Evaluation of Microleakage by Gas Permeability and Marginal Adaptation of MTA and Biodentine™ Apical Plugs: *In Vitro* Study

Evaluación de la microfiltración por permeabilidad de gas y adaptación marginal de tapones apicales de MTA y Biodentine™: Estudio *in vitro*

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ABSTRACT

The endodontic treatment of teeth with incomplete development is always a complex task. Nowadays, biomaterials such as bioceramics offers promising clinical evidence that supports its use. However, the standardization of its use for apexification purpose still needs a deeper understanding of the materials' behavior. The aim of this investigation was to evaluate the marginal adaptability and microleakage by gas permeability of MTA and Biodentine™ apical plugs in an *in vitro* model. Materials and methods: Twenty-four single rooted human teeth were selected according to previously stablished inclusion criteria. All samples were prepared obtaining standard cylindrical internal canals with a diameter of 1.3 mm. Root canals were gently rinsed using 5.25% sodium hypochlorite and EDTA 17%. The apical 3mm and remaining coronal dental structure were sectioned to obtain 10mm roots. Roots were randomly assigned to 3 different groups as follows: GROUP A: MTA (n=10), GROUP B: Biodentine™ (n=10) and Group C: Control (positive n=1, negative n=3). MTA and Biodentine™ were prepared according to manufacturer's indications, and apical plugs of 4mm were passively placed in the correspondent teeth. All samples were stored in saline solution for 7 days at 37°C before evaluation. Samples were mounted in cylindrical sample-holders using epoxy resin. Microleakage was evaluated with an automatic permeability detector that calculates nitrogen diffusion between the material-root interphase. After microleakage evaluation, the samples were recovered and analyzed by scanning electron microscopy (SEM). Microleakage results were analyzed using Chi-square and adaptation was evaluated with a descriptive analysis. Results: None of the evaluated materials completely avoided the nitrogen microleakage (positive leakage of 10% and 20% of samples for MTA and Biodentine™ respectively); with no statistical significant difference between groups ($p=0.527$). All apical plugs showed good adaptation under SEM, at 30x, 200x, 1000x and 2500x; with microscopical structures similar to previous reports. Conclusions: Both bioceramics behave similar when used as apical barriers to avoid permeability, with acceptable marginal adaptation. Further *in vivo* studies are needed to validate these results.

KEYWORDS

Bioceramics; MTA; Biodentine™; Microleakage; Adaptation; Gas permeability.

RESUMEN

El tratamiento endodóntico de dientes con desarrollo incompleto es siempre una tarea compleja. Hoy en día, biomateriales como las biocerámicas ofrecen una evidencia clínica prometedora que apoya su uso. Sin embargo, la estandarización de su uso para fines de apexificación todavía necesita una comprensión más profunda del comportamiento de los materiales. El objetivo de esta investigación fue evaluar la adaptabilidad marginal y microfiltración por permeabilidad de gas de los tapones apicales de MTA y Biodentine™ en un modelo *in vitro*. Materiales y métodos: Veiticuatro dientes humanos uniradiculares fueron seleccionados meticulosamente según criterios de inclusión previamente establecidos. Todas las muestras fueron preparadas con canales cilíndricos internos estandarizados de 1,3 mm de diámetro. Los conductos radiculares fueron gentilmente lavados con hipoclorito de sodio al 5,25% y EDTA al 17%. La estructura dental apical de 3 mm y la coronal restante se seccionó para obtener raíces de 10 mm de longitud. Las raíces se asignaron aleatoriamente a 3 grupos diferentes de la siguiente manera: GRUPO A: MTA (n = 10), GRUPO B: Biodentine™ (n = 10) y Grupo C: Control (n = 1 positivo, n = 3 negativos). El MTA y Biodentine™ se prepararon de acuerdo con las indicaciones de los fabricantes, y se colocaron pasivamente los tapones apicales de 4 mm en los dientes correspondientes. Todas las muestras se almacenaron en solución salina durante 7 días a 37ºC antes de la evaluación. Las muestras se montaron en porta-muestras cilíndricos utilizando resina epóxica. La microfiltración se evaluó con un detector de permeabilidad automática que calcula la difusión de nitrógeno entre la interfase material-raíz. Después de la evaluación de microfiltración, las muestras fueron recuperadas y analizadas por microscopía electrónica de barrido (SEM). Los resultados de microfiltración se analizaron utilizando una prueba estadística de Chi-cuadrado y la adaptación se evaluó con un análisis descriptivo. Resultados: Ninguno de los materiales evaluados evitó completamente la microfiltración de nitrógeno (fuga positiva de 10% y 20% de muestras para MTA y Biodentine™, respectivamente); sin diferencias estadísticamente significativas entre los grupos (p = 0,527). Todos los tapones apicales mostraron una buena adaptación bajo SEM, a 30x, 200x, 1000x y 2500x; con morfologías similares a las previamente reportadas. Conclusiones: ambas biocerámicas se comportan de forma similar cuando se usan como barreras apicales para evitar la permeabilidad de gas, con adaptación marginal aceptable. Se necesitan más estudios *in vivo* para validar estos resultados.

