

In Vitro Comparison of MTA and BC RRM-Fast Set Putty as Retrograde Filling Materials

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Abstract

Background: To compare, *in vitro*, the bioceramic materials (MTA and BC RRM-fast set putty) capacity to prevent microleakage of *Enterococcus faecalis* over time.

Methods: An experimental design was made with forty extracted human teeth, coronally cut, and prepared to be placed in a leakage system under sterile conditions. They were randomly divided into an experimental group: thirty teeth (fifteen for retrograde filling material MTA and BC RRM-fast set putty, respectively) and a control group: ten teeth (five positive control, five negative control). The 3 mm root-ends were submerged in a brain-heart infusion broth with a red phenol indicator. The coronal access of each sample was inoculated with *E. faecalis* every seven days to maintain bacterial viability. The lower chamber was evaluated daily for 30 days to observe the turbidity of the culture medium and establish the presence and day of the filtration. Calculation of the colony-forming units (CFU) was performed for each leaked sample.

Results: Of the total samples prepared for each type of bioceramic material, leaked 60.0% (9/15) of the MTA group and 40.0% (6/15) of the BC RRM-fast set putty group. All positive controls filtered on the first day of evaluation, while 20% (1/5) of the negative control leaked in the second week. There was no significant difference in leakage between the bioceramic material types, nor concerning the bacterial count and the type of cement used ($p = 0.101$).

Conclusions: This study suggests that BC RRM-fast set putty and MTA have a similar sealing capacity when used as a retrograde filling material *in vitro*.

Background

The biological aim of root canal treatment is to prevent or cure apical periodontitis by controlled asepsis or disinfection of the root canal system, thus creating an environment in which the periradicular repair can occur. Surgical intervention is indicated only when orthograde retreatment fails or when orthograde retreatment is contraindicated [1, 2, 3, 4]. Endodontic surgery aims to remove diseased tissues and get an apical seal to prevent the entry of residual irritants into the periradicular area [5].

The retrofilling material must be completely adherent to the dentin walls. It should also maintain its long-term structural integrity and not dissolve or corrode in contact with tissue fluids. The sealing ability is further enhanced if the material is bacteriostatic or preferably bactericidal, and it should promote the formation of dentin and cementum on the resected root surface. Among other properties, it should be radiopaque, easy to handle, have an adequate working time, and a reasonable price. Therefore, the ideal features must satisfy biological, physical, practical, and economic criteria. However, of all the above-mentioned desired characteristics, lack of toxicity and excellent sealing ability are the two principal requirements of an ideal material [5, 6, 7].

Bioceramic materials such as mineral trioxide aggregate (MTA) and root repair material (EndoSequence BC RRM) are two of the most used. Although none of them meet all the requirements of an ideal material, MTA and the second generation of bioceramics have more potential in terms of sealing ability, biocompatibility, and bioactivity [7, 8, 9, 10, 11, 12].

MTA was the first generation of bioceramic materials used in endodontics. It was developed -originally from Portland cement as a gray powder by Dr. Mahmoud Torabinejad. The main compounds of gray MTA are tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide, mineral oxide, and bismuth oxide. Bismuth oxide increases the radiopacity [7, 8, 9, 10, 11, 12, 13].

The sealing ability and biocompatibility of MTA are attributed to tricalcium silicate. However, the major drawbacks of MTA are its handling, long setting time, and discoloration of the remaining tooth structure [7, 14].

Root repair material (EndoSequence BC RRM-fast set putty) is a relatively new bioceramic material. The indications are like those of MTA, including retrofilling of root-endings, pulp capping, apexification, sealing of resorptions, and root perforations [6, 8, 9, 10, 11]. According to the manufacturer, it consists of calcium silicates, zirconium oxide, tantalum pentoxide, and monobasic calcium phosphate, besides bulking agents. The material is ready-to-use, pre-mixed, and comes as a paste in a syringe or putty in a jar. An advantage of EndoSequence BC RRM-fast set putty, based on clinical experience, are its handling properties, like Cavit. EndoSequence BC RRM-fast set putty is biocompatible, hydrophilic, insoluble, dimensionally stable, has a high pH, a set time of 20 minutes from the moment it encounters the tissues [6, 8, 9, 10, 11].

