



UNIVERSIDADE FEDERAL DE UBERLÂNDIA
FACULDADE DE ODONTOLOGIA



GABRIELLE ALVES NUNES FREITAS

**EFEITOS DE DIFERENTES MATERIAIS À BASE
DE SILICATO DE CÁLCIO NA RESISTÊNCIA À
FRATURA DE DENTES PERMANENTES
IMATUROS COM REABSORÇÃO RADICULAR
POR SUBSTITUIÇÃO E NA
OSTEOCLATOGÊNESE:
UM ESTUDO LABORATORIAL**

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Trabalho de conclusão de curso apresentado
a Faculdade de Odontologia da UFU, como
requisito parcial para obtenção do título de
Graduado em Odontologia

Orientadora: Prof^a. Dr^a. Camilla Christian
Gomes Moura

Coorientadora: M.a Gabriela Leite de Souza

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DEDICATÓRIA

*Aos meus pais Luzia e Danilo que
fizeram do meu sonho o deles e não
mediram esforços para que eu
chegasse até aqui. À minha irmã
Isabela, aos meus sobrinhos Lucas e
Anaísa, aos meus avós Antônio e
Hosana (in memoriam), e a todos os
familiares e amigos por todo apoio e
carinho.*

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RESUMO

Objetivo(s): Este estudo avaliou o efeito do Biodentine (BD), Bio-C Repair (BCR) Agregado de Trióxido Mineral (MTA) na resistência à fratura de dentes bovinos nos quais foi simulado rizogênese incompleta e reabsorção radicular (RR), e na osteoclastogênese induzida *in vitro*.

Materiais e Métodos: Sessenta incisivos bovinos simulando dentes imaturos com RR foram divididos em cinco grupos experimentais (n=12 por grupo): BD e BCR, totalmente obturados com os respectivos materiais; MTA, tampão apical de MTA e obturação com guta percha e cimento Bio-C sealer; RR, reabsorção radicular simulada e canal radicular vazio (controle negativo); e ligamento periodontal normal (PL), dente imaturo simulado e canal vazio (controle positivo). Todos os dentes sofreram fadiga mecânica e, em seguida, foi realizado teste de resistência à fratura à compressão utilizando a máquina de ensaio mecânico universal Instron. Macrófagos RAW 264.7 foram tratados com extratos de BD, BCR e MTA diluídos em 1:16 e simultaneamente estimulados com ativador do receptor do ligante do fator nuclear kappa B (RANKL) por 5 dias. A diferenciação de osteoclastos induzida por RANKL foi avaliada por coloração com fosfatase ácida resistente a tartarato. A carga de fratura e o número de osteoclastos foram analisados usando análise de variância unidirecional (ANOVA) e teste de Tukey ($\alpha = 0,05$).

Resultados: Não foi observado diferença significativa na resistência à fratura entre os grupos ($P > 0,05$). A presença de RR não afetou a resistência à fratura dos dentes imaturos simulados e os materiais testados não os fortaleceram. Todos os materiais inibiram similarmente a osteoclastogênese ($P > 0,05$), exceto o BCR, que apresentou menor porcentagem de osteoclastos do que o MTA ($P < 0,0001$).

Conclusão(ões): As opções de tratamento utilizadas para tratar dentes imaturos não vitais com RR não os fortalecem e proporcionaram a mesma resistência à fratura entre si. BD, MTA e BCR apresentaram efeito inibitório na diferenciação de osteoclastos, com o grupo BCR apresentando maior potencial inibitório.

Palavras-chave: resistência à fratura; dente não vital; osteoclastogênese; reabsorção radicular; cimento de silicato.

(Restorative Dentistry and Endodontics: Research Article)

Effects of different bioceramic-based materials on fracture resistance of immature permanent teeth with replacement root resorption and osteoclastogenesis – a laboratory study.

Gabriela Leite de Souza¹, Gabrielle Alves Nunes Freitas², Maria Tereza Hordones Ribeiro³, Nelly Xiomara Alvarado Lemus¹, Carlos José Soares³, Camilla Christian Gomes Moura^{1*}.

¹ Department of Endodontics, School of Dentistry, Federal University of Uberlândia, Uberlândia, Brazil.

² Undergraduation student, School of Dentistry, Federal University of Uberlândia, Uberlândia, Brazil.

³Department of Operative Dentistry and Dental Materials, School of Dentistry, Federal University of Uberlândia, Uberlândia, Brazil.

Souza GL, Freitas GAN, Ribeiro MTH, Lemus NXA, Soares CJ, Moura CCG.