PALABRAS CLAVE

Biocerámicas; MTA, Biodentine™; Microfiltración; Adaptación; Permeabilidad al gas.

INTRODUCTION

Immature teeth presenting pulpal necrosis cease the natural root development, turning the conventional endodontic therapy in a more challenging procedure, due to the necessary additional techniques and materials required to obtain an adequate apical seal (1,2). Apexification is the procedure that promotes the formation of an apical barrier to close the open apex of a non-vital immature tooth (3,4). Many materials have been used to fill the root canal, trying to obtain this apical barrier; from antiseptic pastes (5,6), the regularly used calcium hydroxide (7-10) and the more recent use of bioceramics (11-14).

Bioceramic materials are considered a hot topic in dental biomaterials research, in an effort to increase the clinical success of endodontic treatment procedures (15). These materials have demonstrated the ability to overcome the limitations of earlier generations of endodontic materials, showing to be biocompatible and having good physico-chemical characteristics (16,17). Mineral trioxide aggregate (MTA) was first introduced in 1995 (18). Due to its clinical behavior, it is considered the most appropriate material to seal communications between the pulp cavity and periodontal tissue (19). More recently, Biodentine™, a calcium silicate based material, became commercially available in 2009 (Septodont, http:www.septodontusa.com/) and has gained popularity because of its similarities to MTA and its applicability in endodontics (14). Both materials are well known for their clinical versatility, which allows them to come into contact with different oral environmental conditions (14,20) and has been suggested for use as a root-end filling material (21), for perforation repair (22), pulpotomies (23,24), pulp capping (25) and apexification (26-27).

These bioactive materials have been able to improve the sealing ability of the apical barrier when compared to older materials, however, clinical failures are still a recurrent problem, mostly due to the clinically undetectable passage of bacteria, fluids, molecules or ions between tooth and the restorative or filling material, known as microleakage (28). Dye penetration (29), dye extraction (30), bacteria infiltration (31), and radioisotopes (32), in addition to scanning electron microscopy (33), transmission electron microscopy and micro-computed tomography (34), are some of the proposed methodologies found in literature for evaluating microleakage. From a different approach, some studies have evaluated the leakage of fluids under dynamic conditions, such as the increment of pressure within a closed system. Since 1987, Pashley et.al. proposed a fluid transportation model that measures the movement of an air bubble through a capillary tube filled with liquid (35). Substituting this liquid for an inert gas, allowed an independence of water-wetting properties of the tested materials, and increased the sensitivity of the methodology. Gas permeability, is a non-destructive, quantitative, in vitro method for measuring leakage based on the precise and reliable indirect physical measurement of the interphase width (36). A previously reported gas permeability system (37) was modified using a bi-chamber device and piezoelectric sensors, that converts gas pressure changes into voltage signals analyzed and graphed by a software (38). The development of such system, permits a quantitative measurement of materials microleakage under a dynamic, accurate methodology. The aim of this investigation was to evaluate the gas permeability and marginal adaptation of MTA and Biodentine™ apical plugs using an *in vitro* model.

