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BIOCOMPATIBILIDADE DE CIMENTOS ORTODÔNTICOS MODIFICADOS POR RESINA: ANÁLISE MORFOLÓGICA E IMUNO-HISTOQUÍMICA

JANAINA ALMEIDA MESQUITA

Campina Grande-PB

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Estadual da Paraíba, como parte dos requisitos para a obtenção do título de Mestre em Odontologia.

Orientadora: Profa. Dra. Pollianna Muniz Alves
Co-orientador: Prof Dr. Rogério Lacerda dos Santos

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DEDICATÓRIA

À Deus, pai amado,

a quem eu devo tudo em minha vida.

Aos meus pais, **Gilvan e Mariêde**,

minha base e meu auxílio em todo o tempo.

Ao meu esposo, **Fioravante Prest**,

meu amor e maior incentivador.

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AGRADECIMENTOS ESPECIAIS

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“Para tudo há um tempo para cada propósito debaixo do céu:

Tempo de nascer e tempo de morrer,

Tempo de plantar e tempo de arrancar o que se plantou;

Tempo de matar e tempo de curar,

Tempo de derrubar e tempo de construir;

Tempo de chorar e tempo de rir,

Tempo de prantear e tempo de dançar;

Tempo de espalhar pedras e tempo de ajuntá-las,

Tempo de abraçar e tempo de se conter

Tempo de procurar e tempo de desistir,

Tempo de guardar e tempo de lançar fora;

Tempo de rasgar e tempo de costurar,

Tempo de calar e tempo de falar;

Tempo de amar e tempo de odiar,

Tempo de lutar e tempo de viver em paz.”

Ec 3.1-8

RESUMO

RESUMO

Objetivo: Avaliar a biocompatibilidade de cimentos de ionômero de vidro modificados por resina (CIVMR) em tecido subcutâneo de ratos através de análises morfológicas e imuno-histoquímicas. **Material e Métodos:** Setenta e cinco ratos Wistar machos adultos foram selecionados e distribuídos em cinco grupos: Grupo C (*Controle*); Grupo CK (*Crosslink Orthodontic Band Cement*); Grupo RS (*Resilience Light Cure Band Cement*); Grupo RMO (*RMO Band Cement*) e Grupo TP (*Transbond Plus Light CureBand*). Os materiais foram implantados em tecido subcutâneo e após os intervalos de tempo de 7, 15 e 30 dias foram realizadas biópsias excisionais. Os parâmetros morfológicos avaliados foram: infiltrado inflamatório, edema, necrose, reação de células gigantes multinucleadas, reação de granulação, fibroblastos jovens e colagenização. Na avaliação imuno-histoquímica por meio da imunomarcação com o anticorpo anti CD68, foram quantificados macrófagos e células gigantes multinucleadas. Os resultados obtidos foram analisados estatisticamente através dos testes Kruskal-Wallis e Dunn, (p<0.05). **Resultados:** Na análise morfológica, após 7 dias, os grupos RS, RMO e TP demonstraram infiltrado inflamatório mais intenso que o grupo controle (p=0,004); apenas o grupo RMO apresentou maior reação de células gigantes multinucleadas (p=0,027) e todos os grupos demonstraram reação de granulação mais intensa (p=0,002). O grupo RMO apresentou baixo grau de colagenização após 15 (p=0,008) e 30 dias (p=0,014). Na análise imuno-histoquímica, pode-se observar que os grupos RMO e RS apresentaram maior imunoexpressão do CD68 (p=0,004) no período de 7 dias e apenas o grupo RMO apresentou diferença estatisticamente significativa para este parâmetro após 15 dias quando comparado ao grupo controle (p=0,026). No período de 30 dias, o grupo RMO apresentou maior quantidade de células gigantes multinucleadas (p=0,004). **Conclusão:** Dentre os CIVMR avaliados, Ck e TP proporcionaram melhores respostas teciduais, visto que demonstraram menor intensidade de infiltrado inflamatório e maior grau de colagenização. O RMO demonstrou a menor biocompatibilidade, seguido pelo RS, devido à maior intensidade de infiltrado inflamatório com acúmulo substancial de macrófagos e de células gigantes multinucleadas observado nas análises morfológicas e imuno-histoquímicas.

Palavras-chave: Cimentos ortodônticos; Teste de biocompatibilidade; Inflamação; Macrófagos; Células gigantes multinucleadas.

ABSTRACT

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Objective: To evaluate the biocompatibility of resin-modified glass ionomer (RMGIC) orthodontic cements in rat subcutaneous tissue by morphological and immunochemistry analysis. **Materials and Methods:** Seventy-five (75) adult male Wistar rats were selected and divided into five groups: Group C (Control); Group CK (Crosslink Orthodontic Band Cement); Group RS (Resilience Light Cure Band Cement); Group RMO (RMO Band Cement) and Group TP (Transbond Plus Light Cure Band). The materials were inserted in the subcutaneous tissue of each rat and after time intervals of 7, 15 and 30 days excisional biopsies were performed. The histological parameters assessed were: inflammatory infiltrate intensity; reaction of multinucleated giant cells; edema; necrosis; granulation reaction; young fibroblasts and collagenization. The immunochemistry analyzed immunostaining of CD68 antibody for macrophages and multinucleated giant cells. The results obtained were statistically analyzed by the Kruskal-Wallis and Dunn test, with value of $p<0.05$. **Results**: In the morphological analysis, after 7 days, the groups Resilience, RMO and Transbond showed inflammatory infiltrate more intense than the control group ($p=0.004$); only Group RMO presented greater expression of multinucleated giant cell reaction ($p=0.003$) and all groups demonstrated a more intense granulation reaction ($p=0.002$). The group RMO presented low degree of collagenization after 15 ($p=0.008$) and 30 days ($p=0.014$). The immunochemistry analysis revealed that the RMO and Resilience groups showed a higher immunostaining of CD68 ($p=0.004$) within 7 days and only the RMO group showed a statistically significant difference for this parameter after 15 days compared to the control group ($p=0.026$). In the period of 30 days, the RMO group had a higher number of multinucleated giant cells ($p=0.004$). **Conclusion:** Among the RMGICs evaluated, Crosslink and Transbond provided better tissue response, since it demonstrated a lower level of inflammatory infiltrate and higher degree of collagenization. The RMO demonstrated the lowest level of biocompatibility due to the intense inflammatory with substantial accumulation of macrophage and multinucleated giant cells observed in morphological and immunochemistry techniques.

Key words: Orthodontic cement; Biocompatibility testing; Inflammation; Macrophage; Multinucleated Giant Cells.

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BisGMA	Do inglês *Bisphenol A-glycidyl methacrylate,*
BSA	Albumina de soro bovino
C	Controle
CD68	Do inglês *cluster of differentiation 68,* traduzido como grupamento de diferenciação 68.
CGM	Células gigantes multinucleadas
CIV	Cimento de ionômero de vidro
CIVMR	Cimento de ionômero de vidro modificado por resina
CK	Crosslink® Orthodontic Band Cement
COX-2	Ciclo-oxigenase-2
GIC	Do inglês *glass ionomer cement,* traduzido como cimento de ionômero de v
HCL	Ácido clorídrico
HE	Hematoxilina e eosina
LED	Do inglês *Light Emitting Diode,* traduzido como diodo emissor de luz
MGC	Do inglês *Multinucleated giant* cells, traduzido como células gigantes multinucleadas
MOs	Macrófagos
PGE2	Prostaglandina E2
RMGIC	Do inglês *Resin modified glass ionomer cement,* traduzido como cimento de ionômero de vidro modificado por resina
RMO	RMO® Band Cement
RS	Resilience® Light Cure band Cement
TP	Transbond® Plus Light Cure Band
TRIS	Tris(hidroximetil)aminometano

SUMÁRIO

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CONSIDERAÇÕES INICIAIS

1. CONSIDERAÇÕES INICIAIS

Na ortodontia, as bandas constituem elementos de suporte aos aparelhos fixos e desempenham um importante papel na terapia ortodôntica convencional (SFONDRINIA et al., 2010). A cimentação desses acessórios é rotineiramente empregada em áreas de alto estresse oclusal, principalmente em dentes submetidos a forças mecânicas e mastigatórias intensas, como pré-molares e molares, que servirão de ancoragem para aparelhos intra ou extrabucais, barras transpalatinas, arcos linguais, ou naqueles com coroas clínicas anômalas (KNOX; CHIE; DURNING, 2004; FERREIRA; COTRIM-FERREIRA, 2013).

Os materiais utilizados para cimentação de bandas devem apresentar propriedades físicas, químicas e biológicas adequadas. Alguns cimentos ortodônticos foram utilizados por muito tempo, dentre estes o cimento de fosfato de zinco, o cimento de policarboxilato e o cimento de ionômero de vidro (CIV) (DRAGAS et al., 2012). O CIV é utilizado até hoje, visto que apresenta baixa solubilidade, boa adesividade e capacidade de liberação de flúor. No entanto, apesar dessas características favoráveis, a sua retenção ao esmalte dentário ainda não é suficiente para resistir às forças da mastigação e à mecanoterapia ortodôntica (VARLIK; ULUSOY, 2009).

Atualmente, o material de preferência dos ortodontistas é o cimento de ionômero de vidro modificado por resina (CIVMR), o qual agrega às principais propriedades do CIV o maior tempo de trabalho e aumento da resistência, fornecendo mais segurança e facilidade durante a cimentação (MALKOC et al., 2010). Entretanto, apesar das propriedades mecânicas adequadas, o CIVMR tem demonstrado efeitos citotóxicos mais evidentes que o CIV convencional (ANGELIERI et al., 2012; RODRIGUEZ et al., 2013).

A maior citotoxicidade do CIVMR pode estar relacionada à quantidade de monômeros hidrofílicos ou a outras espécies de baixo peso molecular, tais como aditivos e co-iniciadores liberados durante a polimerização (DRAGAS et al., 2012; RODRIGUEZ et al., 2013). Normalmente, os monômeros presentes nesses materiais são convertidos em polímeros durante o processo de polimerização (JAGDISH et al., 2009). No entanto uma conversão inadequada acarretará em uma maior quantidade de

monômeros residuais que são capazes de provocar efeito citotóxico significativo e afetar a biocompatibilidade destes materiais (KUAN et al., 2012; SANTOS et al., 2012).

A biocompatibilidade é a capacidade de um material atuar em uma aplicação específica e proporcionar uma resposta aceitável do hospedeiro sem provocar ação tóxica, inflamatória, alérgica ou carcinogênica sobre os tecidos (MOUSAVINASAB, 2011). Idealmente, para que o material possa ser utilizado na cavidade oral é necessário que seja inofensivo para os tecidos bucais: gengivas, mucosas, polpa e osso, sendo assim a biocompatibilidade um requisito fundamental a ser avaliado na odontologia (AMINOZARBIAN et al., 2012).

Embora os modelos *in vitro* proporcionem boa indicação e avaliação dos biomateriais, testes *in vivo* são necessários para fornecer uma análise mais crítica em relação à biocompatibilidade (ZHOU et al., 2011; ANDOLFATTO et al., 2012; VOSOUGHHOSSEINI et al., 2012; GOCIU et al., 2013). Estudos envolvendo animais, especialmente em tecido subcutâneo de ratos, têm sido frequentemente realizados e representam uma forma fácil e segura para avaliar a compatibilidade dos biomateriais (ANDOLFATTO et al., 2012; VIOLA et al., 2012; SANTOS et al., 2014; LACERDA-SANTOS et al., 2014). Embora essa metodologia não reproduza com fidedignidade as condições bucais, ela fornece informações preliminares sobre as propriedades biológicas dos materiais e as características da reação tecidual local (BOAVENTURA et al., 2012).

A avaliação da biocompatibilidade baseia-se principalmente no quadro inflamatório do tecido reativo. A inflamação é uma resposta fisiopatológica decorrente de fatores químicos, físicos ou biológicos; dentre estes, os biomateriais são considerados agentes químicos capazes de provocar estas reações teciduais (IBRAGUIMOV et al., 2012; GOMEZ; PERRETTI; SOEHNLEIN, 2013). Substâncias liberadas por cimentos ortodônticos podem desencadear reação inflamatória ou necrose em tecidos adjacentes através de uma interação direta com os tecidos ou da solubilidade dos componentes para a cavidade oral (MALKOC et al., 2010).