Running Title: Bioceramic effects on fracture resistance and osteoclastogenesis.

***Correspondence:** Camilla Christian Gomes Moura, PhD

Department of Endodontics, School of Dentistry, Federal University of Uberlândia. Av Pará 1720, Campus Umuarama, Block 4L, annex A, Uberlândia, MG, Brazil. Zip-Code: 38405-328
E-mail address: camillahistologia@yahoo.com.br Phone number: +55 34 3225-8119

ORCID

Gabriela Leite de Souza <https://orcid.org/0000-0003-3505-5258>

Gabrielle Alves Nunes Freitas <https://orcid.org/0000-0001-7865-2795>

Maria Tereza Hordones Ribeiro <https://orcid.org/0000-0002-5126-7931>

Nelly Xiomara Alvarado Lemus <https://orcid.org/0000-0002-9658-2433>

Carlos José Soares <https://orcid.org/0000-0002-8830-605X>

Camilla Christian Gomes Moura <https://orcid.org/0000-0002-5653-8403>

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ABSTRACT

Objective(s): This study evaluated the effects of Biodentine (BD), Bio-C repair (BCR), and mineral trioxide aggregate plug (MTA) on fracture resistance of simulated immature teeth with replacement root resorption (RRR) and *in vitro* induced osteoclastogenesis.

Materials and Methods: Sixty bovine incisors simulating immature teeth and RRR were divided into five groups: BD and BCR, completely filled with respective materials; MTA, a 3-mm apical plug of MTA; RRR, no root canal filling (negative control); PL, no RRR, and root canal filling performed (positive control). All the teeth underwent mechanical fatigue, and then compression strength testing was performed using an Instron universal testing machine. RAW 264.7 macrophages were treated with 1:16 extracts of BD, BCR, and MTA containing receptor activator of nuclear factor-kappa B ligand (RANKL) for 5 days. RANKL-induced osteoclast differentiation was assessed by staining with tartrate-resistant acid phosphatase. Fracture load and osteoclast number were analyzed using one-way analysis of variance (ANOVA) and Tukey's test ($\alpha=0.05$).

Results: No significant difference in fracture resistance was observed among the groups ($P>0.05$). All materials similarly inhibited osteoclastogenesis ($P>0.05$), except for BCR, which showed a lower percentage of osteoclasts than MTA ($P<0.0001$). The presence of RRR did not affect the fracture strength of the simulated immature teeth and the materials tested did not significantly strengthen them.

Conclusion(s): The treatment options used to treat non-vital immature teeth with RRR do not strengthen them and provide similar resistance to fractures. BD, MTA, and BCR showed inhibitory effects on osteoclast differentiation, with BCR group showing better results.

Keywords: Fracture strength; Non-vital tooth; Osteoclastogenesis; Root resorption; Silicate cement.

INTRODUCTION

Traumatic dental injuries of immature permanent teeth may lead to pulp necrosis, which interrupts root development, making root canal treatment challenging. Although pulp necrosis is the most common post-traumatic complication [1], replacement root resorption (RRR) is the most concerning complication because it may affect the long-term survival of teeth, mainly after lateral luxation and avulsion [2].

In cases involving RRR, new alveolar bone is formed and deposited in the space previously occupied by the PDL fibers that were lost, and the root is progressively resorbed and replaced by bone tissue [3]. Thus, the development of the RRR process invariably leads to exposure of the filling material to periodontal tissues, making it important to choose root canal filling materials with greater biocompatibility [4]. Additionally, as resorption is mediated by osteoclasts/odontoclasts, it is reasonable to assume that filling the root canal with materials that reduce or inhibit osteoclastic activity could delay the progression of root resorption, making it possible to plan adequate rehabilitation for hebiatric patients, in whom the early loss of a tooth has a greater psychosocial impact.

Mineral trioxide aggregate (MTA) has been suggested for filling reimplanted teeth [4] due to its sealing ability, biocompatibility, low solubility, ability to inhibit osteoclastogenesis, adequate radiopacity, and high pH. However, despite all the widely known advantages, MTA has several disadvantages including prolonged setting time, potential for coronal staining, and handling difficulties [5], and other bioceramic materials have been suggested as alternatives.