MATERIALS AND METHODS

This in vitro model used 40 anonymously donated extracted human teeth. A pre-selection of single rooted teeth was performed by clinical observation, excluding teeth with presenting severe loss of dental structure. A digital radiograph was taken for pre-selected teeth for further assessment.

Inclusion criteria for teeth selection were as follows: minimum tooth length of 18 mm and radiographically verified straight root canal. Exclusion criteria were as follows: teeth presenting a previous restorative treatment (i.e. amalgams, composites, or fixed prosthetics), radicular caries, severe dilacerations, hypercementosis, previous root canal treatment, root canal obliteration, isthmuses, calcifications, or presence of bifurcations or lateral canals. All radicular surfaces were finally stained with caries detector for 5 minutes, rinsed and examined using a surgical microscope (Alltion® 4000, Guangxi, China) to discard the presence of surface fissures. After final evaluation, 24 teeth were selected.

SPECIMEN PREPARATION

Selected teeth were stored in individual plastic cases in saline solution at room temperature. The apical 3mm portion of each tooth and the cervical third were sectioned to obtain 10 mm length cylindrical structures using a diamond disc at 250 RPM under copious water irrigation (IsoMet 1000 Precision Cutter, Buehler Co. Illinois, USA). The internal diameter was standardized using Pesso® burs (Dentsply-Maillefer®, Ballaigues, Switzerland) sequence #1 through #4, until a homogeneous diameter of 1.3 mm was obtained. Root canals were gently rinsed with 5.25% sodium hypochlorite and EDTA 17%. Specimens were randomly assigned to 3 groups as follows: group A (MTA, n=10), group B (Biodentine™, n=10) and control group C (positive n=1, negative n=3).

GROUP A: MTA GROUP

MTA (MTA Angelus®, Londrina, Brazil) was prepared according to the manufacturer indications. Using an MTA carrier, the mixed cement was gently placed into the root canal and with a calibrated endodontic plugger RCP 9/11 (Hu-Friedy®, Illinois, USA) until 4mm plugs were obtained. Residual material on the canal walls was removed using #80 paper points (Hygienic Corp., Ohio, USA). All specimens were prepared within a 5 min period, and then stored in plastic vials previously filled with floral foams containing 7 cc of sterile saline solution in an incubator (VWR, Pennsylvania, USA) at 37°C for seven days. Daily placement of fresh saline solution was added to avoid dehydration of samples.

GROUP B: BIODENTINE™ GROUP

Biodentine™ (Septodont Co., St. Maur-Des-Fossés, France) was prepared according to the manufacturer indication. Apical plugs were prepared following the same protocol previously described for the MTA group.

GROUP C: CONTROL GROUP

An empty prepared root (n=1) was used as the positive control. For negative controls (n=3) root canals were etched with Ultra-Etch (Ultradent® Products Inc, Utah, USA) 37% phosphoric acid for 10 seconds, then rinsed thoroughly and excess humidity was removed until a moist surface was obtained. Single Bond (3M ESPE, Minnesota, USA) adhesive system was placed, air thinned and light-cured within the root canals following the manufacturer recommendations. Finally, the prepared root canals were filled with Filtek Flow 3M composite (3M ESPE®, Minnesota, USA) and light-cured.

MICROLEAKAGE EVALUATION

To evaluate the microleakage of the bioceramic-dentin interphase the gas permeability method was selected, using a previously customized device known as Automatic Evaluator of Microleakage (EMA) (patent pending number 01/2017-000075) (39). Briefly, using a stainless-steel ring with a 1cm internal diameter, samples were mounted using an epoxy metal/concrete resin (Loctite, Düsseldorf, Germany). The width of the sample holder was 5mm, therefore the roots were 5 mm inside the chamber. Nitrogen gas entered through Chamber 1 where an exit hose recorded air pressure using piezoelectric sensors. Chamber 2 had a second exit hose for the recording of this chambers air pressure and a gas release valve. Once the system was closed nitrogen at 20 psi was injected in chamber 1 and simultaneously the sensor in chamber 2 recorded pressure changes due to gas leakage through the material-dentin interphase. All specimens were ran for a minimum of 3 minutes in the system.

