benefícios:

O projeto relata que não há riscos e os benefícios mencionam que após finalizado o estudo, a comunidade estudada saberá o comportamento de acúmulo de biofilme sobre distintos sistemas de bráquetes, evitando, em pacientes propensos a distúrbios periodontais, os sistemas que favorecem um maior acúmulo de biofilme.

Comentários e Considerações sobre a Pesquisa:

O projeto de pesquisa possui tema relevante e impacto positivo direto na melhoria da saúde bucal da sociedade.

Considerações sobre os Termos de apresentação obrigatória:

Os termos solicitados anteriormente foram anexados e corrigidos a contento.

Recomendações:

Recomendo a aprovação do projeto de pesquisa.

Conclusões ou Pendências e Lista de Inadequações:

De acordo com a análise ética, todos os aspectos solicitados como correção do TCLE, indicação dos benefícios diretos e o destino final das amostras coletadas foram contemplados.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Conforme reunião do Colegiado do CEP/UESPI e de acordo com as normas de eticidade da Resolução 196/96 (CNS/MS) e seus complementares, o presente projeto de pesquisa apresenta o parecer *APROVADO* por apresentar todas as solicitações indicadas na versão 1.

TERESINA, 29 de Maio de 2009

Luciana Saraiva e Silva

Assinado por:

LUCIANA SARAIVA E SILVA



MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PIAUÍ - CCA
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA EM EXPERIMENTAÇÃO COM ANIMAIS
Campus Universitário Ministro Petrônio Portela, Bairro Ininga, Teresina, Piauí, Brasil; CEP: 64049-550

PARECE DE PROJETO DE PESQUISA

Parecer Nº 01/09
Pesquisador responsável: LEONARD EULER A. G. DO NASCIMENTO
Processo nº 016450/08-57
Protocolo: 004/08
Instituição onde será desenvolvido: Universidade Federal do Piauí
Situação: APROVADO

O Comitê de Ética em Experimentação com Animais (CEEA) da Universidade Federal do Piauí analisou o protocolo do projeto intitulado “**O comportamento biomecânico entre “brackets” autoligáveis e “edgewise” convencional**”, o qual considerando o atendimento aos requisitos fundamentais das Propostas de Diretrizes Éticas Internacionais para Pesquisas Biomédicas Envolvendo Animais (Conselho das Organizações Internacionais das Ciências Médicas - CIOMS/OMS, 1985) e também aquelas definidas a nível nacional pelo Colégio Brasileiro de Experimentação Animal (COBEA), além de apresentar importância social e científica, teve parecer APROVADO.

Solicita-se ao pesquisador o envio, a este CEEA, de relatórios parciais do projeto, bem como de relatório final gravado em CD-ROM.

Teresina, 02 de Julho de 2009

Prof.º Ivete Lopes de Mendonça
Presidente do CEEA-UFPI

8.2 ACEITES DE PUBLICAÇÃO

ACEITE ARTIGO 1

De: sgdpjjo@dentalpressjournals.com.br
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Maringá, Friday, 1 de February de 2013

Ilmo(a) Sr.(a)
Prof(a), Dr(a) LEONARD EULER ANDRADE GOMES DO NASCIMENTO

Referente ao protocolo: 1272
Classificação: Novo Artigo

Temos o prazer de informar que a versão em inglês do manuscrito Os bráquetes autoligados estão relacionados com menor formação de colônias de Streptococcus mutans? Uma Revisão Sistemática foi aprovada pelo Conselho Editorial do Dental Press Journal of Orthodontics e será publicado em breve. Lembramos que algumas modificações poderão ser solicitadas até a publicação do artigo.

Obrigado por submeter seu trabalho ao Dental Press Journal of Orthodontics.

Atenciosamente,

David Normando
Editor Chefe do Dental Press Journal of Orthodontics

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ACEITE ARTIGO 2

De: ees.ajodo.0.1ae0a3.2891b6d7@eesmail.elsevier.com
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Ms. Ref. No.: AJODO-D-12-00398R1

Title: Colonization of Streptococcus mutans on aesthetic
brackets: self-ligating versus conventional.

American Journal of Orthodontics & Dentofacial Orthopedics

Dear Dr do Nascimento,

Thank you for resubmitting the revised version of your manuscript. I sent the most recent revision back to the Associate Editor, who is now satisfied that all necessary changes have been made and recommends acceptance and publication of your research in the AJO-DO. Congratulations.

I look forward to seeing the article in the AJO-DO. When we approach the publication date, I will send you more information about the proofing process and your publication date.

With kind regards,

Vincent G. Kokich
Editor-in-Chief

American Journal of Orthodontics and Dentofacial Orthopedics