Biodentine (BD; Septodont, Saint Maur-des-Fosses, France) is a tricalcium silicate-based restorative cement indicated as a “dentine replacement” material, comparable to MTA in terms of biocompatibility and induction of a calcific barrier [6]. It has been shown to possess additional improvement in several other properties, such as mixing, handling, shorter initial setting time, and less coronal discoloration [6]. Recently, a new ready-to-use bioceramic material, Bio-C Repair (BCR; Angelus, Londrina, Brazil), has become available for reparative or regenerative endodontic treatments. This material has low cytotoxicity [7,8], good biocompatibility [9], and biomineralization ability [9,10].

Although previous studies have evaluated the fracture resistance of immature teeth restored with various intraradicular filling materials [11,12], to the authors’ knowledge, no study has evaluated intraradicular filling materials in immature permanent teeth in conditions that mimic RRR. Thus, in this study, we aimed to evaluate

fracture resistance after cyclic loading of simulated immature teeth with RRR filled with BD, BCR, or MTA plugs and to compare the cytotoxicity and anti-osteoclastic effects of these materials. The null hypothesis was that in immature teeth with RRR, treatment with different intraradicular filling materials would have the following effects: 1) not affect the fracture resistance of immature permanent teeth and 2) not affect cell viability and osteoclast-like maturation.

MATERIALS AND METHODS

Fracture resistance

Sample size calculation

The sample size was based on data from Tanalp *et al.* (2011)[13] and was calculated using the G*Power version 3.1.9.6 software (<http://www.psych.uni-duesseldorf.de/abteilungen/aap/gpower3/>) to allow an analysis with $\alpha = 0.05$, Power ($1-\beta$ prob) = 0.99, and effect size $f = 1,52$. The statistical test ANCOVA (fixed effects, main effects, and interactions) was performed. The type of power analysis was set a priori (to compute the required sample size, given α , power, and effect size). Therefore, a minimum of 12 teeth were allocated to each experimental group, with a total of 60 teeth.

Selection of samples

Bovine central incisors (30-36 months) were obtained from a local abattoir (Real, Uberlândia, MG, Brazil), cleaned and stored in distilled water at 4 °C. Under microscope examination (D. F. Vasconcellos, Valença, RJ, Brazil) at $\times 3.0 \times$ magnification, the teeth were analyzed for the presence of cracks and fractures. Teeth without crack, fracture line or other deformities had the mesiodistal and buccolingual dimensions of their roots measured using a digital caliper (Mitutoyo Sul Americana Ltda., Suzano, SP, Brazil), allowing those samples with a maximum deviation of 10% from the mean to be included in the study. The selected teeth were stored in distilled water at 4°C until further use.

Simulation of immature permanent teeth

To simulate complicated crown fracture in an immature tooth, the samples were sectioned 8 mm above and 12 mm below the cemento-enamel junction under a water-cooled diamond disc [8]. The root canals were instrumented with Peeso reamers (size 1–5, Dentsply Maillefer) followed by 3017 HL diamond bur (KG Sorensen, Barueri, SP, Brazil) to create a standard open apex of 2.5 mm in diameter [14]. Next, the root canals were rinsed with 20 ml of 2.5% sodium hypochlorite followed by final irrigation with 3 mL of 17% EDTA solution and 5 mL of distilled water [8]. The samples were re-evaluated for the presence of cracks or defects using the transillumination method. All teeth with cracks were excluded from this study.

Root canal filling and simulation of replacement root resorption (RRR)

The RRR simulation was performed on the palatal surface of the prepared samples based on the protocol previously described by Vieira *et al.* 2020 [15]. First, perforations were made on the palatal surface in the cervical, middle, and apical root thirds, using cylindrical drills with diameters of 1.2, 1.8 mm, and 0.6, respectively. Subsequently, the root regions containing the perforations were exposed to an acid demineralization process using treatment with 5% nitric acid solution for 12 h, 8% sodium hypochlorite for 10 min, and 5% nitric acid solution for resorption lesions. The teeth were washed and stored in deionized water to arrest the process. Randomization (blocked random scheme) was performed using the website www.sealedenvelope.com. Randomization and allocation of the samples to one of the following five groups (n=12) were performed by the same researcher (CCGM), who was not involved in the implementation (GANF) and evaluation processes (GLS).

- *RRR (negative control group)*: RRR simulated; no apical plug or root canal filling was performed.
- *Normal periodontal ligament (PL; positive control group)*: no RRR and no apical plug or root canal filling was performed.
- *MTA Plug*: RRR simulated, MTA was prepared according to the manufacturer's instructions, placed into the root canals with an MTA carrier, and condensed with hand pluggers to create a 4-mm thickness of MTA. After MTA apexification, the root canal was filled with a BioC sealer (Angelus) and gutta-percha cones (Dentsply-Maillefer).
- *BCR*: RRR was simulated and the root canals were completely filled with BCR. The ready-to-use cement was inserted incrementally and condensed in the root canal with hand pluggers up to the limit of the cemento-enamel junction.