MARGINAL ADAPTATION ANALYSIS

After the microleakage experiment, samples were sectioned using diamond discs. The specimens were prepared for scanning electron microscope (SEM) (Jeol Ltd, Tokyo, Japan) analysis and were mounted on specific metallic stubs to prevent their movement and to allow the evaluation to be made parallel to the long axis of the foramen. Specific parameters of 3 kV and 30x, 200x, 1000x, and 2500x were used and a single trained examiner performed the blind evaluations of SEM images and photomicrographs were taken to evaluate marginal adaptation.

STATISTICAL ANALYSIS

Microleakage results were analyzed using Chi-square statistics (95% CI) and marginal adaptation was evaluated with descriptive analyses.

RESULTS

Twenty-four experimental specimens were evaluated using the EMA gas permeability system. One specimen in each group was eliminated due to inconclusive measurements (sealing failure of epoxy resin detected). The positive control (empty canal) demonstrated synchronic sensitivity to pressure changes in both chambers. The negative controls (light cured composite-filled canals) tested negatively with no gas permeation between chambers. For the experimental groups, one MTA specimen and two Biodentine™ specimens recorded microleakage as shown in Figure 1. No statistical difference was observed between groups ($p=0.527$). Figure 2 shows the behavior of each sample per group during the analysis period.

Representative SEM (30x, 200x, 1000x and 2500x) analysis are shown in figures 3 and 4 for MTA and Biodentine™ plugs respectively. All specimens showed acceptable and homogeneous marginal adaptation between the materials and the dental structure.

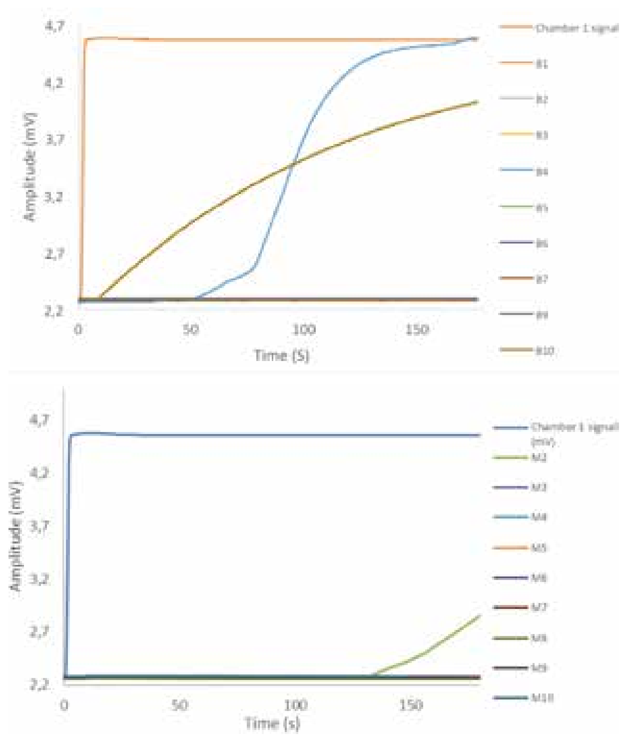

Figure 1. Nitrogen permeability measurements. 1.a. Group A (MTA). 1.b Group B (Biodentine™).