A reação tecidual decorrente da ação de biomateriais caracteriza-se pelo acúmulo de células inflamatórias que migram para o tecido inflamado e em direção à superfície dos materiais exógenos (ZHOU et al., 2011; IBRAGUIMOV et al., 2012; MURRAY; WYNN, 2011). Neste tipo de resposta inflamatória, os macrófagos (MOs) destacam-se por atuarem na apresentação de antígenos, fagocitose de restos celulares,

bem como na liberação de uma variedade de quimiocinas responsáveis pelo recrutamento de outros tipos de células, tais como fibroblastos, que participam na remodelação do tecido (KOCHANOWSKI et al., 2011; IBRAGUIMOV et al., 2012; YAMANAKA et al., 2013). Além disso, os MOs podem se fundir e originar células gigantes multinucleadas (CGM), capazes de potencializar a resposta pró-inflamatória e auxiliar na limitação e remoção de resíduos considerados não-inertes ao organismo (COSTA et al., 2011; ANDOLFATTO et al., 2012).

A análise da resposta inflamatória nos testes de biocompatibilidade é realizada mais comumente por métodos histológicos, nos quais uma reação inflamatória mais intensa é observada na presença de materiais irritantes ou menos compatíveis (CUNHA et al., 2011; VIOLA et al., 2012; ANDOLFATTO et al., 2012; BOAVENTURA et al., 2012; YANG et al., 2012; SAGHIRI et al., 2013). Embora os elevados níveis de MOs presentes na inflamação sejam tipicamente observados através de análises histológicas, critérios quantitativos precisos dificilmente podem ser definidos por esse método. (MANOLEA et al., 2009; ZHOU et al., 2011).

Nesse sentido, entende-se que a quantificação dos MOs recrutados serviria como uma boa avaliação da intensidade da resposta inflamatória local, uma vez que essas reações são caracterizadas por um acúmulo substancial de fagócitos **(**KIRK; MCNALLY; ANDERSON, 2011; ZHOU et al., 2011; IBRAGUIMOV et al., 2012; YAMANAKA et al., 2013). A maioria dos estudos que avaliam as propriedades biológicas dos materiais utilizam abordagens qualitativas e semi-quantitativas para examinar a resposta tecidual. Adicionalmente, abordagens quantitativas são importantes, uma vez que podem proporcionar maior precisão dos resultados em análises *in vivo* da biocompatibilidade (WALSCHUS et al., 2011).

Atualmente, o método imuno-histoquímico destaca-se pela possibilidade de oferecer análises quantitativas de estruturas identificáveis, tornando-se uma importante ferramenta na prática anátomo-patológica em todo o mundo. Assim, a utilização dessa técnica, com marcadores bem definidos da inflamação, pode oferecer melhores resultados para uma avaliação altamente precisa da resposta inflamatória (MANOLEA et al., 2009).

Na análise imuno-histoquímica, as células a serem estudadas apresentam estruturas moleculares que são reconhecidas e imunomarcadas por anticorpos

específicos como o anti-CD68 (AMANZADA et al., 2013). O CD68 é uma proteína de membrana de 110kDa, fortemente glicosilada, que embora possa ser observada em uma pequena fração na superfície da célula, é predominantemente localizada em membranas lisossomais. O anti-CD68 é amplamente considerado como um marcador seletivo para monócitos e macrófagos e, portanto, comumente utilizado em estudos de patologia humana (MANOELA et al., 2009; AMANZADA et al., 2013; YAMANAKA et al., 2013).

O marcador biológico anti-CD68 pode ser considerado um importante indicador da intensidade da resposta inflamatória decorrente de biomateriais. Atualmente, alguns estudos têm utilizado o anticorpo CD68 em testes de biocompatibilidade de diversos materiais (KOCHANOWSKI et al., 2011; WALSCHUS et al., 2011; YAMANAKA et al., 2013). No entanto, até o presente momento ainda não há relatos na literatura de pesquisas que avaliaram a biocompatibilidade de CIVMR através da análise imuno-histoquímica (Pubmed Database – Acesso em 20/07/2014).

Nesse contexto, destaca-se a importância do presente estudo quanto à avaliação da biocompatibilidade dos cimentos ortodônticos de ionômero de vidro modificados por resina através de um teste *in vivo* e análises morfológicas e imuno-histoquímicas.

OBJETIVOS

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar a biocompatibilidade de cimentos ortodônticos de ionômero de vidro modificados por resina em tecido subcutâneo de ratos, através de uma análise morfológica e imuno-histoquímica.

2.2 OBJETIVOS ESPECÍFICOS

- Avaliar, histopatologicamente, as seguintes alterações teciduais: intensidade de infiltrado inflamatório, edema, necrose, reação de células gigantes multinucleadas, reação de granulação, fibroblastos jovens e colagenização causadas pelos diferentes cimentos ortodônticos utilizados, nos períodos de acompanhamento de 7, 15 e 30 dias.
- Comparar o grau de intensidade das alterações histopatológicas ao longo do tempo e entre os diferentes cimentos ortodônticos testados.
- Avaliar, através da técnica da imuno-histoquímica, a presença de macrófagos e células gigantes multinucledas pela expressão do anti-CD68 nos tecidos analisados, nos três diferentes tempos (7, 15 e 30 dias), e compará-las aos diferentes cimentos ortodônticos testados.

MATERIAL E MÉTODOS

3 MATERIAL E MÉTODOS

3.1 CONSIDERAÇÕES ÉTICAS

O estudo foi encaminhado para o Comitê de Ética em Pesquisa Animal da Unidade Acadêmica de Ciências Biológicas do Centro de Saúde e Tecnologia Rural (CSTR) da Universidade Federal de Campina Grande (UFCG). A pesquisa foi aprovada conforme o parecer n° 0102011 (ANEXO A).

3.2 CARACTERIZAÇÃO DO ESTUDO

Caracteriza-se como um estudo laboratorial *in vivo*, consistindo em avaliações histopatológica e imuno-histoquímica da biocompatibilidade de cimentos ortodônticos em tecido subcutâneo de ratos através de uma análise qualitativa, quantitativa e comparativa entre os grupos.

3.3 POPULAÇÃO

A população do estudo foi constituída de 75 ratos Wistar machos adultos com peso entre 200 e 300g pertencentes ao Biotério da Unidade Acadêmica de Ciências Biológicas do Centro de Saúde e Tecnologia Rural (CSTR) da Universidade Federal de Campina Grande (UFCG), Campina Grande-PB, e que estavam de acordo com os critérios de inclusão e exclusão do estudo.

3.4 AMOSTRA

Os 75 ratos selecionados foram distribuídos em cinco grupos de acordo com os CIVMR (Controle, Transbond®, Crosslink®, Resilience® e RMO®) (QUADRO 1). Cada grupo consistiu de 05 ratos para cada um dos períodos de acompanhamento (7, 15 e 30 dias) com o total de 15 ratos por grupo. Cada rato recebeu um implante do material a ser analisado. Após a biópsia, cada espécime foi seccionado em dois, resultando em 30 amostras por grupo e totalizando 150 blocos parafinados para análise (QUADRO 2).

Quadro 1. Composição dos materiais testados. Campina Grande-PB, 2014.

Grupos	Composição	Fabricante
Controle	Polietileno	Embramac, Itapera, Brasil
Tansbond® Plus Light Cure Band	2hidroxi 1,3-dimetacnloxipropano, promotores de cura, fluoroaluninosilicato de vidro, silano e pigmento azul	3MUnitek, Monrovia, CA,USA
Crosslink® Orthodontic Band Cement	Monômeros, fluoroaluninosilicato de vidro, promotores de cura e pigmentos	TP Orthodontics, La Porte, Indiana, USA
Resilience®Light Cure Band Cement	Sistema a base de resina Bis-GMA com catalisador químico.	Ortho Technology, Tampa, Florida, USA
RMO® Band Cement	Monômeros dimetacrilato e fluoroaluninosilicato de vidro	RMO, Denver, CO, USA

Quadro 2. Distribuição da amostra de acordo com os materiais testados. Campina Grande-PB, 2014

Grupos	Quantidade de ratos*	Amostra de tecido para análise
Controle	15	30
Tansbond®	15	30
CrossLink®	15	30
Resilience®	15	30
RMO®	15	30
TOTAL	**75**	**150**

*Cada grupo consistiu de 15 ratos no total (5 ratos para cada período de 7, 15 e 30 dias).

Cada material foi direcionado aos grupos I, II, III, IV e V, de forma que o examinador não teve conhecimento de quais materiais foram implantados em cada grupo.

3.4.1 Critérios de inclusão

Foram incluídos na amostra ratos machos Wistar adultos com peso entre 200 e 300g pertencentes ao Biotério da Unidade Acadêmica de Ciências Biológicas do Centro de Saúde e Tecnologia Rural (CSTR) da Universidade Federal de Campina Grande (UFCG), Campina Grande-PB.

3.4.2 Critérios de exclusão

Foram excluídos os materiais biológicos provenientes das biópsias excisionais que não apresentaram quantidade ou qualidade suficiente de tecido para a realização da análise histomorfológica e imuno-histoquímica.

3.5 ESTUDO CLÍNICO LABORATORIAL

Os ratos foram anestesiados utilizando injeção intraperitonial de tiopental sódico (50 mg/kg) (Cristália, Campinas, São Paulo, Brasil) e realizada tricotomia da região dorsal de cada animal com lâminas de barbear para eliminação dos pêlos (4 x 4 cm).

Para anti-sepsia do campo operatório foi utilizado digluconato de clorexidina a 4% (SANTOS et al., 2010). Na linha média, equidistante da inserção da cauda e da cabeça do animal, foi realizada uma incisão de aproximadamente 8 mm de comprimento utilizando lâmina de bisturi n° 15 adaptada a um cabo de bisturi.

Com o auxílio de uma tesoura de ponta romba, o tecido subcutâneo foi divulsionado lateralmente promovendo uma tunelização no sentido lateral, formando uma loja cirúrgica, com aproximadamente 18 mm de profundidade. Cada rato recebeu um implante de tubo de polietileno (0,8 cm de comprimento e 0,5 cm de diâmetro interno, previamente mantidos em álcool 70% por 120 minutos, lavados com água deionizada e finalmente autoclavados à temperatura de 110^0 C por 20 minutos) que foram utilizados como veículos de inoculação dos materiais testados.

Os CIVMR foram manipulados segundo as instruções dos fabricantes e em seguida introduzidos nas aberturas das extremidades dos tubos, com auxílio de espátula

de inserção antiaderente aluminada. Posteriormente foram fotopolimerizadas com aparelho LED (Radii, SDI, Baywater, Victoria, Australia) de acordo com o tempo de fotopolimerização recomendado pelo fabricante. A intensidade de luz do aparelho fotopolimerizador (1000mw/cm2) foi checada imediatamente antes de cada polimerização usando um radiômetro (Model 100, DemetronResearch Corporation, Danbury, CT, USA). Após os compósitos serem polimerizados os tubos foram implantados. No grupo controle, foi utilizado o tubo de polietileno vazio que corresponde ao trauma induzido e possível contaminação dos mesmos.

Após o implante dos materiais, as lojas cirúrgicas foram suturadas com fio de sutura agulhado 4.0 (Ethicon, Jonhson&Jonhson, São José dos Campos, São Paulo, Brasil) e em seguida os animais receberam injeção de 0,2 ml de pentabiótico veterinário via intramuscular (Wyeth Laboratory, New York, NY, EUA) e uma injeção de dipirona sódica (0,3 ml/100g, Novalgina, São Paulo, SP, Brasil). Todos os procedimentos do presente estudo foram realizados em conformidade com o *Canadian Council on Animal* Care (1981). Os animais foram mantidos em gaiolas individuais a uma temperatura variando de 22 °C a 26 °C sob um ciclo de luz claro-escuro de 12 horas, sob condições adequadas, com ração apropriada e água ad libitum.

Após 7, 15 e 30 dias, os animais foram anestesiados para obtenção da biópsia excisional da área do implante, abrangendo tecido normal circundante suficiente. Posteriormente os animais foram sacrificados pela técnica do deslocamento cervical após sedação de tiopental sódico (50 mg/kg) (Cristália).