- *BD*: RRR-simulated BD was prepared according to the manufacturer's instructions and inserted into the root canal as described in the previous group, up to the limit of the cemento-enamel junction.

The teeth were radiographed again to confirm the quality of the filling, and endodontic access was restored using an adhesive system (Adper Single Bond 2; 3M ESPE, St. Paul, MN, USA) and composite resin (Filtek Z350 XT; 3M, Sumaré, SP, Brazil). The samples were stored at 37 °C and 100% humidity for 24 h.

After this period, the teeth were inserted into resin cylinders to simulate RRR regions and regions with normal periodontal ligaments. The external surfaces of the root were then covered with a 0.2– 0.3 mm thick wax layer until it reached 2 mm below the CEJ. Afterwards, the wax-covered roots were embedded in polystyrene resin cylinders (Classico, Rio de Janeiro, RJ, Brazil). After resin polymerization, the roots were removed from the cylinder, and wax was removed from the root surface, creating a space in the resin cylinder. The polyether impression material, Impregum F (3M ESPE, St. Paul, MN, USA), was mixed and distributed over the buccal root surface. Simultaneously, polystyrene resin was manipulated and distributed over the palatal root surface, filling the regions where the perforations were made and the regions free from perforation. The tooth was re-inserted into the cylinder, and excess material was removed using a scalpel blade.

Mechanical cycling test and fracture testing

Chewing cycles were simulated to induce mechanical fatigue (Biocycle; Biopdi, São Paulo, SP, Brazil) by immersing the samples in water at 37°C and cycling for 1,200,000 times. It was established to apply a load of 0 to 50 N at 45° and a frequency of 2 Hz on the palatal surface of the teeth using a stainless-steel cylindrical-shaped tip. Each specimen was arranged in a mounting jig such that the load was applied to the palatal surface at a 45 °angle with the long axis of the tooth. Fracture was induced in the specimens by applying compressive load with a crosshead speed of 0.5 mm/min in a universal testing machine (Instron model 3344, Instron; Norwood, MA, USA) fitted with a 5000 N load cell [16]. Both the fracture load (N) and the fracture location were recorded. The fracture modes were analyzed using the scale shown in Figure 1.

Cell culture assays

Preparation of material extracts

MTA, BCR, and BD were mixed according to the manufacturer's instructions under sterile conditions and the extracts were prepared as previously described [8]. The materials were shaped in cylindrical polyethylene moulds (diameter, 5 mm; height, 2 mm) and incubated under the humidified condition at 37°C for 24 h to allow complete setting. After this period, the specimens were immersed in α -minimal essential medium (α -MEM) (LGC Biotechnology, Cotia, SP, Brazil) for 24 h at 37°C and 5% CO₂ in a humid atmosphere to create the extracts. Following the ISO 10993-5 guidelines [17], a material surface area/medium volume ratio of approximately 1.5 cm²/mL was obtained.

Cell cytotoxicity assay

A murine RAW 264.7 macrophage cell lineage was obtained from the Cell Bank of Rio de Janeiro (Rio de Janeiro, RJ, Brazil). Cells were incubated in α -MEM supplemented with 10% fetal bovine serum (FBS; Gibco, Invitrogen, Carlsbad, CA, USA) and 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA) and cultured until confluence. After this period, RAW264.7 were seeded in a 96-well plate and exposed to diluted extracts (1:1-1:16) or not exposed (control group) for 24 h. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Thermo Fisher Scientific, Waltham, MA, USA) was prepared at a concentration of 5 mg/mL and 10% MTT diluted in α -MEM was added to each well for a period of 4 h as specified in the manufacturer's instructions. After this period, 100 μ L/well of dimethylsulfoxide (Sigma-Aldrich) was added once a purple precipitate was observed. Optical density (OD) at 570 nm was measured using a microplate reader (Biochrom, Cambridge, UK). Cell viability was expressed as the percentage of OD of the experimental wells relative to that of the control, which was taken to represent 100% cell viability according to the following formula: Percentage of viable cells = (A/B) x 100 where, A = viable cells in the experimental well and B = viable cells in the control well. The assay was performed in quadruplicate (n=4) and the trial was repeated twice under the same conditions. A dilution that demonstrated a cell viability higher than 80% was used for the osteoclastogenesis assay (1:16).