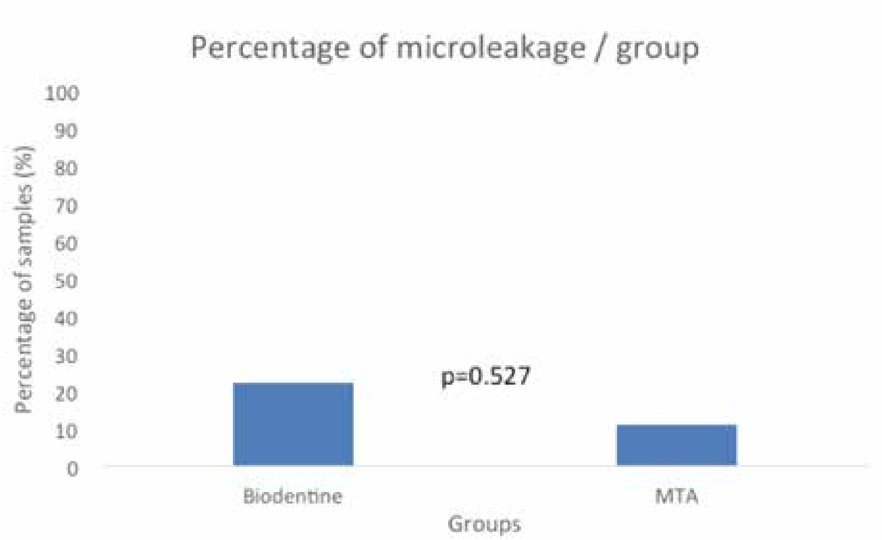

Figure 2. Microleakage results per group. Results are shown in the percentage of positive gas permeability for each group. No statistical difference was observed between MTA and Biodentine™ groups (p=0.527).

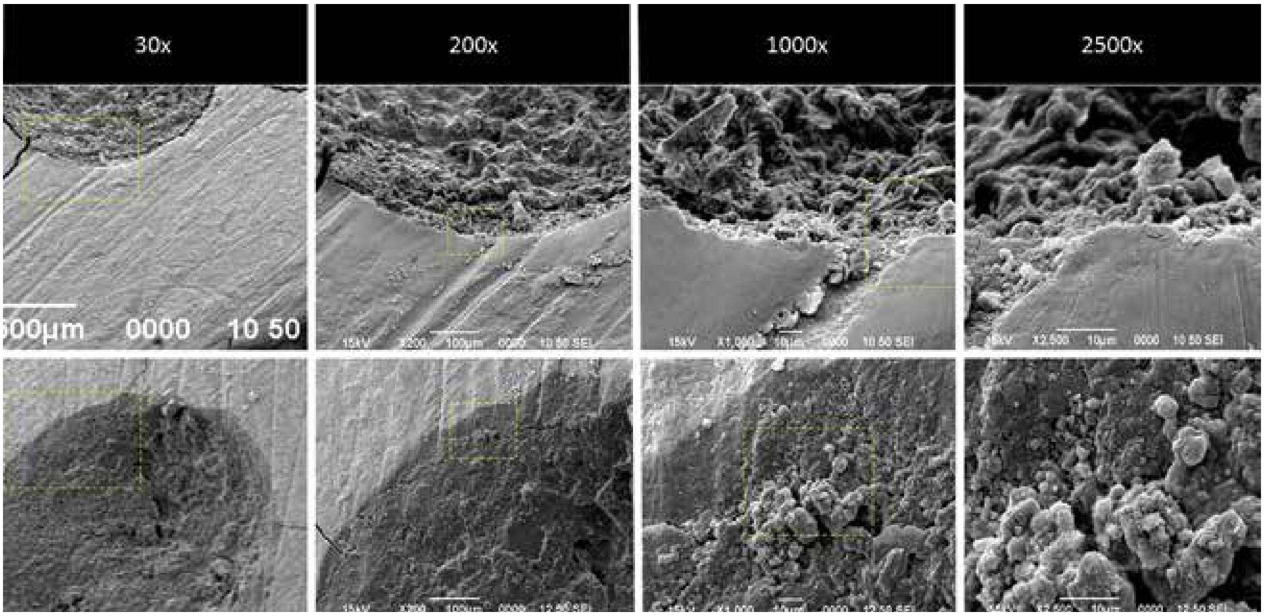

Figure 3. Adaptation of MTA plugs.