3.6 ESTUDO MORFOLÓGICO

A amostra selecionada, fixada em formol a 10% e incluída em parafina, foi submetida a cortes de 5µm de espessura. Em seguida os cortes foram estendidos em

lâminas de vidro e submetidos à coloração de hematoxilina e eosina, de acordo com o protocolo descrito a seguir:

⇒ Xilol I (15 minutos)
⇒ Xilol II (15 minutos)
⇒ Álcool etílico 90° I (5 minutos)
⇒ Álcool etílico 90° II (5 minutos)
⇒ Álcool etílico 70° I (5 minutos)
⇒ Álcool etílico 70° II (5 minutos)
⇒ Água corrente (5 minutos)
⇒ Hematoxilina de Harris (1 min e 20 segundos)
⇒ Água corrente (5 minutos)
⇒ Eosina de Lison (8 minutos)
⇒ Álcool etílico absoluto I (5 minutos)
⇒ Álcool etílico absoluto II (5 minutos)
⇒ Álcool etílico absoluto III (5 minutos)
⇒ Xilol I (5 minutos)
⇒ Xilol II (5 minutos)
⇒ Xilol III (5 minutos)
⇒ Montagem da lâmina com resina *Permount*® (Fisher Scientific Inc., Fair lawn, NJ, USA).

Os espécimes foram avaliados sob microscopia de luz (Leica DM500®, Leica Microsystems Vertrieb GmbH, Wetzlar, DE) em aumentos de 100x, 400x e 1000x. De acordo com a metodologia proposta por Garcia et al (2010) e Santos et al. (2010) foram avaliados os seguintes parâmetros histopatológicos: intensidade de infiltrado inflamatório, edema, necrose, reação de células gigantes multinucleadas, reação de granulação, fibroblastos jovens e colagenização, sendo considerado os escores: 0 – escasso; 1- leve; 2 – moderado; 3 – intenso para todos os parâmetros. A avaliação histopatológica foi procedida por dois avaliadores previamente calibrados (Kappa=0.8).

3.7 ESTUDO IMUNO-HISTOQUÍMICO

A amostra selecionada, fixada em formol a 10% e incluída em parafina, foi submetida a cortes com 3μm de espessura, os quais foram estendidos em lâminas de vidro devidamente preparadas com adesivo à base de organosilano (3-aminopropiltrietoxisilano, Sigma Chemical Co., St. Louis, MO, USA). Posteriormente, o material foi submetido ao método da imunoperoxidase pela técnica baseada em polímeros de dextrano (*ADVANCE^{TM} HRP*, Dako North America Inc., Carpinteria, CA, USA), utilizando monoclonal CD68 (QUADRO 3).

Como controle interno positivo, foram utilizados cortes histológicos do fígado do rato. O controle negativo consistiu na substituição do anticorpo primário por albumina de soro bovino (BSA) a 1% em solução tampão.

A técnica utilizada seguiu o protocolo descrito abaixo:

⇒ Desparafinização: 2 banhos em xilol, à temperatura ambiente (10 minutos cada);

⇒ Re-hidratação em cadeia descendente de etanóis:

- Álcool etílico absoluto I (5 minutos);
- Álcool etílico absoluto II (5 minutos);
- Álcool etílico absoluto III (5 minutos);
- Álcool etílico 95°GL (5 minutos);
- Álcool etílico 80°GL (5 minutos);

⇒ Remoção de pigmentos formólicos com hidróxido de amônia a 10% em etanol 95°, à temperatura ambiente (10 minutos);

⇒ Lavagem em água corrente (10 minutos)

⇒ Passagens rápidas em água destilada (2 trocas);

⇒ Recuperação antigênica (Quadro 3);

⇒ Lavagem em água corrente (10 minutos);

⇒ Passagens rápidas em água destilada (2 trocas);

⇒ Duas incubações dos cortes em solução de peróxido de hidrogênio 3% 10 volumes, em proporção de 1/1, para o bloqueio da peroxidase endógena tecidual (10 minutos cada);

⇒ Lavagem em água corrente (10 minutos);

⇒ Passagens rápidas em água destilada (2 trocas);

⇒ Duas passagens em solução de Tween 20 a 1% em TRIS-HCl pH 7,4 (5 minutos cada);

⇒ Incubação dos cortes com anticorpos primários, em solução diluente (*Antibody diluent with background reducing components*, Dako North America Inc., Carpinteria, CA, USA), à temperatura ambiente (60 minutos);

⇒ Duas passagens em solução de Tween 20 a 1% em TRIS-HCl pH 7,4 (5 minutos cada);

⇒ Incubação com anticorpo secundário (*ADVANCE™ HRP Link*, Dako North America Inc., Carpinteria, CA, USA), à temperatura ambiente (30 minutos);

⇒ Duas passagens em solução de Tween 20 a 1% em TRIS-HCl pH 7,4 (5 minutos cada);

⇒ Incubação com anticorpo polimerizado à peroxidase (*ADVANCE™ HRP Enzyme*, Dako North America Inc., Carpinteria, CA, USA), à temperatura ambiente (30 minutos);

⇒ Duas passagens em solução de Tween 20 a 1% em TRIS-HCl pH 7,4 (5 minutos cada);

⇒ Revelação da reação com solução cromógena de 3,3-diaminobenzidina (*Liquid DAB+ Substrate*, Dako North America Inc., Carpinteria, CA, USA) (7 minutos);

⇒ Lavagem em água corrente (10 minutos);

⇒ Passagens rápidas em água destilada (2 trocas);

⇒ Contracoloração com hematoxilina de Mayer, à temperatura ambiente (5 minutos);

⇒ Desidratação em cadeia ascendente de etanóis:

- Álcool etílico 80°GL (2 minutos);
- Álcool etílico 95°GL (2 minutos);
- Álcool etílico absoluto I (5 minutos);
- Álcool etílico absoluto II (5 minutos);
- Álcool etílico absoluto III (5 minutos);

⇒ Passagens rápidas em xilol (3 trocas);

⇒ Montagem em resina *Permount*® (Fisher Scientific Inc., Fair Lawn, NJ, USA)

Quadro 3. Especificidade, nº catálogo, fabricante, diluição, recuperação antigênica e tempo de incubação do anticorpo primário utilizado no estudo. Campina Grande-PB, 2014

Especificidade	Clone	Fabricante	Diluição	Recuperação Antigênica	Incubação
CD68	ED1	Abcam	1:1500	Citrato, pH 6,0 Steamer, 100ºC, 60 minutos.	60 minutos

Após o processamento dos cortes histológicos e tratamento imuno-histoquímico, cada espécime foi analisada, sob microscopia de luz por um avaliador previamente treinado e calibrado. A análise foi do tipo quantitativa. Sob aumento de 100x foram selecionados 5 campos de maior imunorreatividade ao anticorpo CD68. Sob aumento de 400x cada um destes campos foi fotomicrografado (Leica DM500®, Leica Microsystems Vertrieb GmbH, Wetzlar, DE) e as imagens obtidas foram transferidas para um computador de uso pessoal. Com o auxílio do programa ImageJ® (*Imaging Processing Analysis in Java*, National Institute of Menthal Health, Bethesda, Maryland, USA), em cada um destes campos foi realizada a contagem de células mononucleadas e de células gigantes multinucleadas CD68+. Os valores obtidos foram somados, estabelecendo-se o número total de células CD68+, e posteriormente calculada a média por campo, para cada caso.

3.8 ANÁLISE ESTATÍSTICA

Os resultados obtidos foram organizados em um banco de dados com o auxílio do programa Microsoft Excel, versão 2007. Os dados foram tabulados e analisados no programa BioEstat versão 5.0 (Mamirauá, Manaus, Brasil). O método estatístico foi escolhido com base no modelo de distribuição e variância dos dados avaliada pelo Teste de Kolmogorov-Smirnov e Teste de Levene, respectivamente. Os resultados dos eventos celulares foram submetidos ao teste de Kruskal-Wallis, seguido pelo teste de Dunn para determinar as diferenças entre os grupos ($p < 0.05$).

ARTIGOS

4 ARTIGOS

4.1 APRESENTAÇÃO

O projeto de pesquisa foi apresentado e devidamente aprovado na qualificação desenvolvida pelo Programa de Pós-Graduação em Odontologia da UEPB.

Como resultado de sua execução, dois artigos são apresentados nessa dissertação:

- Artigo 1: **Do Resin-modified Cements for Bonding Orthodontic Bands Express Similar Biocompatibility?** O artigo será submetido ao periódico *The Journal of Adhesive Dentistry* (Fator de Impacto: 0,905 *Qualis* A2), cujas normas de formatação e submissão do manuscrito estão elencadas no ANEXO B.

- Artigo 2: **Análise Morfológica versus Imuno-histoquímica na Biocompatibilidade de Cimentos Modificados por Resina**. O artigo será submetido ao periódico *Dental Materials* (Fator de Impacto: 3.773 *Qualis* A1), cujas normas de submissão estão no ANEXO C.

ARTIGO 1

4.2 ARTIGO 1

DO RESIN-MODIFIED CEMENTS FOR BONDING ORTHODONTIC BANDS EXPRESS SIMILAR BIOCOMPATIBILITY?

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Abstract

Purpose: To evaluate the biocompatibility of resin-modified glass ionomer (RMGIC) orthodontic cements in rat subcutaneous tissue by morphological analysis. **Materials and Methods:** Sixty male Wistar rats were selected and divided into four groups: Group C (Control); Group CK (Crosslink Orthodontic Band Cement); Group RMO (RMO Band Cement) and Group TP (Transbond Plus Light Cure Band). The materials were inserted in rat subcutaneous tissue. After time intervals of 7, 15 and 30 days morphological analyses were performed. The histological parameters assessed were: Inflammatory infiltrate intensity; reaction of multinucleated giant cells; edema; necrosis; granulation reaction; young fibroblasts and collagenization. The results obtained were statistically analyzed by the Kruskal-Wallis and Dunn test ($p<0.05$). **Results**: After 7 days, Groups RMO and TP showed intense inflammatory infiltrate ($p=0.004$), only Group RMO presented greater expression of multinucleated giant cell reaction ($p=0.003$) and all groups demonstrated a more intense granulation reaction ($p=0.002$) compared with the control group. After the time intervals of 15 and 30 days, there was evidence of light/moderate inflammatory infiltrate, lower level of multinucleated giant cell reaction and thicker areas of young fibroblasts in all the groups. Only Group RMO presented low degree of collagenization after 15 ($p=0.008$) and 30 days ($p=0.014$) in comparison with control group. **Conclusion:** CK cement provided good tissue response, since it demonstrated a lower level of inflammatory infiltrate and higher degree of collagenization, while RMO demonstrated the lowest level of biocompatibility due to the intense inflammatory response in the initial periods and lowest degree of tissue repair.

Key words: Orthodontic cement; Biocompatibility testing; Inflammation.

Introduction

The resin modified glass ionomer cement (RMGIC), hybrid version of conventional glass ionomer cement (GIC), is composed of glass particles, acids, initiators, additives and a resinous system of organic monomers[4]. This material has been increasingly used by orthodontists for cementing orthodontic bands, due to the favorable physical and chemical properties capable of providing good retentiveness, fluoride release capacity and being practical to use[15]. However, there are indications that this cement may present cytotoxicity, genotoxicity and inadequate biocompatibility[2,14,19].

Studies *in vitro* have demonstrated that the RMGICs are capable of inducing cytotoxic effects on oral tissues, due to the presence of substances released during polymerization, such as hydrophilic monomers or other low molecular weight species[2,4,15,19,24]. Nevertheless, in spite of these studies providing evaluation of the biologic properties of RMGICs, *in vivo* models are required to provide a more critical analysis with regard to the biocompatibility of these resin cements[26].

The biocompatibility evaluation is due to the analysis of the different cell reactions after contact of the materials with the vascularized tissues[17]. This is one of the most important properties to be evaluated, because these biomaterials may trigger inflammatory reactions or necrosis in adjacent tissues, such as the oral mucosa and gingiva, by the direct interaction with the tissues, or the solubility of the components in the oral cavity[7,15,19].

The study of tissue reactions in biocompatibility tests is most frequently performed by morphological analyses, in which the most intense inflammatory reactions or inadequate tissue repair processes may be observed in the presence of irritant or less compatible materials[3,16,18,22,25]. However, in spite of the importance of analyzing the biologic effect of RMGICs, little is known about the level of compatibility of these materials with tissues.

In this context, the aim of the present study was to evaluate the biocompatibility of RMGIC by an *in vivo* test and perform a morphological analysis of the tissue inflammatory response after contact with these materials.

Material and Methods

The study sample consisted of 60 adult male Wistar rats, weighing between 200 and 300g, which were divided into four groups according to the materials tested: Group TP (Transbond Plus Light Cure Band); Group RMO (RMO Band Cement); Group CK (Crosslink Orthodontic Band Cement); and Group C (Control, Polyethylene tube) (Table 1). This *in vivo* laboratory study was previously approved by the Ethics Committee on Research Involving Animal Experimentation, Protocol/CSTR, No.0102011.