Studies comparing the antibacterial sealing ability of EndoSequence BC RRM-fast set putty to MTA have shown conflicting results [9, 10, 13]. When evaluating retrofilling materials, microleakage remains a priority. Based on this premise, the purpose of this study was to compare *in vitro* the ability of bioceramic materials (MTA and EndoSequence BC RRM-fast set putty) to prevent microleakage of *Enterococcus faecalis* over time.

Methods

This investigation was conducted using forty extracted human teeth, caries-free, monoradicular, single-canaled with fully formed roots; teeth with caries were excluded to minimize the possibility of preoperative bacterial contamination of the root canal. No data were collected on age, sex, or reason for extraction.

The teeth were divided into two groups, an experimental group of thirty teeth (15 for each retrograde filling material to be evaluated: MTA and EndoSequence BC RRM-fast set putty) and a control group of ten teeth (5 for positive control and five for negative control).

The extracted teeth were stored in PBS (phosphate-buffered solution) to avoid dehydration. Remains of bone, calculus, or soft tissue were gently removed from the root surface of each tooth with a sterile curette. Subsequently, they were immersed in 5% sodium hypochlorite (NaClO) for approximately 15 minutes to remove organic debris from the root surface. Preoperative radiographs were taken of each tooth and were evaluated using an operating microscope (D.F. Vasconcellos, Brazil) at 20X magnification to ensure that there were no root caries or vertical fractures. The teeth were cut with a water-cooled diamond disk (D&N, Darmstadt, Germany) at the coronal level of the root, at an approximate root length of 14 mm, to standardize the working length of the samples.

The samples were randomly divided into two groups of fifteen teeth each and two control groups of five teeth each. For the root canal preparation, the working length was first determined using #15 files, K-Flexofile (Dentsply Maillefer SA, Ballaigues, Switzerland), which were placed inside the canal until the apex was slightly exceeded and 1 mm was subtracted from the previous measurement. Instrumentation was performed using the crown-down technique. The canal preparation in the coronal and middle third of the root was completed with Gates Glidden drills (Dentsply Maillefer SA, Ballaigues, Switzerland), sizes 3, 2, 1. The WaveOne Gold reciprocating, rotating system, Primary 025/0.06 (Dentsply Maillefer SA, Ballaigues, Switzerland), was used to continue the canal shaping process.

Instrumentation was performed under copious irrigation with 2.5% NaClO using a sterile syringe with a 27-gauge Endo-Eze irrigation needle (Ultradent products, INC, Spain). All canals received final irrigation with 3 ml of 17% EDTA followed by 3 ml of 2.5% NaClO to remove the smear layer and dried with Hygenic sterile absorbent paper points (Coltene/Whaledent inc., Germany).

Under continuous water irrigation, the apical 3 mm of each root was cut at 90 degrees to the long axis of the tooth, with a multiblade bur (Dentsply Maillefer SA, Ballaigues, Switzerland) on a high-speed handpiece. At the root-end, cavities were prepared with retrograde diamond-coated tips to a depth of 3 mm using a DTE ultrasound unit at a low power setting and water coolant. The teeth were steam sterilized for 30 min at 121°C. Before using the retrofilling material, the root-end was fitted with a red Machtou condenser (Dentsply Maillefer SA, Ballaigues, Switzerland) 3 mm anterior to the sectioned root-end, which was verified with a radiograph. At this point, the condenser provided an intercanal matrix against which the filling materials were compacted.

Experimental group

It comprised two subgroups corresponding to the retrofilling material to be used. All materials were prepared according to the manufacturer's instructions.

Subgroup #1: the cavities were retrofilled with MTA-Angelus (Angelus, Londrina, PR, Brazil), which was mixed in a 3:1 powder-to-water ratio, using sterile water, and incrementally placed in the root preparations using an MTA carrier. The material was then compacted using a Machtou straight condenser (Dentsply Maillefer SA, Ballaigues, Switzerland). The root surface was cleaned with moistened sterile cotton swabs

and then coated with two layers of Valmy® nail varnish (Drocosca CA, Caracas, Venezuela) except for the 3mm in the apical portion containing the obturation material.