Osteoclastogenesis induction and exposure to the extracts

RAW 264.7 cells were seeded (2×10^4 cells/well) in a 96-well plate in α -MEM supplemented with 10% FBS and 1% penicillin-streptomycin, and allowed to adhere overnight. For osteoclast-like formation, the cells were stimulated with 1:16 diluted extracts of materials and 100 ng/mL RANKL (PeproTech, Rocky Hill, NJ, USA) for 5 days. On day 3, the medium and RANKL were changed [18,19]. The negative control group contained cells maintained in 10% α -MEM (not induced to differentiation), and the positive control group contained cells kept in RANKL without extract treatment. Samples were prepared for quantification of tartrate-resistant acid phosphatase (TRAP) activity. The assay was performed in quadruplicate (n=4) and the trial was repeated twice under the same conditions.

Tartrate-resistant acid phosphatase (TRAP) staining

Cells were washed with phosphate-buffered saline (PBS; Gibco) and fixed for 30 s with a liquid containing citrate, acetone, and formaldehyde. Next, the cells were stained using an acid phosphatase leukocyte TRAP kit (Sigma-Aldrich) in accordance with the manufacturer's recommendations. TRAP-positive cells had cytoplasmic granules marked by dark red to purple deposits. The number of osteoclasts formed per well was considered TRAP-positive multinucleated cells with three or more nuclei [18,19]. Four images of each well were taken at the top left, top right, bottom left, and bottom right, respectively, at 10x magnification using a Leica DM IRB-inverted microscope coupled to a DFC490 camera (Leica, Wetzlar, HE, Germany). The results are expressed as a percentage of TRAP-positive cells identified per camp in each well compared with that of the positive control group [18].

Statistical analysis

Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Fracture load and osteoclast number were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test to ascertain any significant differences between groups. The fracture modes were qualitatively evaluated. For cell viability, two-way ANOVA and Tukey's test were used to compare the data

between the treated groups. Dunnett's test was used to compare the experimental groups with the control group. Statistical significance was set at $\alpha = 0.05$.

RESULTS

Fracture testing

The results of the fracture testing are presented in Figure 2. There were no statistically significant differences in fracture resistance among the five groups evaluated (one-way ANOVA, $F = .872$, $P = .487$). All teeth filled with MTA plug, BCR, and BD fractured in the cervical and middle thirds, except for three samples in the BCR group, which showed enamel fractures. RRR group tended to fracture mainly in the cervical and middle thirds; however, two samples had vertical root fractures (Table 1).

Cell cytotoxicity assay

The viability by MTT formazan method of RAW 264.7, BD and MTA groups with different extract dilutions, is shown in Figure 3. The “dilution” and “material” factors significantly influenced viability of macrophages ($P < .0001$). In addition, the interaction of the factors had a significant effect on the MTT assay result (two-way ANOVA, $F = 77.97$, $P < .0001$). There were no statistically significant differences in the viability of cells exposed to the 1:1 and 1:2 dilutions, regardless of the material evaluated ($P > .05$). The MTA group showed the highest viability values at the 1:4, 1:8, and 1:16 dilutions ($P < .0001$). For all materials evaluated, the highest percentage of viable cells was found at the 1:16 dilution ($P < .0001$). All groups showed lower viability than the control group ($P < .0001$), except for MTA at the 1:16 dilution, which showed similar values ($P > .05$).

TRAP staining

Compared with negative control RAW 264.7, RANKL-induced RAW 264.7 differentiated into TRAP positive multinucleated osteoclasts, which served as a positive control. However, the number of TRAP-positive osteoclasts decreased after treatment with the extract of each agent ($P < .0001$). The percentage of TRAP-positive

cells was similar among all experimental groups ($P > .05$), except for the BCR group, which showed a lower percentage of osteoclasts than MTA ($P < .0001$) (Figure 4).

DISCUSSION

In the present study, the null hypothesis was rejected as significant differences were found between calcium silicate-based materials in cell culture assays. However, the evaluated materials did not affect the fracture resistance of immature permanent teeth subjected to RRR. Previous studies have emphasized the importance of strengthening teeth that are in the early stages of development [12,14], since dentin deposition on the root canals is not enough to reinforce the teeth, which are consequently predisposed to fracture [20]. However, to date, no studies have evaluated the fracture resistance of immature teeth with simulated RRR. Bone support and the periodontal ligament are fundamental structures for the mechanisms of stress distribution over teeth [21]. Thus, it is important that *in vitro* tests can reproduce, even in adverse situations such as RRR, bone capacity to absorb masticatory load, and support compressive and tangential forces in a fracture resistance test. The RRR was simulated by associating the protocol with cylindrical drill wear and acid demineralization [15], to the selective inclusion of roots with elastomeric impression materials and polystyrene resin. Although the biomechanical characteristics of the polystyrene resin are not the same as those of bone tissue, which represents a limitation of the study, the simulation was performed in the same manner in all groups, seeking to closely resemble the clinical situation.