The rats were anesthetized with an intraperitoneal injection of sodium thiopental (50 mg/kg) (Cristália, Campinas, SP, Brazil). After this, trichotomy was performed in the dorsal region of each animal, using razor blades (4 x 4 cm) and anti-sepsis of the operative field with 4% chlorhexidine digluconate (17). On the midline, equidistant from the insertion of the animal's tale and head, an incision approximately 8 mm long was made using a No.15 scalpel blade adapted to a scalpel handle. The subcutaneous tissue was laterally divulsed with blunt tipped scissors, promoting tunneling in the lateral direction for insertion of implants of the materials. Each rat received one polyethelyne tube implant (0.8 mm long and 0.5cm internal diameter, which previously kept in 70% alcohol for 120 minutes, washed with deionized water and finally autoclaved at a temperature of 110^0 C for 20 minutes and used as inoculation vehicles for the materials tested.

The RMGICs were manipulated in accordance with the manufacturers' instructions, and then introduced into the openings of the polyethylene tube extremities, by means of an aluminated insertion spatula. Afterwards they were polymerized with a LED appliance (Radii, SDI, Baywater, VIC, Australia) according to the light polymerization time of 40 seconds. The light intensity of the light polymerizing appliance ($1000mw/cm^2$) was checked immediately before each polymerization procedure using a radiometer (Model 100, Demetron Research Corporation, Danbury, CT, USA). After the RMGICs were polymerized, the tubes were implanted in the subcutaneous tissue of the rats. In the control group, an empty polyethylene tube was used, which corresponded to the trauma induced and possible contamination of the tubes.

After the materials were implanted, the surgical recesses were sutured with a 4.0 suture needle with thread (Ethicon, Jonhson & Jonhson, São José dos Campos, SP,

Brazil) and after the procedure, the animals received an intramuscular injection of 0.2 ml of veterinary pentabiotic (Wyeth Laboratory, New York, NY, USA), and an injection of sodium dipyrone (0.3 ml/100g, Novalgina; SP, Brazil). All the procedures were performed in compliance with the guidelines of the Canadian Council on Animal Care (1981). The animals of each group were kept in individual cages at a temperature ranging from 22°C to 26°C under a 12-hour light-dark cycle, under adequate conditions with appropriate rations and water *ad libitum*.

After time intervals of 7, 15 and 30 days, the animals were anesthetized to obtain excisional biopsies of the implant area, including sufficient normal surrounding tissue. Each group consisted of 5 rats per each time interval, totaling 15 samples per group. Afterwards the rats were sacrificed by the cervical dislocation technique after having been sedated with sodium thiopental (50 mg/kg) (Cristália).

The specimens were prepared on glass slides by routine Hematoxylin and Eosin (HE) staining, and afterwards evaluated under an optical microscope, Leica DM500® (Leica Microsystems Vertrieb GmbH, Wetzlar, Germany), at 100x, 400x and 1000x magnifications. According to the methodology proposed by Garcia et al. (2010)[11] and Santos et al. (2010)[17] the histological parameters assessed were as follows: Inflammatory infiltrate intensity; edema; necrosis; multinucleated giant cell reaction, granulation reaction; young fibroblasts and collagenization, with the scores being considered: 0 – scarce; 1- light; 2 – moderate; 3 – intense, for all the histopathological parameters[11,17]. The histopathological evaluation was performed by only one previously calibrated examiner (Kappa=0.8).

The data were tabulated and analyzed in the statistical software program BioEstat version 5.0 Mamirauá, Manaus, AM, Brazil). The statistical method was chosen based on the model of distribution and variance of data evaluated by the Kolmogorov- Smirnov and Levene tests, respectively. Thus, the results of the cellular events were submitted to the Kruskal-Wallis test, and afterwards the Dunn test to determine the differences between the groups ($P< 0.05$).

Results

In the time interval of 7 days, the presence of chronic inflammatory infiltrate was observed in all the groups, however, it was shown to be more intense in the RMO and TP groups (P=0.004). Granulation reaction was shown to be more intense in all the groups analyzed in comparison with the control group (P=0.002) and the multinucleated

giant cell reaction was more evident only for Group RMO in this time interval (*P*=0.003) (Figure 1). As regards the histopathological parameters, edema and necrosis, in spite of edema having been observed in all the groups, and necrosis in Groups CK and TP after 7 days, no significant differences were observed (Table 2).

In the time interval of 15 days, there was evidence of moderate inflammatory infiltrate, lower quantity of multinucleated giant cells, areas of young fibroblasts and light collagenization level in all the groups (Figure 2). In the time interval of 30 days, light inflammatory infiltrate and a thick layer of collagen fibers could be visualized as tissue reaction against the cements (Figure 3). As regards the parameter collagenization, only Group RMO presented low degree of collagenization after 15 (*P*=0.008) and 30 days (*P*=0.014) in comparison with control group (Table 2).

Over the course of the time evaluated, it was observed that the parameters of inflammatory infiltrate intensity, granulation reaction, and multinucleated giant cell reaction diminished progressively, while the presence of young fibroblasts and areas of collagenization increased for all the groups evaluated.

Discussion

Various studies have sought to evaluate the biologic properties of materials used in dentistry[3,6,7,16,17,18,22,25], however, there is still a scarcity of researches involving orthodontic cements, in the literature. In this sense, the option was to analyze the biocompatibility of three types of RMGICs frequently used by orthodontists for band cementation: Transbond®, RMO® and Crosslink®.

In this study, the method used for evaluating the biocompatibility of the composites in rat subcutaneous tissue was conducted in accordance with the ISSO Standards- 6876 and 10993-5, which propose the implantation of biomaterials in the tissues of laboratory animals[1]. Although this methodology does not faithfully reproduce the conditions in the oral cavity, it provides important preliminary information about the biologic properties of dental materials[23] and characteristics of tissue reactions[3,6,16,22,25]. The histological parameters were evaluated after time intervals of 7, 15 and 30 days, as observed in other studies[6,7,14,23].

For Zhou et al. (2011)[26], the different histopathological characteristics in rat subcutaneous tissue after contact with the material, when observed in the short, medium and long term, allow a more precise evaluation because of the dynamism of inflammatory reactions**.** In this study, the first analysis was performed after 7 days

because, it is only after this period that one expects there to be a more organized inflammatory reaction, seeing that in the first few hours/days right after the implant, a more intense and less specific reaction may be observed due to the surgical procedure[17,18]. Whereas the analyses in longer periods (30 days) are for the purpose of verifying the reparative capacity of the tissue after the aggressive challenge initially caused by the materials tested[5].

After the period of 7 days, the RMO and Transbond Plus cements demonstrated intense inflammatory infiltrate when compared with the control group (P=0.004). This initial inflammatory response reflects the attempt by the tissue to induce the process of degradation of the materials through the inflammatory cells recruited to the surrounding tissue and in the direction towards the surface of the cements[12,15]. In addition, it suggests that the elution of toxic substances released by these cements is capable of stimulating the production of reactive oxygen species (ROS), as well as increasing the expression of COX-2 / PGE 2, which are potent chemical mediators derived from arachidonic acid, capable of potentiating the inflammatory response[5].

As regards the presence of multinucleated giant cells after 7 days, RMO cement presented intense macrophagic reaction in the attempt to limit/remove the residues liberated by this cement and considered non inert to the body (P=0.003). This suggests that this material may have released more irritant substances in the initial periods than the other cements[6]. The presence of rests of cement close to the cavity observed in some samples in subsequent periods, demonstrates that these biomaterials were not easily digested by the macrophages or removed by local lymphatic drainage[21].

Furthermore, all the groups demonstrated intense granulation reaction with the presence of a larger number of blood vessels, the majority of them congested, which clinically suggests the presence of hyperemic areas (P=0.002). The granulation reaction induced by the cements was predictable in the initial period, since it functions as a pathway for the macrophages to reach the implant site[12].

After 15 and 30 days, progressive reduction in the inflammatory response, and increase in the production of young fibroblasts and deposition of collagen fibers were observed in all the groups. This occurs due to the tissue remodeling process, which frequently occurs after 15 days[20]. In this study, RMO cement exhibited a larger quantity of collagenization compared with the control group both after the time interval of 15 days (P=0.008) and 30 days (P=0.014). It is suggested that substances released by this cement had induced intense inflammatory response in the initial periods and because of

this, the tissue had not been able to achieve adequate remodeling even after 30 days of follow-up.

In general, it could be observed that RMO cement presented intense inflammatory response after 7 days and a lower capacity of tissue repair up until the late stages. In spite of Transbond Plus cement presenting a similar level of inflammatory infiltrate to that of RMO, over the course of time, a more favorable degree of tissue repair was obtained. Whereas Crosslink cement demonstrated moderate inflammatory infiltrate in the initial periods, and consequently a good tissue response after 15 and 30 days. This difference between the cements may be related to the quantity of additives, co-initiators and mainly hydrophilic monomers released during the process of polymerizing these materials[19,24].

The RMO contains BisGMA, a monomer that presents low degree of conversion values due to the presence of strong hydrogen bond and π - π interactions provided by bisphenol-A[10]. Normally, the monomers present in these materials are converted into polymers during the polymerization process, however, inadequate conversion would result in a larger quantity of residual monomers that are capable of causing a significant cytotoxic effect and affect the compatibility of these materials with the oral tissues[8,9,13,14]. Santos et al. (2014)[14] evaluated the relationship between biocompatibility and degree of conversion of RMGICs and demonstrated that the cement that presents the lowest degree of conversion was related to a more intense inflammatory response after 7 days, and lower quantity of young fibroblasts and collagenization after 30 days in comparison with cements that presented higher degrees of conversion.

Whereas the Transbond Plus cement, composed of 2- hydroxyl-1, 3-Dimethacryloxypropane, has a good degree of conversion, which may justify the fact that this material obtained a better response when compared with the RMO group over the course of time[4,15]. Malkoc et al. (2010)[15] when evaluating the cytotoxicity of three RMGICs, demonstrated that in spite of all the materials presenting significant cytotoxicity in comparison with the control group, the cement that contained BisGMA presented the worst results when compared with Transbond Plus.

With regard to Crosslink cement, it is suggested that the monomers of which it is composed have a good degree of conversion capable of providing greater chemical stability, and consequently, less aggression on the tissue, similar to that observed with Transbond Plus cement because, based on the histopathological parameters evaluated, Crosslink presented good biologic compatibility.

The gradual decrease in inflammatory response and increase in tissue repair over time, observed in all the groups tested corroborate the findings of other studies[14,18]. It is suggested that this characteristic is related to the pattern of monomer conversion into polymers, and consequently, the release of residual monomers in the first four weeks[4,8].

At present, with the improvement in the manufacturing process of RMGICs, a lower quantity of residual monomers has been found, however, the free monomers are still sufficient to contribute to the cytotoxic effects and interfere in the biocompatibility of these cements[19]. Studies *in vivo* may also help to elucidate the biologic compatibility of MRGICs, considering that orthodontic bands are continually in contact with the gingival tissue for long periods of time.

It could be concluded that the Crosslink cement provided the best tissue response, with less inflammatory infiltrate and a greater degree of collagenization being observed, followed by Transbond Plus due to its tissue repair capacity. The RMO cement demonstrated the lowest level of biocompatibility, due to the intense inflammatory infiltrate and MGC in the initial periods, and lower degree of collagenization after 15 and 30 days.

Acknowledgments

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Clinical Relevance: Among the RMGICs evaluated, the RMO cement showed the lowest level of biocompatibility in tissue compared to Crosslink and Transbond cements. This suggests that the RMO can generate a more lasting inflammatory process on gingival tissues and therefore be clinically harmful.

