Original Research Article

Histological response and bone neoformation of bioceramic cements in rat calvaria

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ABSTRACT

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| **Aims:** This study evaluated the level of inflammatory infiltrate, vasodilation, granulation tissue, and bone regeneration of two repair materials: White MTA (Angelus, Londrina, Brazil) and Bio-C Repair (Angelus, Londrina, Brazil) in rat calvaria.  **Study design:** This is an in vivo study with a sample of adult Wistar rats, approximately three months old and weighing between 250 and 300 grams.  **Place and Duration of Study:** The selected analysis periods were 7, 30, and 60 days, corresponding to the following divisions: Group 1 (7 days): 5 rats; Group 2 (30 days): 5 rats; Group 3 (60 days): 5 rats.  **Methodology:** A total of 15 Wistar rats were divided into three groups (n=5) based on the euthanasia period (G7, G30, and G60 days). After sedation, two bone defects, approximately 1.5 mm in depth and 5 mm in diameter, were created in the calvaria of each animal. After euthanasia, the calvaria were removed, histologically prepared in 6 µm sections, and stained with hematoxylin-eosin (H/E). The material was quantitatively analyzed using the Statistical Package for the Social Sciences (SPSS), adopting a significance level of 5% (p < 0.05).  **Results:** At 7 days, both materials showed an acute inflammatory response. After 30 days, there was a reduction in the inflammatory process in both bioceramics cements. At 60 days, both White MTA and Bio C Repair did not induce severe inflammation. During this period, Bio C Repair showed greater bone tissue formation, with inflammatory infiltrate levels at 60 days being equivalent between the two materials.  **Conclusion:** Both bioceramics cements preserved the integrity of the bone tissue and stimulated bone neoformation. |

*Keywords: Dental Cements. Skull. Biocompatible Materials. Bone Regeneration. Material Testing.*

1. INTRODUCTION

Bioceramics are defined as biocompatible ceramic materials, containing alumina, zirconia, bioactive glass, glass ceramics, hydroxyapatite, resorbable calcium phosphate, and calcium silicate in their composition (Dong; Xu, 2023). These materials have been used in medicine since the 1960s as substitutes for joints, bone plates, bone cement, artificial ligaments and tendons, blood vessel prostheses, heart valves, skin repair devices (artificial tissue), cochlear substitutes, and contact lenses (Tomer et al., 2020).

In dentistry, their use primarily occurs in endodontics since the early 1990s, with the development of Mineral Trioxide Aggregate (MTA) by Mahmoud Torabinejad, the first to use a bioceramic as an apical repair material (Margunato et al., 2015). MTA is formed by the combination of Portland cement with the addition of bismuth oxide as a radiopacifier (Guimarães et al., 2018). Due to its pioneering nature, MTA is the most thoroughly studied bioceramic material, considered the gold standard in endodontic applications for its physicochemical properties and biological characteristics (Song et al., 2020).

Based on its interactions with surrounding tissues, bioceramics can be classified as bioinert (which do not interact with biological tissues, such as alumina and zirconia), bioactive (which interact with adjacent tissues, such as bioactive glass), and biodegradable (which are absorbed or replaced by tissues, such as calcium silicates) (Motwani et al., 2021). The most commonly used bioceramics in endodontics are those based on calcium silicate, which have a wide range of applications, being used as cements, root repair materials, root canal sealers, and filling materials (Raghavendra et al., 2017).

Their superior biocompatibility, potential to increase root strength after filling, antibacterial properties, and sealing capability make them extremely advantageous in pulp and root therapy (Rawat et al., 2022). The strength of calcium silicate-based materials is due to the tricalcium silicate (Ca3SiO5) component. This reacts with water to form hydrated calcium silicate, which polymerizes to form networks, contributing to increased mechanical strength and self-setting of the material over time (Madadi; Wei, 2022). The dicalcium silicate (Ca2SiO4) component also reacts with water, forming calcium hydrate networks, but at a slower rate than tricalcium silicate (Jimenez-Sanchez; Segura-Egea; DíazCuenca, 2020).

When hydrated, either during manipulation or through contact with bodily fluids, bioceramics react by producing compounds (such as hydroxyapatite) that have the ability to induce regenerative reactions in the human body (Tanvir et al., 2024). When in contact with bone, for example, the mineral hydroxyapatite exhibits osteoconductive properties, resulting in the formation of new bone at the interface (Palczewska-Komsa; Kaczor-Wiankowska; Nowicka, 2021).