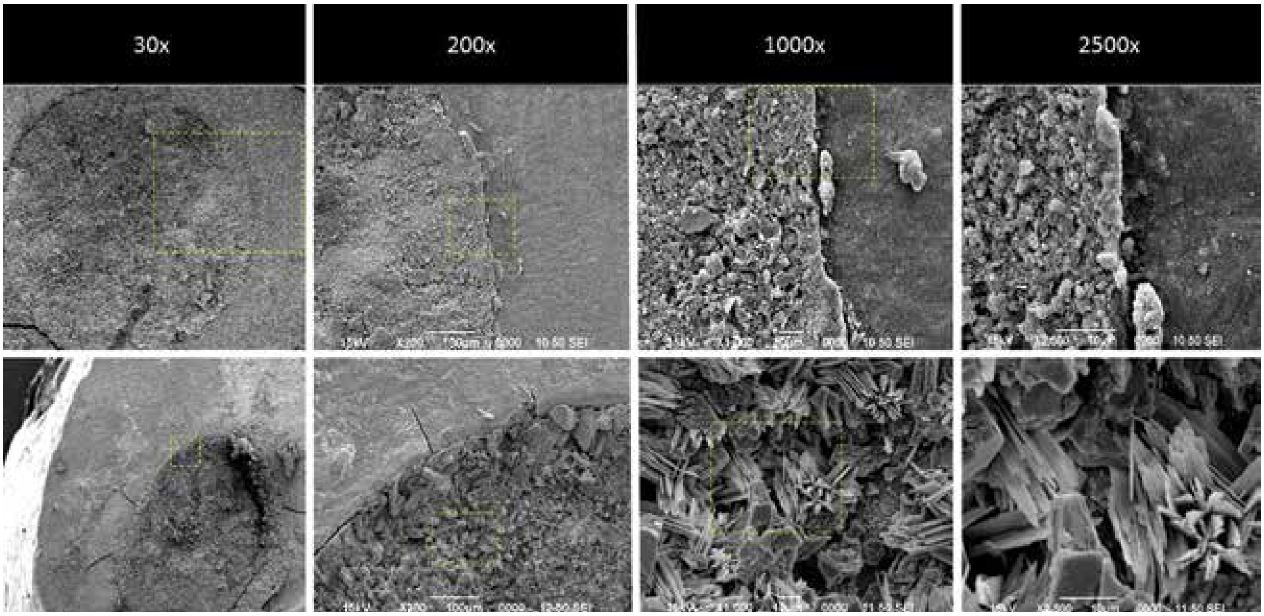

Figure 4. Adaptation of Biodentine™ plugs

DISCUSSION

The aim of this investigation was to evaluate the adaptability and sealing ability of MTA and Biodentine™ apical plugs *in vitro*. The selection of biomaterials for apexification treatments is as complex as the procedure itself. Many clinicians prefer biomaterials, like calcium hydroxide, to enhance the gradual biological sealing of the open apex (40), however, several factors such as inter-appointment contamination (41) or obtaining a porous barrier (3) are still remarkable concerns. Young patients depend of their parents or caregivers to complete a multiple-visits treatment and control appointments, and many may gradually abandon the treatment. Also, the use of temporary restorative materials between multiple appointments provides no certainty on their mechanical and sealing properties. These factors may converge to balance the equation towards failure and finally all efforts could be worthless. To avoid these problems, both MTA and Biodentine are recommended options to perform apexification plugs (26).