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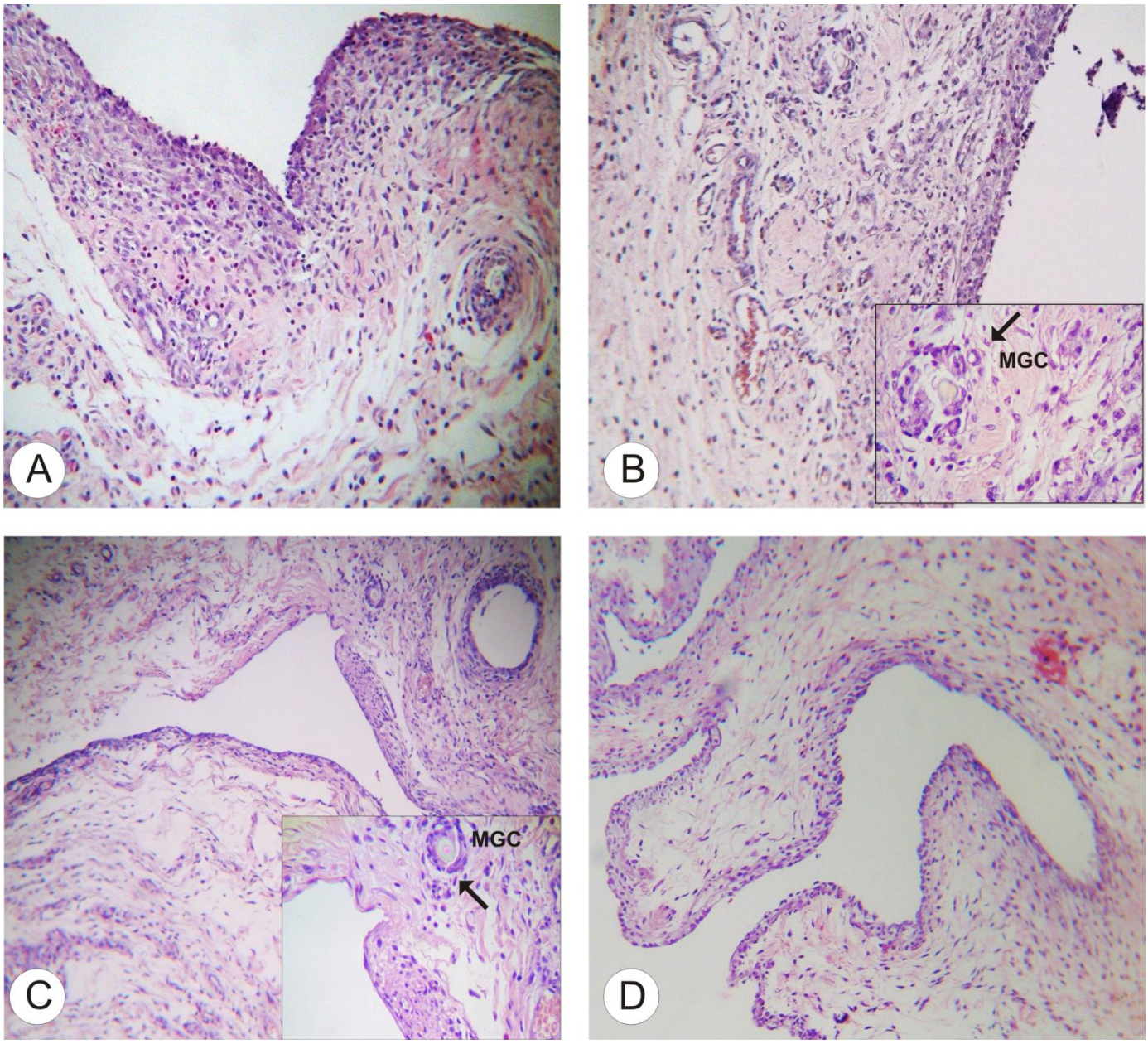
Figure Legends

Figure 1. Time interval of 07 days: **A)** In Group CK the presence of moderate chronic inflammatory infiltrate surrounding the cavity may be observed (HE, 100X). **B)** In Group RMO there is evidence of intense inflammatory infiltrate, predominantly mononuclear, as well as presence of granulation reaction (HE, 100X). At greater magnification, one observes the reaction of multinucleated giant cells enveloping the exogenous material, compatible with RMO (black arrow) (HE, 400X). **C)** In Group TP observes chronic light inflammatory infiltrate with diffuse distribution in proximity to the cavity (HE, 100X) and highlighted, the presence of multinucleated giant cells (black arrow) (HE, 400X). **D)** In the control group, chronic light inflammatory infiltrate and scare granulation reaction may be seen at the cavity margins (HE, 100X).

Figure 2. Time interval of 15 Days: **A)** In Group CK there is evident presence of intense multinucleated giant cell reaction enveloping residues of material compatible with CK. **B)** In Group RMO may visualize the presence of intense chronic inflammatory infiltrate close to the cavity, as well as presence of young fibroblasts and light collagenization (HE, 100X). Highlighted, observes the presence of multinucleated giant cells (black arrow) (HE, 400X). **C)** In Group TP there is evident presence of numerous collagen fibers enveloping the cavity (HE, 100X). **D)** In the control group,

may see a thick layer of collagen fibers with the presence of fibroblasts and light co llagenization reaction (HE, 100X).

Figure 3. Time interval of 30 Days: **A)** In Group CK the presence of a thick area of collagenization enveloping the cavity may be observed (HE, 100X). **B)** In Group RMO there is evident moderate collagenization, and there is still presence of chronic light inflammatory infiltrate in the bottom area of the cavity (black arrow) (HE, 100X). **C)** In Group TP one observes the cavity completely surrounded by a thick layer of collagen fibers (HE, 100X). Highlighted, there is evident intensity of collagen fibers and scarcity of fibroblasts (HE, 400X). **D)** In the Control Group there is also evidence of intense collagenization enveloping the cavity (HE, 400X).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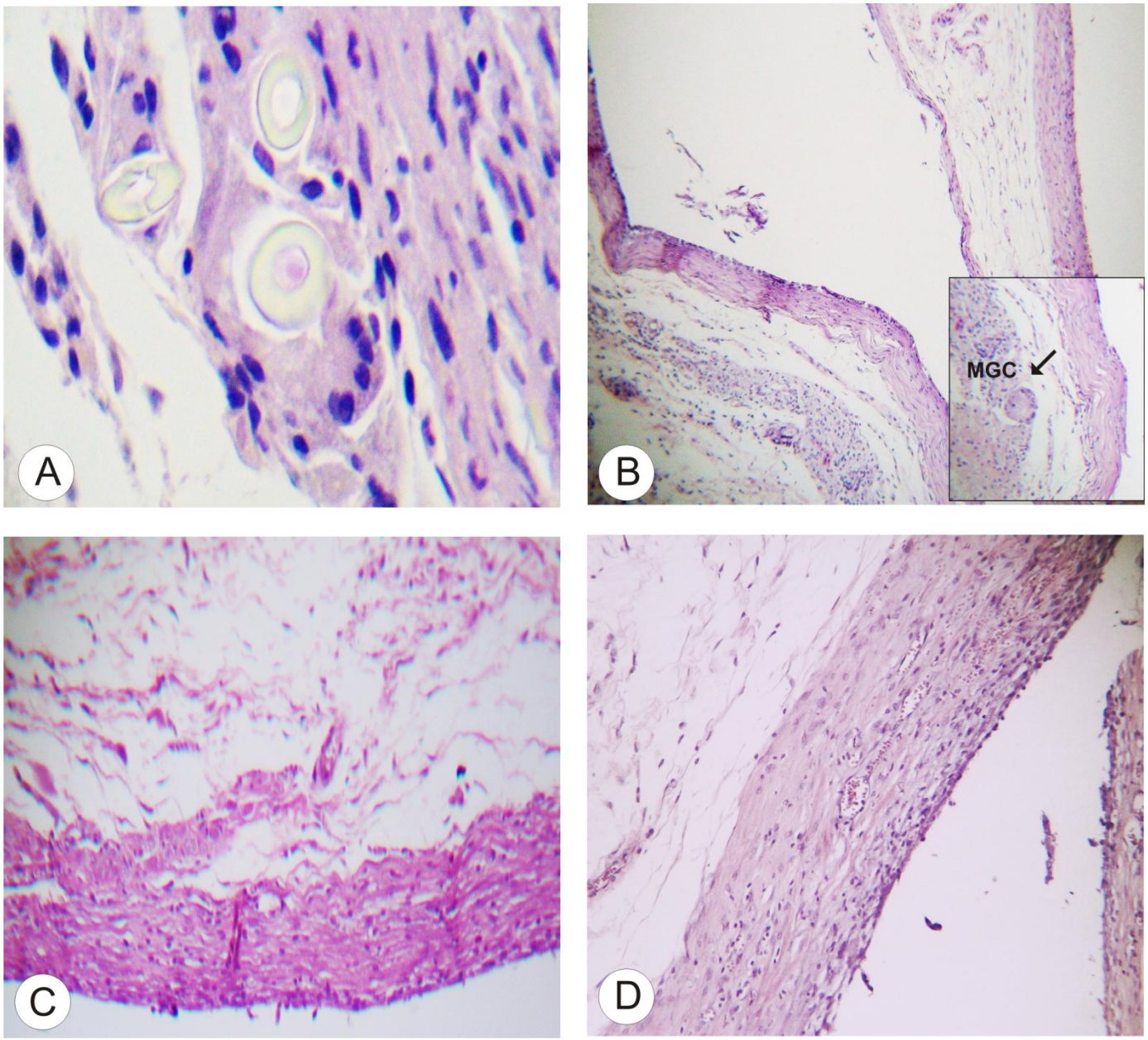
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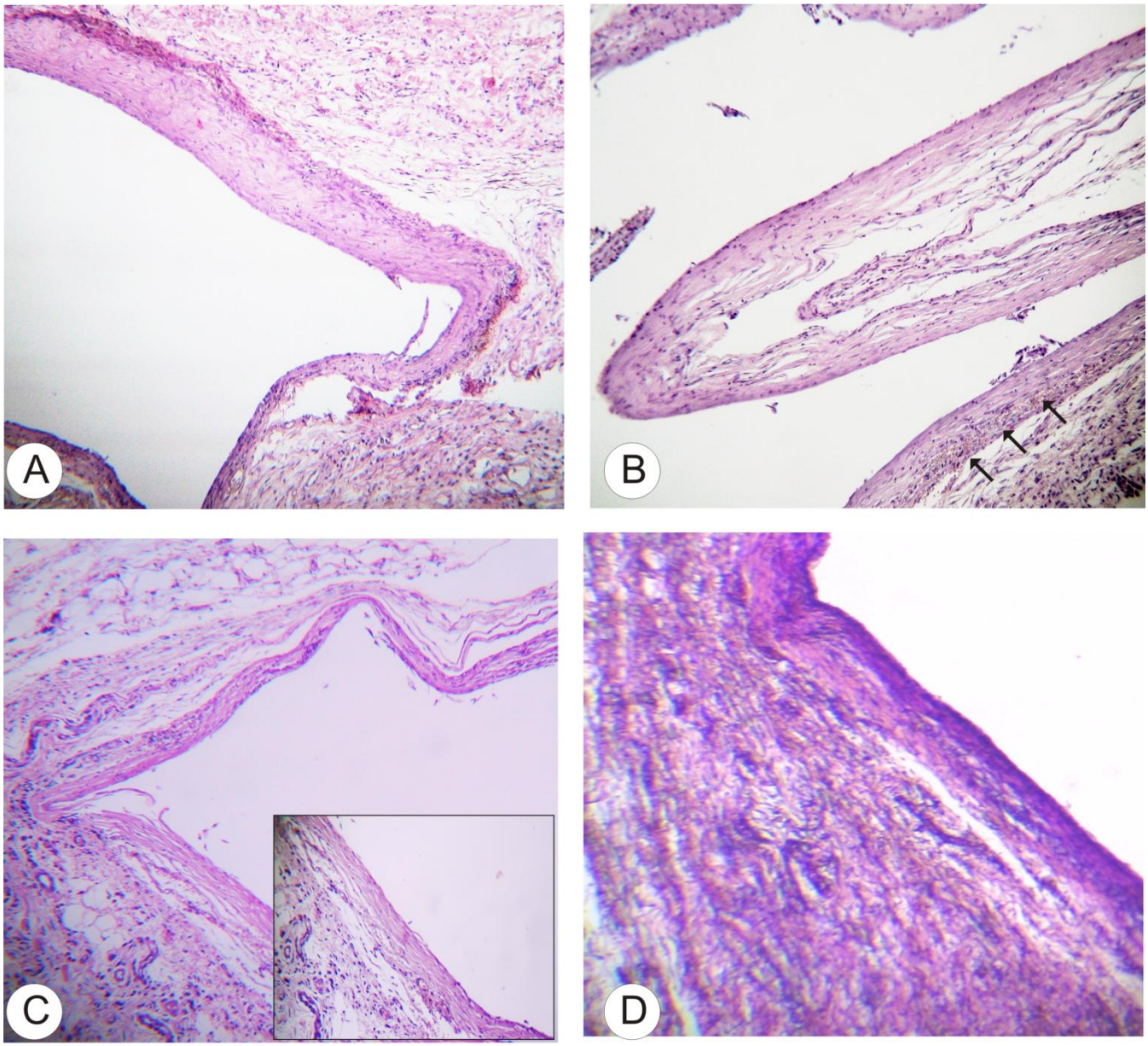
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Table 1. Composition of Transbond, Crosslink e RMO cements evaluated in this study.