According to Ashique et al. (2002), the calvarial region is where the intramembranous ossification process can be observed through immune receptors, a factor directly involved in bone condensation. In this context, this study evaluated the behavior of MTA Angelus White® and Bio C Repair in tissue response and bone repair using the critical defect model in rat calvaria.

2. material and methods

**2.1 Ethical aspects and sample preparation**

The research was approved by the Animal Ethics Committee (Ceua) of the University of Fortaleza (CEP/UNIFOR) under opinion No. 8477130819. This is an in vivo study with a sample of 15 adult male Wistar rats, approximately three months old and weighing between 250 and 300 grams.

The animals were kept under care and supervision for the required period at the UNIFOR Animal Facility, each housed individually with the essentials for habitation. The cages were cleaned and filled with appropriate bedding, and the animals were fed solid food with ad libitum access to water. The environment maintained a regulated temperature (21ºC to 25ºC), as well as controlled lighting, with 12 hours of darkness and an equal period of artificial light.

**2.2 Materials and Groups**

The animals were divided into three groups based on the progression of the inflammatory process and tissue repair: White MTA (Angelus, Londrina, Brazil), Bio-C Repair (Angelus, Londrina, Brazil), and control. The selected analysis periods were 7, 30, and 60 days, corresponding to the following divisions: Group 1 (7 days): 5 rats; Group 2 (30 days): 5 rats; Group 3 (60 days): 5 rats (list 1). To facilitate identification, the animals from each group were marked with a non-toxic pen on their tails. This strategy ensured proper control and tracking during the experiment. At each calvaria, three cavities were made: one for the control group and two for the materials under study.

list 1: Experimental groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Bioceramic material | Group 1 – 7 days | Group 2 -30 days | Group 3 – 60 days | Total |
| MTA | n=5 | n=5 | n=5 | 15 |
| Bio-C Repair | n=5 | n=5 | n=5 | 15 |
| Control Group | n=5 | n=5 | n=5 | 15 |

**2.3 Sample Preparation**

Before creating the cavities for material insertion, the rats were anesthetized with a combination of Ketamine Hydrochloride (Virbac, São Paulo, Brazil) at a dosage of 0.08 mL/100 g of body weight and Xylazine Hydrochloride (Virbaxyl 2%, Virbac, São Paulo, Brazil) at a dosage of 0.04 mL/100 g of body weight. The mixture was administered intraperitoneally using an insulin syringe and a 6 x 0.25 mm needle (Sol Millennium, São Paulo, Brazil).

After confirming the anesthetic effect, clinically assessed by performing a pressure test on the lower limbs, the animals underwent trichotomy in the calvarial region. The area was cleaned with povidone-iodine antiseptic (Rioquímica, São Paulo, Brazil), and then an incision was made using a #15 scalpel blade (Solidor, São Paulo, Brazil).

A bicoronal incision, involving both skin and muscle, was made in a triangular shape, approximately 3 cm in lateral length, preserving the base intact. This shape ensured adequate access to the cranial structures while respecting the depth limit down to the periosteum. After the incision, the tissues were carefully separated to expose the bone tissue, allowing the experimental procedure to be performed (Figure 1).

**Figure 1. Representation of the access surgery and positioning of the materials in the calvarial region of the rats.**



In the bone tissue, two cavities were created in the parietal bone, located near the apex of the coronal suture, each approximately 1.5 mm in depth and 5 mm in diameter. The procedure was performed using an LS drill (MIG, Campo Largo, Brazil) in an indirect and continuous manner with saline solution to avoid overheating. After cavity formation, the defects were filled with the repair cements previously prepared on a glass plate, following the manufacturer's specifications, using a sterile standard 24 (Golgran, São Caetano do Sul, Brazil).

During the procedure, strict care was taken to avoid unwanted injuries, such as damage to the dural layer. After filling the defects using a Lucas curette #21 (Golgran, São Caetano do Sul, Brazil) without overflow, the bone tissue was covered by the periosteum and the soft tissues that were displaced during the incision and dissection. Suturing was performed at the incision vertices using Vicryl sutures (Johnson & Johnson, São Paulo, Brazil) and 4.0 silk sutures (Johnson & Johnson, São Paulo, Brazil).