RRR occurs progressively, and it irregularly advances to the point of complete replacement of the root by bone [3]. Considering this pattern, and the fact that ankylosis is significantly more often on the rounded labial and lingual surfaces than on the flat or proximal concave surfaces [22], the present study simulated ankylosis and RRR only on the palatal root surface. In addition, previous studies have demonstrated that immature maxillary incisors exhibit a high concentration of tensile stress on the palatal root surface, located within the structure that is more prone to failure [14].

The group with simulated ankylosis and RRR had a fracture load similar to that of the normal periodontal ligament group. A previous study showed that teeth embedded directly in polystyrene resin have lower fracture resistance than those in which PDL simulation is performed [21]. PDL acts as a sustaining pad, reducing alveolar

bone stress value [23]. When a force is applied, the tooth moves away from the rotation center compressing periodontal fibers and causes bone distortion in the direction of the root movement [24]. Initially, the resistance of periodontal fibers against tooth displacement is low, but as the tooth is forced within its alveolus, the load progressively increases [21]. In the present study, the periodontal ligament was partially simulated, which could explain the similarity between the groups.

Although it has been reported that bioceramic materials enhance the resistance to horizontal root fractures when used as obturating materials for immature teeth [12], our results suggest that they are not able to increase the fracture strength of immature teeth with RRR. There were also no differences between the MTA plug group and the groups filled with the repair materials. These findings are consistent with those of previous studies [25,26] and may be related to the limited ability of the bioceramic materials to bond to root dentin [27]. In addition, a retrospective clinical study demonstrated that the rate of root fracture in immature teeth treated with MTA apexification and filled with gutta percha or completely filled with MTA exhibited a similar rate of root fracture [28]. However, it is important to emphasize that the results of laboratory studies must be considered with caution and cannot be directly compared to those of clinical studies.

BCR is a bioceramic material that was launched in the second half of 2019 as a ready-to-use proposal, which included calcium silicate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, and a dispersing agent [9]. The absence of significant differences between the fracture values of the BD and BCR groups in this study could be attributed to the chemical/molecular similarities between them. Although, to the best of our knowledge, there are no studies evaluating the fracture resistance of teeth filled with BCR, this material has cytotoxicity [7] and mineralization-inducing [10] abilities similar to those of BD. However, a recent study showed that the compressive strength and adhesion strength of this material are lower than those of BD [29].

Fractures were mainly concentrated in the cervical and middle third of the root. However, it is observed that in the group simulating a normal periodontal ligament, the fracture pattern is more homogeneous compared to the groups with RRR, since the periodontal ligament transfers the stresses produced by load application over the tooth coronal structure to all root surfaces [21]. Therefore, the teeth of the RRR, BCR, BD and MTA plug groups, as they are inserted in the palatal face simulating RRR, presented a more heterogeneous fracture pattern. Despite similar fracture patterns between teeth filled with bioceramics and teeth filled with gutta percha, previous studies have shown that complete root canal filling with calcium silicate-based materials can be advantageous for an optimal biomechanical response [30]. This is because filling with bioceramics creates a monoblock with an

interface that extends between the material and the canal walls [31]. BD is an ideal dentin replacement material, and it has been reported to decrease stress at the cervical root region by keeping the stress in its body while forwarding stress to the mid-coronal region [30].

The murine macrophage lineage RAW 264.7 was used for the induction of osteoclastogenesis since it expresses RANK and can indefinitely grow as osteoclast precursors or differentiate into osteoclasts when co-cultured with RANKL. Although the choice of permanent cell lines as study models is controversial [32], RAW osteoclasts exhibit the essential characteristics of osteoclasts, notably the expression of TRAP and cathepsin K [33]. Furthermore, the use of immortalized cell cultures in a preliminary study assessing new materials, such as BCR, has been recommended in the literature [18,19].