Group	Cements	Composition	Manufacturer	Lot No.
CK	**Crosslink Orthodontic Band Cement**	Monomers, Fluroaluminosilicate glass, Cure promoters and pigments	TP Orthodontics, La Porte, IN, USA	32012000
RMO	**RMO Band Cement**	Aromatic and Aliphatic Dimethacrylate Monomers and Fluroaluminosilicate glass	RMO, Denver, CO, USA	A041813
TP	**Tansbond Plus Light Cure Band**	2 hydroxy, 1,3-dimethacryloxypropane, cure promoters, fluoroaluminosilicate glass, silane and blue pigment	3MUnitek, Monrovia, CA,USA	N4 05305

Table 2. Mean of scores attributed to cements and control group, after time intervals of 7, 15 and 30 days, for the 7 events evaluated.

Events	Time	Groups				*P*- value
		CK	**RMO**	**TP**	**C**	
Inflammatory Infiltrate	7 days	1.6^{AB}	2.4^{A}	2.2^{A}	1.0^{B}	0.004
	15 days	1.2	1.0	1.4	0.6	0.084
	30 days	0.6	0.6	0.8	0.4	0.663
Edema	7 days	1.2	0.8	1.0	0.4	0.059
	15 days	0.4	0.0	0.0	0.0	0.096
	30 days	0.0	0.0	0.0	0.0	1.000
Necrosis	7 days	0.4	0.0	0.2	0.0	0.251
	15 days	0.0	0.0	0.0	0.0	1.000
	30 days	0.0	0.0	0.0	0.0	1.000
Granulation Tissue	7 days	2.0^{A}	2.0^{A}	2.0^{A}	1.2^{B}	0.002
	15 days	0.8	0.6	1.2	0.4	0.132
	30 days	0.4	0.2	0.2	0.2	0.859
Giant Cells	7 days	1.0^{AB}	1.6^{A}	1.0^{AB}	0.2^{B}	0.003
	15 days	0.4	0.6	0.8	0.2	0.283
	30 days	0.2	0.4	0.8	0.2	0.191
Young Fibroblasts	7 days	1.0	1.4	1.2	1.8	0.062
	15 days	1.8	1.8	1.8	2.6	0.055
	30 days	1.8	1.8	1.8	2.4	0.169
Collagenization	7 days	0.0	0.2	0.0	0.4	0.251
	15 days	1.2^{AB}	1.0^{A}	1.6^{AB}	2.2^{B}	0.008
	30 days	2.0^{AB}	1.8^{A}	2.2^{AB}	2.8^{B}	0.014

These values represent the mean of scores of the sum of five representative histological sections of the tissue evaluated (n=5, per group). Means followed by different letters express statistically significant difference ($P<.05$) based on non-parametric Kruskal-Wallis Test, followed by the Dunn multiple comparisons test.

ARTIGO 2

4.2 ARTIGO 2

ANÁLISE MORFOLÓGICA VERSUS IMUNO-HISTOQUÍMICA NA BIOCOMPATIBILIDADE DE CIMENTOS MODIFICADOS POR RESINA

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Resumo

Objetivo: Avaliar a biocompatibilidade de cimentos de ionômero de vidro modificados por resina (CIVMR) através de análises morfológicas e imuno-histoquímicas. **Métodos:** CIVMRs foram selecionados e distribuídos em quatro grupos: Grupo CK (Crosslink Orthodontic Band Cement); Grupo RS (Resilience Light Cure Band Cement) Grupo RMO (RMO Band Cement), Grupo TP (Transbond Plus Light Cure Band) e Grupo C (polietileno). Os materiais foram implantados em tecido subcutâneo de ratos e após os intervalos de tempo de 7, 15 e 30 dias os tecidos foram submetidos a análise morfológica. Na análise imuno-histoquímica, avaliou-se a imunomarcação do anticorpo CD68. Os resultados obtidos foram analisados estatisticamente pelo teste de Kruskal-Wallis e Dunn, (P<0,05). **Resultados:** Na análise morfológica, após 7 dias, os grupos RS, RMO e TP demonstraram infiltrado inflamatório mais intenso (P=0,004) e apenas o grupo RMO apresentou maior intensidade de células gigantes multinucleadas (P=0,027). Na análise imuno-histoquímica, pode-se observar que os grupos RMO e RS apresentaram maior quantidade de CD68+ (P=0,004) no período de 7 dias e apenas o grupo RMO apresentou diferença estatisticamente significativa para este parâmetro após 15 dias (P=0,026). No período de 30 dias, o grupo RMO apresentou maior quantidade de células gigantes multinucleadas (P=0,004). **Significado:** Os CIVMRs CK e TP proporcionaram significativamente melhor biocompatibilidade tecidual que os cimentos Resilience e RMO.

Palavras-chaves: materiais dentários, cimentos ortodônticos, teste de biocompatibilidade, macrófagos, células gigantes multinucleadas.

1. Introdução

Os materiais utilizados para cimentação de bandas ortodônticas devem apresentar propriedades físicas, químicas e biológicas adequadas [1-3]. Dentre os cimentos ortodônticos, o cimento de ionômero de vidro modificado por resina (CIVMR) tem sido amplamente utilizado por ortodontistas devido as suas propriedades mecânicas favoráveis. No entanto, há indícios que estes materiais possam liberar substâncias capazes de desencadear respostas tóxicas e inflamatórias aos tecidos [4, 5].

A resposta local decorrente da ação de biomateriais consiste no acúmulo de células inflamatórias, principalmente macrófagos (MOs) e células gigantes multinucleadas (CGM) [6, 7]. Os MOs destacam-se pela capacidade de ingerir e processar materiais estranhos e por atuarem na liberação de quimiocinas, responsáveis pelo recrutamento de células inflamatórias [6, 8, 9]. Além disso, podem se fundir e originar às CGM, as quais são capazes de potencializar a resposta pró-inflamatória e auxiliar na limitação e remoção de resíduos considerados não-inertes ao organismo [10-12].

A avaliação da reação inflamatória nos testes de biocompatibilidade é mais comumente realizada por métodos histológicos que, quando associados a métodos imuno-histoquímicos, podem oferecer resultados altamente precisos da resposta tecidual [9, 13, 14]. Nesse contexto, diversos marcadores biológicos têm sido utilizados, dentre estes, o CD68 se destaca por desempenhar um importante papel na detecção e quantificação de macrófagos, os quais estão substancialmente presentes em reações decorrente de biomateriais [15].

Atualmente, estudos *in vivo* que analisam a resposta tecidual após a implantação de materiais desempenham um importante papel na avaliação da biocompatibilidade [9, 14, 16, 17]. No entanto, até o momento ainda não há relatos na literatura de estudos imuno-histoquímicos que avaliaram a compatibilidade biológica de CIVMRs (Pubmed Database – Acesso em 20/07/2014).

O presente estudo buscou avaliar a biocompatibilidade de diferentes CIVMR através da análise da intensidade da resposta inflamatória e células gigantes multinucleadas por meio de métodos histológicos e avaliação quantitativa de MOs e CGM, pelo método imuno-histoquímico.

2. Material e Métodos

A amostra do estudo foi constituída de 75 ratos Wistar machos adultos com peso entre 200 e 300g, os quais foram distribuídos em cinco grupos de acordo com os materiais testados: Grupo TP (Transbond® Plus Light Cure Band); Grupo RS (Resilience® Light Cure Band Cement); Grupo RMO (RMO® Band Cement); Grupo CK Crosslink® (Orthodontic Band Cement) e Grupo C (Controle, Tubo polietileno) (Tabela 1). Este estudo laboratorial *in vivo* foi previamente aprovado pelo Comitê de Ética em Pesquisa Animal sob o protocolo n° 0102011.

Os ratos foram anestesiados utilizando injeção intraperitonial de tiopental sódico (50 mg/kg) (Cristália, Campinas, São Paulo, Brasil). Em seguida, foi realizada a tricotomia da região dorsal de cada animal com lâminas de barbear (4 x 4 cm) e anti-sepsia do campo operatório com digluconato de clorexidina a 4% (18). Na linha média, eqüidistante da inserção da cauda e da cabeça do animal, foi realizada uma incisão de aproximadamente 8 mm de comprimento utilizando lâmina de bisturi n° 15 adaptada a um cabo de bisturi. O tecido subcutâneo foi divulsionado lateralmente com auxílio de uma tesoura de ponta romba, promovendo uma tunelização no sentido lateral para a inserção dos implantes dos materiais. Cada rato recebeu um implante de tubo de polietileno (0,8 cm de comprimento e 0,5 cm de diâmetro interno, previamente mantidos em álcool 70% por 120 minutos, lavados com água deionizada e finalmente autoclavados à temperatura de 110^0 C por 20 minutos) que foram utilizados como veículos de inoculação dos materiais testados.

Os CIVMRs foram manipulados segundo as instruções dos fabricantes e em seguida introduzidos nas aberturas das extremidades dos tubos de polietileno, com auxílio de espátula de inserção antiaderente aluminada. Posteriormente foram fotopolimerizados com aparelho LED (Radii, SDI, Baywater, Victoria, Austrália) de acordo com o tempo de fotopolimerização durante 40 segundos. A intensidade de luz do aparelho fotopolimerizador (1000mw/cm^2) foi checada imediatamente antes de cada polimerização usando um radiômetro (Model 100, Demetron Research Corporation, Danbury, CT, USA). Após a polimerização dos CIVMRs, os tubos foram implantados no tecido subcutâneo dos ratos. No grupo controle, foi utilizado o tubo de polietileno vazio que corresponde ao trauma induzido e possível contaminação dos mesmos.

Após o implante dos materiais, as lojas cirúrgicas foram suturadas com fio de sutura agulhado 4.0 (Ethicon, Jonhson & Jonhson, São José dos Campos, São Paulo, Brasil) e em seguida os animais receberam injeção de 0,2 ml de pentabiótico veterinário via intramuscular (Wyeth Laboratory, New York, NY, EUA), e uma injeção de dipirona sódica (0,3 ml/100g, Novalgina, São Paulo, SP, Brasil). Todos os procedimentos foram realizados em conformidade com o *Canadian Council on Animal Care* (1981). Os animais de cada grupo foram mantidos em gaiolas individuais a uma temperatura variando de 22 °C a 26 °C sob um ciclo de luz claro-escuro de 12 horas, sob condições adequadas, com ração apropriada e água ad libitum.

Após 7, 15 e 30 dias, os animais foram anestesiados para obtenção da biópsia excisional da área do implante, abrangendo tecido normal circundante suficiente. Cada grupo consistiu em cinco ratos por cada período de tempo, totalizando 15 amostras por grupo. Posteriormente os animais foram sacrificados pela técnica do deslocamento cervical após sedação tiopental sódico (50 mg/kg) (Cristália).

2.1 Estudo Histopatológico

Os espécimes foram preparados em lâminas de vidro através da coloração de rotina Hematoxilina e Eosina (HE) e posteriormente avaliados em um microscópio óptico Leica DM500® (Leica Microsystems Vertrieb GmbH, Wetzlar, DE), em aumentos de 100x, 400x e 1000x. De acordo com a metodologia proposta por Garcia et al. (2010) [19] e Santos et al. (2010) [18], foram avaliados os sequintes parâmetros histopatológicos: intensidade de infiltrado inflamatório e reação de células gigantes multinucleadas, sendo considerados os escores: 0- escasso; 1-leve; 2-moderado; 3-intenso. A avaliação histopatológica foi procedida por um único avaliador previamente calibrado (Kappa=0,8).