In the postoperative period, the animals received a single dose of antibiotic (Pentabiótico veterinary small animal, Zoetis Dodge, São Paulo, Brazil, subcutaneously: 0.03 mL/kg) and were treated with the opioid analgesic Tramadol (Ketoflex, Mundo Animal, São Paulo, Brazil, intramuscular: 1.5 mL/kg) every 12 hours for 72 hours, ensuring proper pain management and infection prevention.

The sacrifice of the animals was performed according to the established periods for the G7, G30, and G60 groups. For euthanasia, a lethal dose of anesthetic was administered intraperitoneally using an insulin syringe with a 6 x 0.25 mm needle (Sol Millennium, São Paulo, Brazil). The anesthetic solution consisted of a mixture of Ketamine Hydrochloride (Virbac, São Paulo, Brazil) and Xylazine Hydrochloride (Virbaxyl 2%, Virbac, São Paulo, Brazil) in a 3:1 ratio, with a total concentration of 400 mg/kg.

Subsequently, the animals were subjected to decapitation using a guillotine. The cranial samples were immediately placed in a 10% paraformaldehyde solution for 48 hours to ensure tissue preservation and halt any potential hemorrhages. The bodies were properly discarded in accordance with specific regulations for biological waste. Using a No. 15 scalpel blade (Solidor, São Paulo, Brazil) and appropriate scissors, the calvariae were carefully isolated and stored in glass tubes for further processing and histological slide preparation.

**2.4 Histological Analysis**

The samples underwent a decalcification process using 10% nitric acid, followed by fixation in 10% formalin. They were then washed in absolute alcohol and xylene for 1 hour and embedded in paraffin at 60°C. After this step, the samples were embedded in paraffin blocks and subjected to coronal sectioning with a thickness of 2 µm. The sections were placed in an oven at 60°C for 40 minutes and washed again with absolute alcohol and xylene.

The slides were stained using Hematoxylin and Eosin (H&E) and mounted with Entellan and coverslips. Subsequently, they were examined under an optical microscope to select the best samples. The selected slides were digitized using the Pannoramic Desk scanner (3D HISTECH, Budapest, Hungary) and analyzed with the Case Viewer software (3D HISTECH, Budapest, Hungary).

Histological images were captured at scales of 100 µm, 200 µm, 1000 µm, and 2000 µm, allowing for a detailed analysis of tissue alterations. The histological evaluation was conducted by a calibrated pathologist blinded to the experimental groups, based on criteria described in the classification tables for bone and cellular reactions established by Kui et al. (2014) and Akhavan et al. (2016).

The inflammatory infiltrate was assessed using a scoring system ranging from 1 to 4: score 1 indicated the absence of inflammatory infiltrate; score 2 represented mild inflammatory infiltrate; score 3 corresponded to moderate inflammatory infiltrate; and score 4 characterized intense inflammatory infiltrate. The degree of vasodilation was classified on a scale of 1 to 3, with score 1 indicating mild vasodilation, score 2 representing moderate vasodilation, and score 3 indicating the presence of an abscess.

The presence of reparative tissue was categorized into three levels using a scoring system: score 1 identified fibrous tissue, score 2 characterized granulation tissue, and score 3 indicated signs of necrosis. Bone tissue progression was evaluated based on a scoring system from 1 to 3, where score 1 indicated mild to moderate bone regeneration, score 2 represented bone destruction, and score 3 described extensive bone destruction.

**2.5 Statistical Analysis**

The data from the histological analysis were quantitatively organized in Microsoft Excel spreadsheets and analyzed using the Statistical Package for the Social Sciences (SPSS), version 24 (IBM, Armonk, New York, USA). Descriptive statistics were applied to quantitative variables, using absolute and percentage frequencies. Comparisons between variables were performed using Fisher’s exact test. The results were presented in graphs and tables, and a significance level of 5% (p < 0.05) was adopted for inferential tests.

3. results

Among the obtained results, for Bio-C Repair, in the G7 group, 40% of cases exhibited mild and intense inflammatory infiltrate, while 20% showed moderate infiltrate. In the G30 group, 60% of cases displayed mild infiltrate, and 40% presented moderate infiltrate, with no occurrence of intense infiltrate or absence of cells. In the G60 group, 40% showed an absence of cells and mild infiltrate, while 20% had moderate infiltrate. No statistically significant differences were observed between the Bio-C Repair groups (p = 0.524 for G7, p = 0.454 for G30, and p = 0.683 for G60).