In the present study, the gas permeability test was selected to identify microleakage through the bioceramic-dentin interphase. Mainly, the reason to choose this technique was to control or reduce the chance of possible biases related to other experimental methods, such as dye or bacterial penetration. Dye penetration is a very popular method use by several authors, which consist in the passive penetration of visible dye through the biomaterial-dentin interphase. Several weaknesses for these models have been reported by important dental journals (42), and some authors strongly suggest to avoid the publication of studies using this method as the only test to measure microleakage (43). As reported by Savadkouhi et.al., bacterial leakage also shows important limitations that should be considered (44), especially the management of controls and possible bacterial penetration through secondary pathways (45). The validity of the employed system (EMA system) was tested by using positive and negative controls (open roots and roots sealed with light-cured composite respectively). When open roots are used, an immediate stabilization of the pressure in both chambers is identified, whilst composite sealing demonstrate the absence of side-leakage within the system; indicating that possible gas passage into chamber 2 may be only through the biomaterial tested.

Previous investigations have evaluated the microleakage of apical barriers of MTA and Biodentine. Samuel et.al. compared the microleakage of MTA and Biodentine in primary molars by using SEM, showing similar results for both biomaterials. However, this last study must be interpreted cautiously, since the data evaluated was marginal adaptation and not the physical presence of microleakage (46). By using bacterial leakage through perforations in primary molars, Ramazani and Sadeghi (47) showed also a similar behavior for MTA and Biodentine. In accordance with these reports, our study showed similar results for both groups when evaluated with the gas permeability test (p=0.527). During the evaluation process, only 1 and 2 samples showed positive leakage for the MTA and Biodentine™ group respectively. It is important to highlight that gas leakage was not immediate, but a gradual response after several seconds of running the specimen through pressured nitrogen. However, as shown on figure 1, the MTA sample that failed, resisted longer before revealing nitrogen leakage. In the case of Biodentine, both failed samples showed gas leakage during the first minute of the experiment.

Regarding SEM analysis, observations were done at 4 different magnifications. All samples showed a good adaptation of the materials, and well define microstructures within both cores. MTA showed improved homogeneity, and less abrupt changes in the dentin-MTA interphase. Biodentine instead, showed in all samples an heterogenous surface full of crystalline structures.

Although several variables were controlled in order to diminish possible bias, important limitations must be discussed before extrapolating these preliminary results to clinical scenarios. This study's methodology evaluated the apical plugs after 7 days of incubation in a humid environment with saline solution. The marginal adaptation of these biomaterials to dentin, could be increased as more incubation days are allowed before testing microleakage, as some studies suggest (48, 49). They reported and increment in bond strength of apical plugs after two months of incubation immersed in a PBS solution. The formation of MTA tag-like structures inside dentinal tubes was attributed as the factor which provided the bonding strength increment. These structures were not found in the 72-hours samples with a wet cotton pellet inside the canal. They also suggest that the interaction of MTA with phosphate-containing solutions may promote this mineralization process inside dentinal tubes. Nonetheless for clinical purposes, it is recommended that if the clinician plans on placing a PBS or saline solution wetted cotton pellet on the intra-dental side of calcium-silicate cements for an extended period of time to obtain the materials' bioactivity properties, measures should be taken to prevent losing the coronal seal. Moreover, all *in vitro* conditions used during this study, cannot be generalized to a clinical setting. Transmission of occlusal forces to the apical area may play an important role on the formation of the apical barrier and the mineralization process. More ex vivo and in vivo studies are necessary to confirm the results from this study and to better understand the behavior of these bioceramics as apexification materials.

Many perspectives can be derived from this investigation. Further works must evaluate new samples by gas permeability employing preparation methods that improve the behavior of the material. For instance, increasing the time of materials setting (i.e. months instead of days), to include an *in vivo* model that favors the mineralization process in biological scenarios instead of an *in vitro* model and to correlate de data from gas microleakage with complementary models such as fluids diffusion or bacteriological assay. Also, different protocols to enhance mineralization of bioceramics when used as apical plus (such as internal PBS canal dressings) can be validated by gas permeability tests (49).

CONCLUSION

MTA and Biodentine™ showed similar results when tested *in vitro* with a gas permeability microleakage method. None of the tested materials were able to form an apical barrier completely preventing microleakage. Both materials showed good marginal adaptation to dental tissue, with different microscopical morphologies.

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