2.2 Estudo Imuno-histoquímico

O material foi submetido ao método da imunoperoxidase utilizando o anticorpo monoclonal anti CD68 (Abcam; Oxford,UK; diluição 1:1500). Os espécimes foram submetidos à recuperação antigênica com citrato pH 6.0 em Steamer, durante 60 minutos. Foi realizado o bloqueio da peroxidase endógena tecidual com peróxido de hidrogênio 3%, durante 20 minutos, e o anticorpo primário foi incubado em solução diluente (*Antibody diluent with background reducing components*, Dako North America Inc., Carpinteria, CA, USA) à temperatura ambiente, durante 60 minutos. Os espécimes

foram sequencialmente incubados com anticorpo secundário (*ADVANCE™ HRP Link*, Dako North America Inc., Carpinteria, CA, USA), à temperatura ambiente, durante 30 minutos e com o anticorpo polimerizado à peroxidase (*ADVANCE™ HRP Enzyme*, Dako North America Inc., Carpinteria, CA, USA), à temperatura ambiente, durante 30 minutos. A imunorreatividade foi visualizada utilizando a solução cromógena de 3,3-diaminobenzidina (*Liquid DAB+ Substrate*, Dako North America Inc., Carpinteria, CA, USA) e a contra-coloração realizada com a hematoxilina de Mayer à temperatura ambiente. O controle positivo para o anticorpo anti CD68 foi realizado com cortes histológicos do fígado de ratos. O controle negativo consistiu na substituição do anticorpo primário por albumina de soro bovino (BSA) a 1% em solução tampão.

A análise imuno-histoquímica foi realizada por um único avaliador previamente treinado e calibrado. Cada espécime foi analisado sob um microscópio óptico Leica DM500® (Leica DM500®, Leica Microsystems Vertrieb GmbH, Wetzlar, DE) e sob o aumento de 100x foram selecionados 5 campos de maior imunorreatividade ao anticorpo CD68. Sob aumento de 400x, em cada um destes campos foi realizada a contagem de células mononucleadas e de células gigantes multinucleadas CD68+. Os valores obtidos foram somados, estabelecendo-se o número total de células CD68+, e posteriormente calculada a média por campo, para cada caso.

Os dados foram tabulados e analisados no programa BioEstat versão 5.0 (Mamirauá, Manaus, Brasil). O método estatístico foi escolhido com base no modelo de distribuição e variância dos dados avaliada pelo Teste de Kolmogorov-Smirnov e Teste de Levene, respectivamente. Desta forma, os resultados dos eventos celulares foram submetidos ao teste de Kruskal-Wallis, seguido pelo teste de Dunn para determinar as diferenças entre os grupos ($P< 0.05$).

3. Resultados

Diante dos achados histopatológicos, pode-se observar que, em todos os cimentos ortodônticos testados, o infiltrado inflamatório diminuiu ao longo do tempo. Em todos os materiais, a resposta tecidual foi caracterizada pela presença de infiltrado inflamatório crônico, predominantemente mononuclear nos períodos iniciais avaliados. No período de 7 dias, os grupos RS, RMO e TP demonstraram infiltrado inflamatório mais intenso (p=0,004) (Tabela 2). Nos períodos de 15 e 30 não foram observadas diferenças estatisticamente significativas em todos os grupos avaliados.

Para a análise imuno-histoquímica, os grupos RMO e RS apresentaram maior quantidade de macrófagos (p=0,004) no período de 7 dias e apenas o grupo RMO apresentou diferença estatisticamente significativa para este parâmetro após o período de avaliação de 15 dias (p=0,026) (Fig. 1). No período de 30 dias, o grupo RMO apresentou maior quantidade de células gigantes multinucleadas quando comparado aos demais materiais e ao grupo controle (p=0,041) (Tabela 3) (Fig. 2).

4. Discussão

Nesse estudo, a avaliação da biocompatibilidade dos CIVMRs foi realizada através da análise da intensidade da resposta inflamatória por métodos histológicos e da identificação específica de MOs e CGM através de métodos imuno-histoquímicos. Semelhantemente, estudos que avaliaram biocompatibilidade utilizaram o anticorpo CD68 para a identificação especifica de MOs nas reações inflamatórias decorrente destes materiais [6, 9, 14].

Observou-se nesse estudo que os cimentos ortodônticos RS, RMO e TP apresentaram infiltrado inflamatório mais intenso quando comparado ao cimento CK e ao grupo controle no período de 7 dias. Essa diferença observada sugere-se a eluição de monômeros residuais durante a polimerização dos CIVMRs. Os monômeros são capazes de desencadear citotoxicidade significativa e interferir diretamente na biocompatibilidade destes compósitos [1, 20]. Os cimentos RS e RMO contém BisGMA, monômero que apresenta baixos valores de grau de conversão e consequentemente tendem a permanecerem residuais mesmo após a completa polimerização [10, 21, 22]. Já o cimento TP apresenta o monômero 2 - hidroxi - 1, 3 – Dimethacryloxypropane que, apesar de possuir um bom grau de conversão, também pode desencadear citotoxicidade aos tecidos bucais [23].

Na análise da quantificação de macrófagos presentes nas reações teciduais, pode-se observar uma maior intensidade dessas células nos períodos de 7 e 15 dias. Essa resposta inflamatória inicial reflete a tentativa do tecido de induzir o processo de degradação dos materiais através de MOs recrutados para os tecidos circundantes e em direção à superfície dos cimentos [21]. Os MOs ativados desempenham um importante papel na fagocitose desses materiais estranhos, no recrutamento de fibroblastos, bem como na liberação de mediadores inflamatórios [6]. Para Yu Gong et al. (2013) [24] os

compostos resinosos são capazes de aumentar a proliferação e a função dos MOs, podendo levar ao progresso da reação inflamatória tecidual.

No período de 7 dias, os cimentos ortodônticos RMO e RS apresentaram maior quantidade de MOs, quando comparados aos demais grupos. A maior intensidade dessas células pode estar relacionada à presença do monômero BiSGMA nesses materiais. Há indícios que este monômero seja capaz de induzir um maior recrutamento e ativação de MOs [9]. Para Kuan et al. (2012) [25] o monômero BisGMA é capaz de induzir a ativação de MOs e a produção de óxido nítrico, espécies reativas de oxigênio e citocinas pró-inflamatórias que atuam na amplificação da resposta inflamatória. No período de 15 dias, apenas o grupo RMO permaneceu com uma intensa quantidade de MOs quando comparado ao grupo controle. Sugere-se que a quantidade de monômeros ou a presença de outros componentes como aditivos e co-iniciadores possam ter interferido na resposta tecidual e proporcionado a persistência da inflamação [1, 26].

Na análise imuno-histoquímica das células gigantes multinucleadas, a quantidade de CGM foi significativamente maior para o grupo RMO após o período de 30 dias quando comparado ao grupo controle. Sugere-se que monômeros ou outras partículas liberadas pelo grupo RMO são mais difíceis de serem digeridas pelos MOs, que tendem a se fundir e formar um maior número de CGM com o intuito de facilitar a degradação de resquícios deste material [11, 16].

O número elevado de células gigantes multinucleadas no período de 30 dias corrobora outros estudos que avaliaram a biocompatibilidade *in vivo* e verificaram que as CGM persistiram mesmo após um longo período de avaliação [10, 11]. O método imuno-histoquímico utilizado em nosso estudo para avaliação de MOs e CGM é considerado um sistema mais sensível de quantificação de células inflamatórias do que os métodos tradicionais [13, 14], visto que esta técnica facilita a identificação celular, permitindo avaliações específicas das respostas celulares com precisão e confiabilidade [13].

Conclusão

Dentre os cimentos testados, CK e TP proporcionaram melhores respostas teciduais para todos os eventos analisados. O cimento RMO apresentou a menor

biocompatibilidade, seguido pelo RS devido à maior intensidade de infiltrado inflamatório com acúmulo substancial de MOs e de células gigantes multinucleadas.

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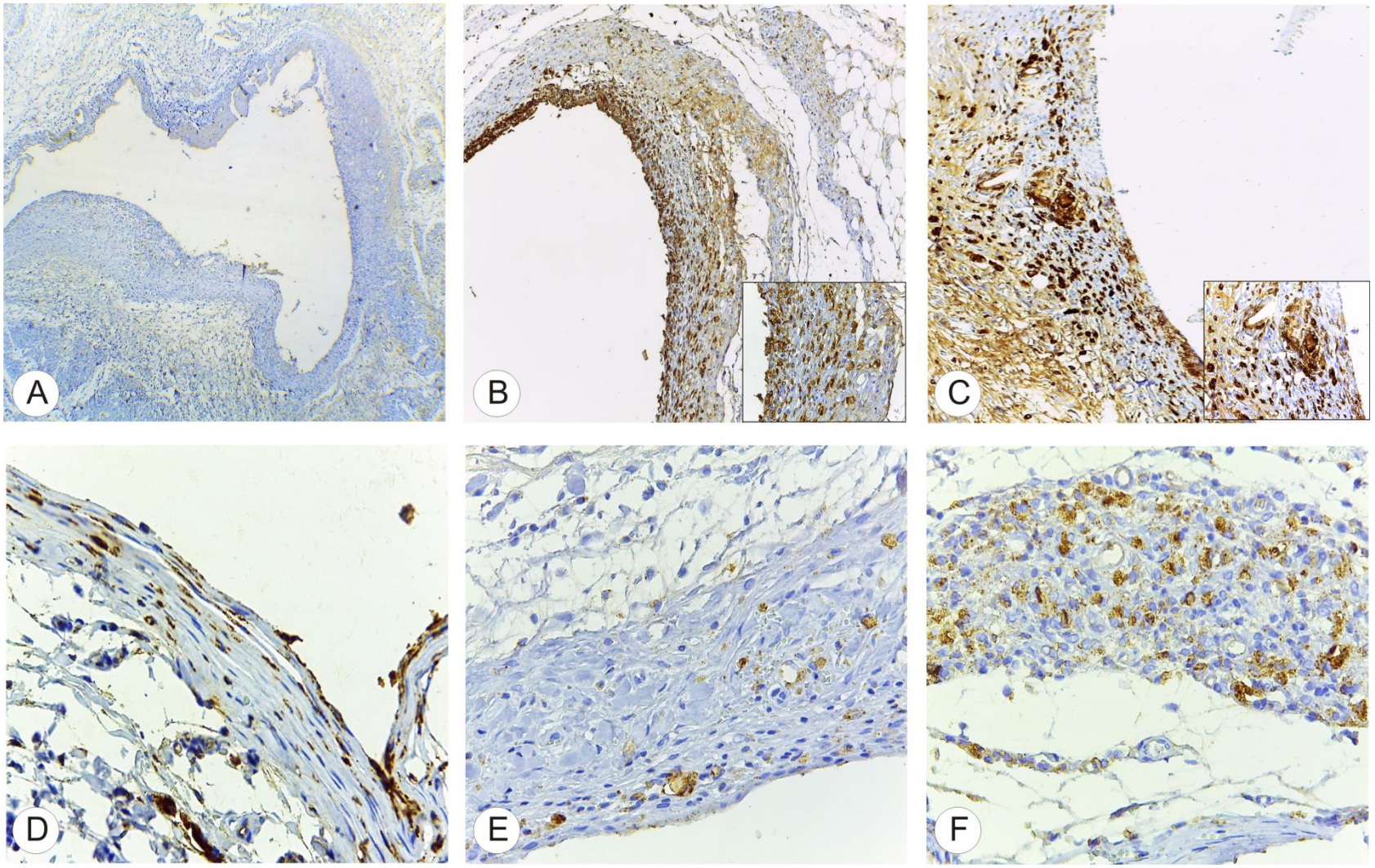
Legendas das figuras

Figura 1. Imunomarcação para anticorpo CD68. No período de 7 dias: A) No grupo CK observa-se escassa imunoexpressão do CD68 circundando toda a cavidade (100x). B) No grupo RS visualiza-se maior intensidade da imunomarcação (100x) e, em maior aumento, grande quantidade de macrófagos (40x). C) No grupo RMO evidencia-se numerosas células CD68+ (100x) e, em destaque, e células gigantes multinucleadas (400x). No período de 15 dias: D e E) Nos grupos CK e RS, respectivamente, observa-se leve quantidade de células $CD68^{+}$. F) No grupo RMO verifica-se grande quantidade de células $CD68^{+}$ (40x).