Regarding MTA, in the G7 group, most cases (75%) exhibited moderate infiltrate, while 25% showed intense infiltrate. In the G30 group, there was a balanced distribution between mild (50%) and intense (50%) infiltrate. In the G60 group, moderate infiltrate was observed in 60% of cases, while 20% exhibited mild and intense infiltrate. As with Bio-C Repair, no statistically significant differences were found between the MTA groups (p = 0.134).

In the control group, 100% of cases in G7 showed an absence of cells. In G30, 50% of cases exhibited mild infiltrate, while the other 50% showed an absence of cells. In G60, all cases (100%) displayed mild infiltrate. Thus, for both Bio-C Repair and MTA, the inflammatory infiltrate profile varied across experimental time points, but without statistically significant differences (Table 1).

**Table 1. Inflammatory Infiltrate**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Material | Group | Cell absence | Discreet inflammatory infiltrate | Moderate inflammatory infiltrate | Intense inflammatory infiltrate | *P value\** | |
|  |  | N | % | N | % | N | % | N | % |  | |
| Bio-C | G7  G30  G60 | 0 | 0,0  0 | 0,0  2 | 40,0 | 2 | 40,0  3 | 60,0  2 | 40,0 | 1 | 20,0  2 | 40,0  1 | 20,0 | 2 | 40  0 | 0,0  0 | 0,0 | .454¹ | .524²  .079²  .683² |
| MTA | G7  G30  G60 | 0 | 0,0  0 | 0,0  0 | 0,0 | 3 | 60,0  1 | 20,0  3 | 60,0 | 2 | 40,0  0 | 0,0  1 | 20,0 | 0 | 0,0  4 | 80,0  1 | 20,0 | .134¹ |  |
| Control | G7  G30  G60 | 0 | 0,0  0 | 0,0  0 | 0,0 | 1 | 25,0  1 | 50,0  2 | 100,0 | 3 | 75,0  0 | 0,0  0 | 0,0 | 0 | 0,0  1 | 50,0  0 | 0,0 |  |  |

*\*Fisher's exact test; ¹Compares the different groups according to type of material; ²Compares the different types of material according to groups.*

Regarding vasodilation in the Bio-C Repair groups, in G7, 40% of cases exhibited mild vasodilation and 60% moderate vasodilation, with no recorded abscesses. In the G30 group, 80% of cases showed mild vasodilation, while 20% presented moderate vasodilation. In G60, mild vasodilation also predominated (80%), followed by moderate vasodilation in 20%, with no abscess formation. No statistically significant differences were observed among the Bio-C Repair groups (p = 0.167 for G7, p = 0.286 for G30, and p = 0.524 for G60).

For MTA, in the G7 group, 40% of cases exhibited moderate vasodilation, while 60% presented abscesses, with no cases of mild vasodilation. In the G30 group, 20% of cases displayed mild vasodilation, while 40% presented moderate vasodilation and abscesses. In the G60 group, 40% of cases showed mild and moderate vasodilation, while 20% exhibited abscesses. Similar to Bio-C Repair, no statistically significant differences were found among the MTA groups (p = 0.765).

The results indicate that both Bio-C Repair and MTA showed variations in vasodilation patterns across experimental time points, with a higher prevalence of mild vasodilation in Bio-C Repair and moderate vasodilation or abscess formation in MTA. In the control group, moderate vasodilation was more frequent in the G7 and G30 groups, while mild vasodilation predominated in G60 (Table 2).

**Table 2. Vasodilation**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Material | Group | Discreet vasodilation | Moderate vasodilation | Abscess | *P value\** | |
|  |  | N | % | N | % | N | % |  | |
| Bio-C | G7  G30  G60 | 2 | 40,0  4 | 80,0  4 | 80,0 | 3 | 60,0  1 | 20,0  1 | 20,0 | 0 | 0,0  0 | 0,0  0 | 0,0 | .500¹ | .167²  .286²  .524² |
| MTA | G7  G30  G60 | 0 | 0,0  1 | 20,0  2 | 40,0 | 2 | 40,0  2 | 40,0  2 | 40,0 | 3 | 60,0  2 | 40,0  1 | 20,0 | .765¹ | -  -  - |
| Control | G7  G30  G60 | 0 | 0,0  0 | 0,0  2 | 100,0 | 4 | 100,0  1 | 50,0  0 | 0,0 | 0 | 0,0  1 | 50,0  0 | 0,0 | -  -  - | -  -  - |