Although previous studies have evaluated the cytotoxicity of these materials, it is known that this parameter is influenced by the cell type evaluated. In the present study, RAW 264.7 exposed to the materials extract at a dilution of 1:16, showed high rates of cell viability (above 80%), demonstrating that this cell line can be sensitive to the components present in the materials, differing from other studies using the same methodology, in which the human dental pulp cells presented viability greater than 80% even at 1:1 dilutions [8,34]. Another study evaluated the biocompatibility and osteogenesis-inducing potential of BD, BCR, and MTA in cultures of human osteoblastic cells (SAOS-2) demonstrating similar cell viability rates to those of the control [10]. Different cell lineages may present divergent biological responses to each other, highlighting the relevance of cell culture studies [10]. In addition to the specificities of each cell line, the setting time, direct or indirect contacts, preparation of the material extract, and elutes containing toxic elements from the cement composition can all directly influence the biocompatibility and bioactivity of the material [10], preventing direct comparisons between studies

RAW 264.7 osteoclasts were confirmed to express active TRAP enzyme, a key marker involved in osteoclastic bone resorption. The results of this study showed TRAP-positive multinucleated osteoclasts were induced at the highest rate in the positive control, which matched previous results [35]. All calcium silicate cements evaluated showed a reduction in the number of differentiated osteoclasts. This is in agreement with recent studies, which have reported that calcium silicate cements prevent osteoclastogenesis and bone resorption, consequently enhancing bone remodeling [35,36]. Significant differences were not observed in the number of osteoclasts between BD and MTA, as observed in other studies [35,37]. This may be associated with the similarity in the composition of the two materials [7]. Despite these results, it is important to emphasize that structural differences in osteoclasts, such as osteoclast size, the number of nuclei per cell, and the relationship between osteoclast

contacts with the bone surface, are parameters directly associated with resorptive activity [38]. With regard to this, laboratory studies in rats have demonstrated an accentuated bone loss observed in regions of furcation perforation sealing when using BD, compared to that in MTA, even without statistical differences in the number of osteoclasts [37].

BCR, in turn, was the material that presented the lowest number of osteoclasts, which may be associated with the lower concentration of calcium present in its structure [7] and the lower amount of calcium ions released in solution compared to MTA and BD [8]. It is known that high extracellular calcium concentration modulates osteoclast differentiation and function, and osteoclastic bone resorption is directly regulated by calcitonin and, locally, by ionized calcium (Ca^{2+}) generated as a result of osteoclastic bone resorption [39]. Parallel to this, high concentrations of extracellular calcium cause cellular changes that markedly inhibit the TRAP enzyme and bone resorption [19].

CONCLUSIONS

Under the conditions of this study, it can be concluded that the treatment options used to treat nonvital immature teeth with RRR do not strengthen them and provide the same resistance to fracture. BD, MTA, and BCR showed inhibitory effects on osteoclast differentiation, with BCR group showing better results.

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Table

Table 1: Fracture modes of the specimens after load tests.

Experimental group	Mode of fracture of the samples			
	I. Enamel fracture	II. One-third cervical root fracture	III. One-third middle root fracture	IV. Vertical root fracture
PL	0	6	6	0
RRR	0	7	3	2
MTA Plug	0	4	8	0
BCR	3	3	6	0
BD	0	5	7	0

FIGURES AND LEGENDS

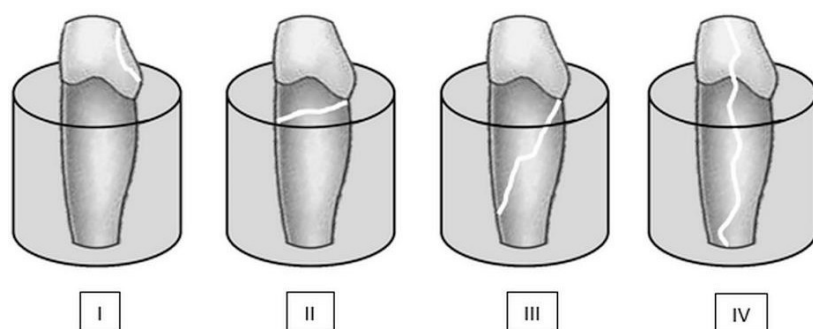


Figure 1: Classification of the fracture modes – (I) enamel fracture; (II) one-third cervical root fracture; (III) one-third middle root fracture; (IV) vertical root fracture.