Figura 2. Imunomarcação do CD68 no período de 30 dias. A) No grupo CK observa-se grande quantidade de CGMs CD68+ na região inferior da cavidade (100x). Em destaque, CGMs imunomarcadas (400x). B) No grupo RS pode ser visualizada moderada quantidade de CGMs CD68+ (setas amarelas). C) No grupo RMO evidencia-se intensa quantidade de CGMs CD68+ (100x) (setas amarelas) e, em destaque, macrófagos e CGMs (40x). D) No grupo TP, observa-se escassa quantidade de CGMs imunomarcadas (100x).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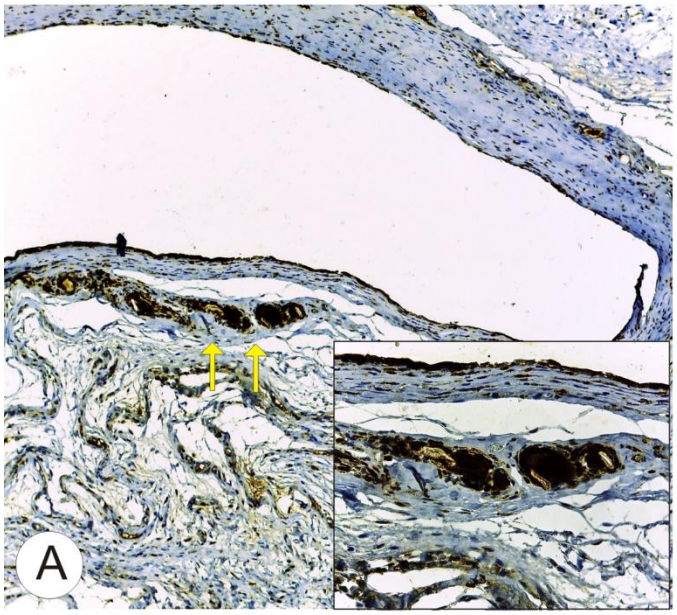
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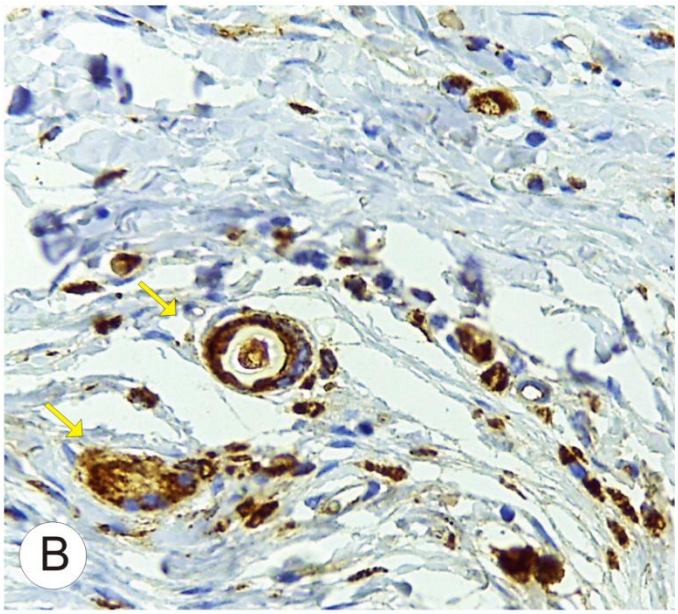
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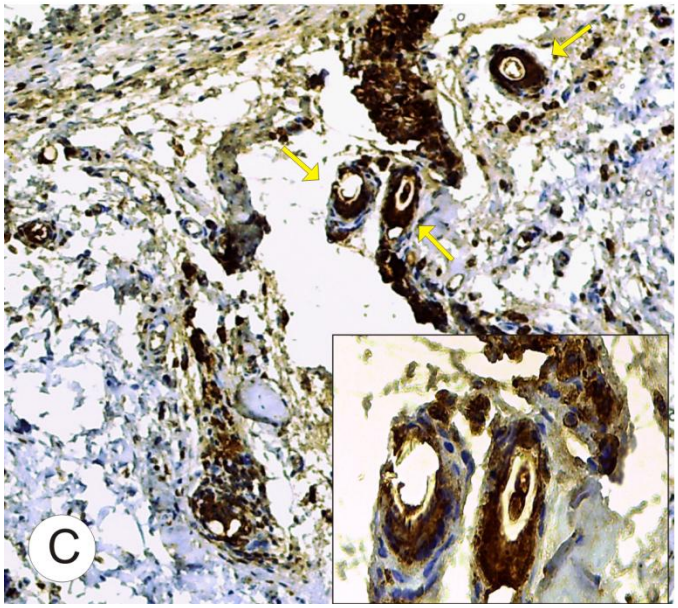
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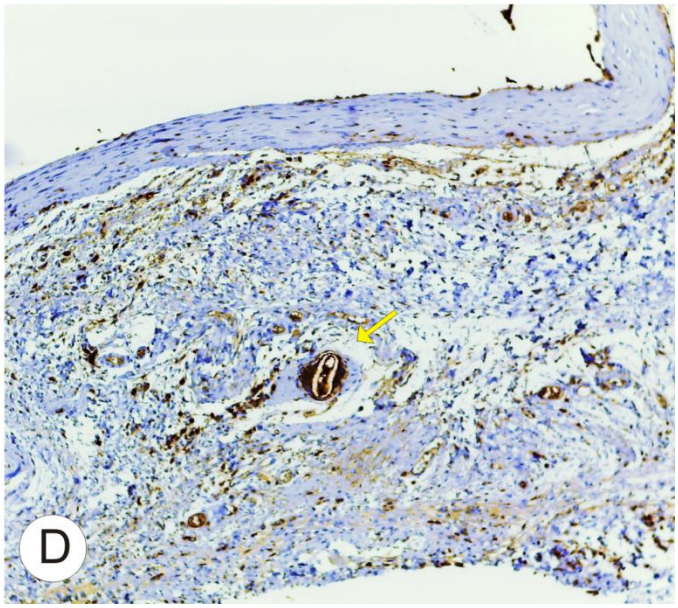
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Tabela 1. Composição dos cimentos Transbond, Crosslink, RMO e Resilience avaliados no estudo.

Grupos	Cimentos	Composição	Fabricante	Lot n.
CK	**Crosslink® Orthodontic Band Cement**	Monomers, Fluroaluminosilicate glass, Cure promoters and pigments	TP orthodontics, La Porte, Indiana, USA	32012000
RS	**Resilience®Light Cure Band Cement**	Bis-GMA based resin system with a chemical catalyst system.	Ortho Technology, Tampa, Florida, USA	32112
RMO	**RMO® Band Cement**	Aromatic and Aliphatic Dimethacrylate Monomers and Fluroaluminosilicate glass	RMO, Denver, CO, USA	A041813
TP	**Transbond® Plus Light Cure Band**	2 hidroxi, 1,3-dimethacry-loxypropane, cure promoters, fluoroaluninosilicate glass and blue pigment	3MUnitek, Monrovia, CA,USA	N4 05305

Tabela 2: Média dos escores atribuídos aos cimentos ortodônticos, após os intervalos de tempo de 7, 15 e 30 dias, para o evento inflamatório.

Eventos	**Tempo**	**Grupos**					
		CK	**RS**	**RMO**	**TP**	**C**	**P valor**
Infiltrado inflamatório	7 dias	1.6^{AB}	2.4^{A}	2.4^{A}	2.2^{A}	1.0^{B}	0.004
	15 dias	1.2	0.8	1.0	1.4	0.6	0.091
	30 dias	0.6	0.8	0.6	0.8	0.4	0.674

Estes valores representam a média dos escores da soma das cinco secções histológicas representativas do tecido avaliado (n=5, por grupo). Médias seguidas por letras diferentes expressam diferença estatisticamente significativa ($p < .05$) com base no teste não paramétrico de Kruskal-Wallis, seguido pelo teste de comparação múltipla de Dunn.

Tabela 3: Análise imuno-histoquímica do anticorpo CD68 após os intervalos de tempo de 7, 15 e 30 dias.

Células	**Tempo**	**Grupos**					
		CK	**RS**	**RMO**	**TP**	**C**	**P valor**
Células Gigantes	7 dias	0.60	1.80	2.20	1.00	0.00	0.056
	15 dias	1.20	0.20	1.40	1.00	0.00	0.339
	30 dias	1.20^{AB}	0.60^{AB}	1.40^{A}	0.20^{AB}	0.00^{B}	0.041
Macrófagos	7 dias	24.7^{AB}	37.3^{A}	37.9^{A}	28.4^{AB}	20.2^{B}	0.004
	15 dias	23.8^{AB}	20.0^{AB}	26.2^{A}	20.9^{AB}	13.6^{B}	0.026
	30 dias	15.4	17.8	23.9	19.5	10.6	0.204

Estes valores representam a quantidade média das células encontradas nas cinco secções histológicas representativas do tecido avaliado (n=5, por grupo). Médias seguidas por letras diferentes expressam diferença estatisticamente significativa ($p < .05$) com base no teste não paramétrico de Kruskal-Wallis, seguido pelo teste de comparação múltipla de Dunn.

CONSIDERAÇÕES FINAIS

5 CONSIDERAÇÕES FINAIS

A biocompatibilidade dos materiais odontológicos tem sido amplamente avaliada através de testes *in vivo* em tecido subcutâneo de ratos. Entretanto, estudos que avaliam a biocompatibilidade de cimentos de ionômero de vidro modificados por resina ainda são escassos na literatura. O estudo das propriedades biológicas dos CIVMRs, através de métodos específicos de avaliação da reação inflamatória tecidual, é de fundamental importância, uma vez que este assunto ainda permanece controverso.

Na avaliação da biocompatibilidade dos CIVMRs, os achados do presente estudo sugerem que os cimentos ortodônticos podem determinar diferentes respostas inflamatórias a depender do grau de citotoxicidade dos monômeros liberados por estes materiais. Os cimentos Crosslink e Transbond proporcionaram melhores respostas teciduais, visto que demonstraram menor intensidade de infiltrado inflamatório e maior grau de colagenização ao longo do tempo. O grupo RMO demonstrou a menor biocompatibilidade, seguido pelo Resilience, uma vez que estes materiais possuem monômeros de BisGMA que, ao entrarem em contato com os tecidos, são capazes de intensificar a resposta inflamatória com consequente acúmulo de MOs e de células gigantes multinucleadas.

Nesse sentido, sugere-se que os cimentos ortodônticos que apresentaram menor biocompatibilidade em nosso estudo, especialmente o RMO e RS, podem induzir um processo inflamatório mais duradouro sobre os tecidos gengivais, sendo prejudiciais clinicamente. Adicionalmente, pode-se observar que a análise imuno-histoquímica associada à análise morfológica dos CIVMR conferem maior fidedignidade aos testes de biocompatibilidade e fornecem informações preliminares das características das reações teciduais decorrente destes materiais.

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APÊNDICES

APÊNDICE 1

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DEPARTAMENTO DE ODONTOLOGIA

MESTRADO EM ODONTOLOGIA

ORIENTADORA: Profa Dr[a] Pollianna Muniz Alves

MESTRANDA: Janaina Almeida Mesquita

FICHA PARA ANÁLISE HISTOPATOLÓGICA

PERÍODO DE ACOMPANHAMENTO – ( ) DIAS							
Grupos	PARÂMETROS A SEREM AVALIADOS						
	Infiltrado inflamatório	Cél. Gigantes multinucleadas	Edema	Reação de granulação	Fibroblastos Jovens	Colagenização	Necrose
Grupo I – 1							
Grupo I – 2							
Grupo I – 3							
Grupo I – 4							
Grupo I – 5							
Grupo II – 1							
Grupo II – 2							
Grupo II – 3							

Grupo II – 4							
Grupo II – 5							
Grupo III – 1							
Grupo III – 2							
Grupo III – 3							
Grupo III – 4							
Grupo III – 5							
Grupo IV – 1							
Grupo IV – 2							
Grupo IV – 3							
Grupo IV – 4							
Grupo IV – 5							
Grupo V – 1							
Grupo V – 2							
Grupo V – 3							
Grupo V – 4							
Grupo V – 5							

*Análise da intensidade do infiltrado inflamatório: 0 – escasso; 1- leve; 2 – moderado; 3– intenso.

*Reação de células gigantes multinucleadas: 0 -escasso; 1- leve; 2 - moderado; 3-intenso.

* Edema: 0 – escasso; 1- leve; 2 – moderado; 3– intenso.

*Reação de granulação: 0 – escasso; 1- leve; 2 – moderado; 3 – intenso.

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*Colagenização: 0 – escasso; 1- leve; 2 – moderado; 3 – intenso.

*Necrose: 0 – escasso; 1- leve; 2 – moderado; 3 – intenso.

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Ofício CEP nº 70/2011

Protocolo CEP nº 0102011

Ao. Sr. Rogério Lacerda dos Santos

Sr. Santos;

A pesquisa coordenada por V.Sa. intitulada "**Estudo *in vivo* da biocompatibilidade de cimentos ortodônticos reforçados com resina**", recebeu parecer FAVORÁVEL após avaliação dos relatores da Comissão de Ética em Pesquisa do Centro de Saúde e Tecnologia Rural – CSTR/UFCG.

Atenciosamente.

Patos, 28 de outubro de 2011.

Onaldo Guedes Rodrigues
Coordenador do CEP

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