*\*Fisher's exact test; ¹Compares the different groups according to type of material; ²Compares the different types of material according to groups.*

At the 7-day mark, the bone regeneration pattern was 40% for both bioceramics. However, MTA exhibited 60% extensive bone destruction, whereas Bio-C Repair showed 60% mild to moderate bone destruction. At 30 days, both materials were predominantly classified under the criterion of mild to moderate bone destruction, though MTA still presented cases of extensive bone destruction. At 60 days, Bio-C Repair showed better results, with 60% bone regeneration. MTA also demonstrated improvement compared to the 30-day post-surgery evaluation, although 20% of cases still presented extensive bone destruction (Table 3).

**Table 3. Bone Regeneration**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Material | Group | Bone regeneration | Discrete and moderate bone destruction | Extensive bone destruction | *\*P value* | |
|  |  | n/% | n/% | n/% |  | |
| Bio-C | G7  G30  G60 | 2 | 40,0  0 | 0,0  3 | 60,0 | 3 | 60,0  5 | 100,0  2 | 40,0 | 0 | 0,0  0 | 0,0  0 | 0,0 | .251¹ | .095²  1.000²  .524² |
| MTA | G7  G30  G60 | 2 | 40,0  0 | 0,0  1 | 20,0 | 0 | 0,0  4 | 80,0  3 | 60,0 | 3 | 60,0  1 | 20,0  1 | 20,0 | .134¹ |  |
| Control | G7  G30  G60 | 0 | 0,0  0 | 0,0  0 | 0,0 | 2 | 50,0  1 | 50,0  2 | 100,0 | 2 | 50,0  1 | 50,0  0 | 0,0 |  |  |

*\*Fisher's exact test; ¹Compares the different groups according to type of material; ²Compares the different types of material according to groups.*

At the 7-day mark, both materials showed the absence of fibrous tissue; however, MTA exhibited 60% signs of necrosis. At 30 days, MTA still presented 40% necrosis and 60% granulation tissue. In contrast, Bio-C Repair showed 80% granulation tissue, with better results in fibrous tissue formation (20% of the samples). At 60 days, both materials demonstrated improvement. MTA presented 40% fibrous tissue and 60% granulation tissue, while Bio-C Repair showed 60% fibrous tissue and 40% granulation tissue. Notably, at no time point did the Bio-C Repair bioceramic exhibit signs of necrosis (Table 4).

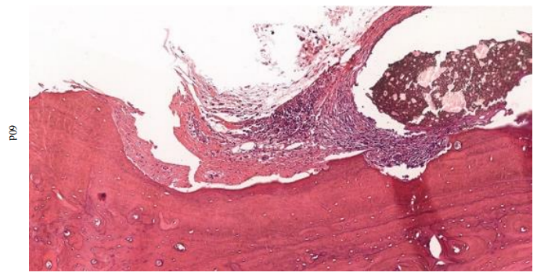
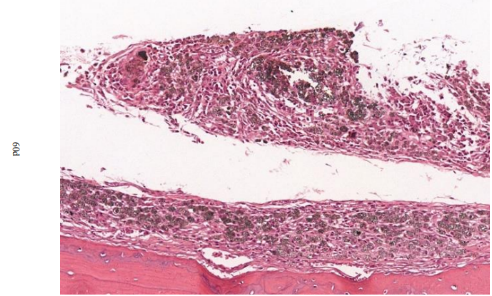
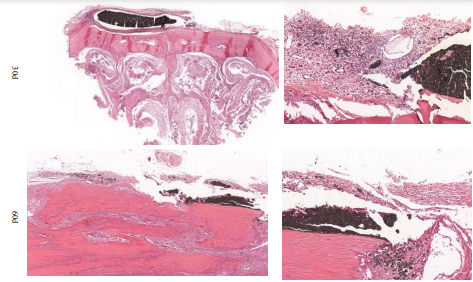
**Table 4. Granulation Tissue**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Material | Group | Fibrous tissue | Granulation fabric | Signs of necrosis | *\*P value* | |
|  |  | N | % | N | % | N | % |  | |
| Bio-C | G7  G30  G60 | 0 | 0,0  1 | 20,0  3 | 60,0 | 5 | 100,0  4 | 80,0  2 | 40,0 | 0 | 0,0  0 | 0,0  0 | 0,0 | . 231¹ | .167²  .444²  .000² |
| MTA | G7  G30  G60 | 0 | 0,0  0 | 0,0  2 | 40,0 | 2 | 40,0  3 | 60,0  3 | 60,0 | 3 | 60,0  2 | 40,0  0 | 0,0 | .190¹ |  |
| Control | G7  G30  G60 | 0 | 0,0  0 | 0,0  0 | 0,0 | 3 | 75,0  2 | 100,0  2 | 100,0 | 1 | 25,0  0 | 0,0  0 | 0,0 |  |  |