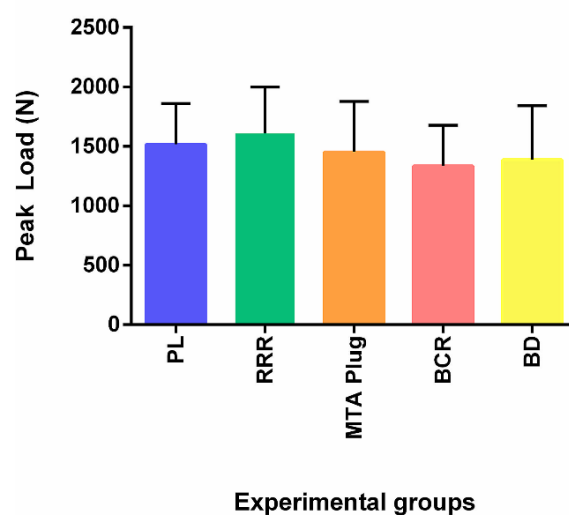


Figure 2: Mean peak load (N) and standard deviations required to cause root fracture for each experimental group. PL, normal periodontal ligament; RRR, replacement root resorption; MTA plug, apical plug with MTA and root canal filling with BioC sealer and gutta-percha; BCR, BioC repair root canal filling; BD, Biodentine root canal filling.

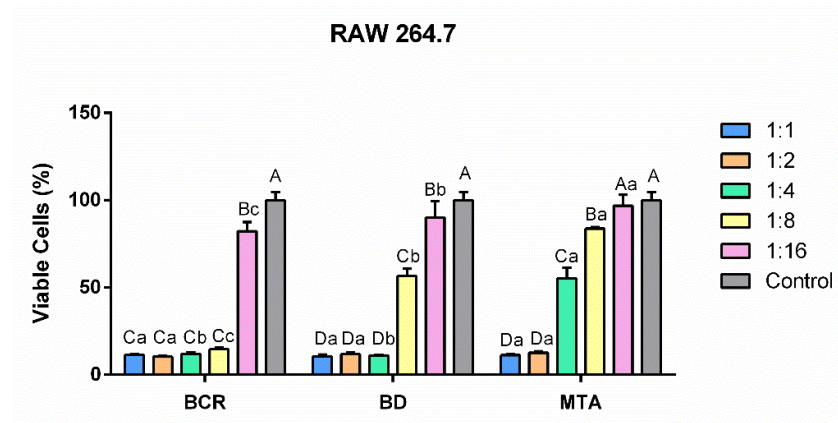


Figure 3: Cell viability percentage of RAW 264.7 after exposure to extracts according to material tested and dilution by the MTT formazan method. BD: Biodentine exposure; BCR: Bio-C Repair exposure; MTA: MTA exposure. Capital letters indicate comparison amongst different dilutions of extracts and the control group for each material. Lowercase letters indicate comparisons of a material amongst the different dilutions. Two-way ANOVA and Tukey's test ($p < .05$).

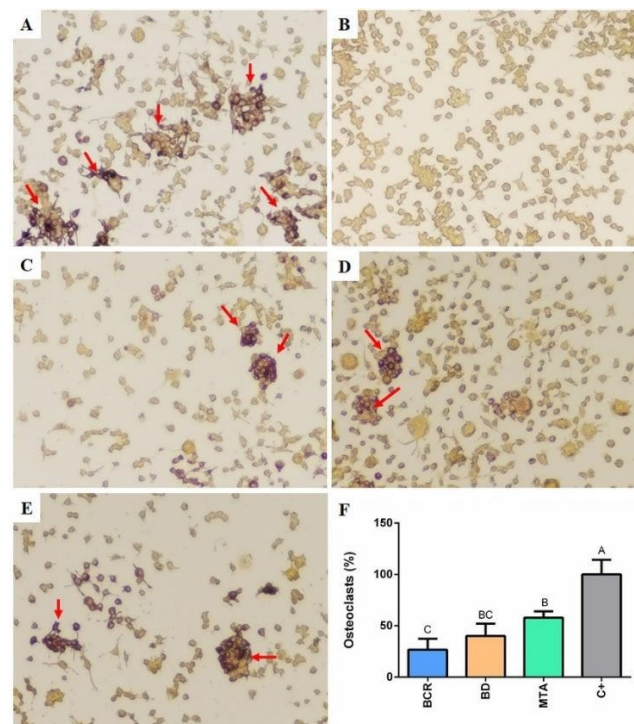


Figure 4: TRAP staining (A) Positive control (RANKL); (B) Negative control (α -MEM); (C) Cells treated with BioC repair extract; (D) Cells treated with Biodentine extract; (E) Cells treated with MTA extract. TRAP-positive multinucleated cells (arrow); x10 magnification. (F) TRAP activity: Capital letters indicate comparison amongst materials tested.