*\*Fisher's exact test; ¹Compares the different groups according to type of material; ²Compares the different types of material according to groups.*

The following histological images illustrate the observed conditions in bone tissues, including the presence of inflammatory infiltrate, vasodilation, repair tissue formation, and bone evolution. These characteristics are crucial for evaluating the biocompatibility and bone regeneration potential of each material.

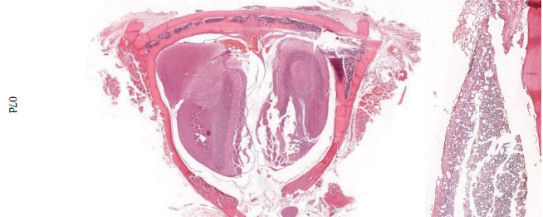
**Figure 2. Compiled Histological Images of Bio C Repair and MTA Groups**

 Bio-C Repair

MTA

**G30**

**G07**



**G60**

G7: Significant presence of inflammatory cells and a certain degree of tissue reaction to the material, characterizing an early phase of acute or subacute inflammatory response. G30: Reduction in the inflammatory response. The inflammatory infiltrate seems to be better controlled, with more organized tissue structure. G60: After 60 days. Greater tissue organization with little or no sign of inflammatory infiltrate, and no evident signs of necrosis or abscesses.

**4. DISCUSSION**

This study evaluated the intensity level of the inflammatory infiltrate, vasodilation, granulation tissue, and bone regeneration in White MTA (Angelus, Londrina, Brazil) and Bio-C Repair (Angelus, Londrina, Brazil). Although bone defects in rat calvaria are widely used in studies on materials employed in dentistry (Vajgel et al., 2014; Schmidt et al., 2019; Zhang et al., 2020; De Oliveira Junior et al., 2021; Camacho-Alonso et al., 2022; Fazeli et al., 2023; Frigerio et al., 2023), their application in specific methodologies for evaluating repair materials is still limited (Kim; Ku, 2023).

In this context, Camacho-Alonso et al. (2022) evaluated new bone formation in critical-sized mandibular bone defects (CSBDs) in healthy, diabetic, osteoporotic, and diabetic-osteoporotic rats, filled with bioceramics with or without bone marrow-derived mesenchymal stem cells (BMSCs). The results showed that, across all groups, CSBDs filled with bioceramics + BMSCs exhibited greater radiological bone union, bone mineral density (BMD), histological bone union, and higher expression of vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP-2) compared to CSBDs treated with bioceramics alone.

Bioceramics stand out among repair materials, mainly due to their biological properties, notably biocompatibility (Shekhawat et al., 2021). Notably, most of the research evaluating the biological properties of these materials consists of in vitro studies (El-Hadad et al., 2017; Aghaei et al., 2014; Szymonowicz et al., 2017). In vivo studies often prefer the implantation of polyethylene tubes with the material in rat subcutaneous tissue (Abou ElReash et al., 2019; Cosme-Silva et al., 2019; Ferreira et al., 2019; Talabani et al., 2020).

The choice of these methodological parameters between polyethylene tubes and rat calvaria is important since the inflammatory response and the ability to stimulate bone growth are crucial factors for the success of bone repair procedures, as demonstrated by Silva et al. (2015) The justification for using the calvaria model, where the biomaterial is in direct contact with the bone bed, lies in its ability to provide a more realistic evaluation of the interaction between the biomaterial and the bone. This is particularly important when assessing the use of bioceramic materials for repair, as it allows for a more accurate understanding of their biocompatibility, osteointegration, and the overall bone response to the material. In this regard, Klein-Junior et al. (2021) highlights that MTA is more biocompatible in the process of stimulating bone tissue.

The majority of studies evaluating the biocompatibility of repair materials used in endodontics employ the rat experimental model, but they assess the inflammatory response in subcutaneous connective tissue (Abou El Reash et al., 2019; Cosme-Silva et al., 2019). The cavities created in rats in the present study had approximately 5mm in diameter, considered to be the size that characterizes a critical defect, which is advantageous for the analysis because, at this dimension, spontaneous regeneration does not occur during the experimental period. Bone defects in the calvaria have been widely used as a model to study the response and regeneration of bone tissue, including experiments with bioceramics (Kim et al., 2023).

The literature reports adversities during the creation of bone defects, such as necrosis, dura mater perforation, and bone overheating during defect creation (Camacho-Alonso et al., 2022; Fazeli et al., 2023; Frigerio et al., 2023; Kim; Ku, 2023; Ferreira et al., 2023). Another method for testing new bone formation was performed by Gandolfi et al. (2017) in the tibia of rabbits, following a protocol with non-critical bone defects measuring 2x2mm. According to Galal et al. (2019), intimate contact between the material and bone tissue is essential for the regenerative process to occur. In the present study, the proposed method was used to test the direct reaction of the material with bone tissue, simulating a treatment situation for perforation, which justifies the idea of directly placing MTA on the cavities.

Few studies in the literature evaluate MTA at different periods, such as 7 and 60 days, associated with critical defects. However, literature mentions bone repair and regeneration processes over these time periods through in vivo studies (Ahuja et al., 2020; Mythraiye et al., 2019). Thus, the present study defined 7, 30, and 60 days as the parameters for histological evaluation. Galal et al. (2019), who conducted a study on rabbit mandibles with critical defects but for a shorter period of 7 days compared to the present study, highlighted the need to extend the time for more reliable results.

On the other hand, Gomes-Filho et al. (2011) used 7, 14, and 21 days with polyethylene tubes in rat alveoli. Silva et al. (2015) worked with 15 and 60 days on rat calvaria, with the material in direct contact with bone tissue. Tronstad et al. (1981) explained in their histological findings at the 7-day period that the presence of a superficial necrotic layer was due to the material's ability to alkalinize the adjacent tissues, a finding also observed in the present study, which partially justifies this necrotic process.

The results also showed that the presence of particles from the repair cement may complicate the bone repair process, but the resorption of these particles may increase over time, leading to more similar outcomes. In this context, the authors Hogan et al. (2014) highlighted the importance of evaluating the effectiveness of these materials in critical defects, as this method is more reliable in assessing bone tissue formation.

In this regard, Gomes-Filho et al. (2011), in their research using polyethylene tubes in rat alveoli, observed mineralized tissue formation in the tubes filled with MTA Angelus at the 7-day period. The material was responsible for stimulating areas of dystrophic calcification. After 60 days, the authors noted a reduction in the inflammatory infiltrate when MTA Angelus was used in polyethylene tubes, which can also be observed in the present study. Similar data were observed in the study by Silva et al. (2015), where, 60 days after the surgical procedure, greater filling of the defect with osteoid tissue was observed, especially along the cavity borders in the Bio-C Repair group.

Based on this discussion, it can be observed that both materials present themselves as viable strategies for bone repair in critical bone defects over a 60-day evaluation period, which supports findings in the scientific literature. However, it is important to note that this study has its limitations, such as the use of an animal model and the analysis over short periods of time. Therefore, the results should be interpreted with caution, serving as an initial basis for future research and clinical studies with larger sample sizes and longer evaluation periods to observe the consolidation of bone tissue repair with these biomaterials. Although both cements show regenerative potential, further studies are recommended to evaluate their long-term properties.

5. Conclusion

Both bioceramics cements demonstrated efficacy in preserving the integrity of the bone tissue and promoting bone neoformation within 60 days, showing a positive tissue response. Bio-C Repair stood out due to its progressive reduction in inflammatory infiltrate and the induction of bone formation, suggesting a more efficient healing process and successful adaptation. On the other hand, MTA also showed significant improvement compared to the 30-day post-surgery period, although it is important to highlight that 20% of bone destruction was observed, which warrants attention for future analyses.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

The authors declare that the AI technology, GPT-4o, was used exclusively to rewrite and edit this manuscript, with the specific purpose of correcting the English grammar of the translated text, which was originally written in Brazilian Portuguese. No additional information was inserted into the text; the AI's role was limited to checking and refining the accuracy of the translation.

Ethical approval

All authors declare that all experiments were reviewed and approved by the appropriate ethics committee and therefore were performed in accordance with ethical standards.

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