Subgroup #2

the cavities were retrofilled with EndoSequence BC RRM-fast set putty (Brasseler, Savannah, GA, USA), which comes in paste or putty form previously mixed in an injectable syringe. It was applied to the cavities using the B&L Jetip microsurgical instrument tip (JM2, B&L Biotech, Fairfax, VA, USA) and condensed with a Machtou straight condenser (Dentsply Maillefer SA, Ballaigues, Switzerland). For surface finishing, a microbrush (FGM, Joinville, SC, Brazil) was used on the BC RRM-fast set putty as recommended by the manufacturer. The root surface was coated with two layers of Valmy® nail varnish (Drocosca CA, Caracas, Venezuela), except for the 3 mm of the apical portion containing the obturation material.

Control group

The control group, in turn, comprised two subgroups of five teeth described below.

Positive control

the root-end cavities were left without retrofilling material.

Negative control

utility wax (Asfer, Caetano do Sul, SP, Brazil) was inserted to obturate the root-end cavity, and the entire root surface was coated with two layers of Valmy® nail varnish (Drocosca CA, Caracas, Venezuela), including the apical area containing utility wax.

After retrofilling the roots, the barrier placed inside the canal was removed, and radiographs were taken to verify the density and depth of the retrofilling material. The samples were stored in 100% humidity at 37°C for one week.

Sample preparation in the leakage system

The coronary part of each tooth was connected to the end of a 2.5 ml polyethylene tube (Nelaton Catheter; MD Products, Venezuela), using cyanoacrylate and sticky wax to prevent leakage from the connection. The root surface and the tubes were coated with two layers of Valmy® nail varnish (Drocosca CA, Caracas, Venezuela), except for the apical 2 mm of the roots. The five teeth used as negative controls were completely covered with two coats of nail varnish. All samples were sterilized using ethylene oxide gas for 12 hours. The polyethylene tube was attached to the screw cap of the sterile 30 ml glass bottle, in which 10 ml of brain heart infusion broth (lower chamber) was placed and maintained at 37°C in an oven for 48 hours, for quality control before placing the bacterial inoculum in the upper chamber. With a sterile Pasteur pipette, 2 ml of brain heart infusion medium with *E. faecalis*, at 0.5 McFarland turbidity (10^8 bacteria/ml), were pipetted. Root-ends were immersed in brain heart infusion

broth containing one drop of 0.04% phenol red indicator solution. The complete set was maintained at 37°C, during the 30-day observation period. (Figure 1)

Bacterial leakage control

The leakage system used in the present study was that proposed by Barthel et al., [14] Chailertvanitkul et al., [15], and Torabinejad et al., [16]. The lower chamber was observed daily to determine bacterial leakage, which was evidenced when the culture medium changed from red (alkaline) to yellow (acidic) because of acid production due to bacterial growth, or by the turbidity of the medium; the time for this to occur indicated contamination of the entire root canal and then it was evaluated whether it was the same bacteria. For this purpose, a sample was taken and sown on Columbia blood agar base plates and incubated at 37°C for 48 hours for macroscopic evaluation by colony morphology; Gram staining was performed for microscopic evaluation to confirm the presence of *E. faecalis*. The bacterial count of each leaked sample was performed using the serial dilution technique. Replacement of the inoculum in the upper chamber was performed aseptically with Pasteur pipettes every seven days to ensure the viability of the bacteria at a density of 0.5 McFarland.

Protocol for the processing, quantification, and identification of microorganisms by serial dilutions

The brain heart infusion broth with the leaked culture was homogenized with agitation, and then serial dilutions (10^{-2} to 10^{-6}) were performed in a sterile saline solution (0.9%). 10µl of the culture, from which the dilutions were made, were taken and plated on blood agar. The plates were incubated at 37 °C for 24 hours.

For the quantification of microorganisms, plate colonies corresponding to the dilutions that presented between 30 and 300 colonies were counted, and the results were expressed in Colony-Forming Units per milliliter of sample (CFU/ml), according to the following formula:

Bacterial titer: No. of colonies x dilution factor/ml of culture plate

Data collection and processing technique

A data collection sheet was used; it included the sample number, the retrofilling material, date of inoculum placement, date of leakage, time in which the turbidity of the medium occurred, and verification of *E. faecalis* in the lower chamber. This information was obtained through daily observation of the specimens. The change in color or turbidity of the culture medium in the lower chamber, and the verification of *E. faecalis* through its morphological identification under the optical microscope and in the culture medium, showed microleakage of *E. faecalis*.

The tabulation of all sample information was collected in a sheet prepared for this purpose that included the day of leakage, the specimens that presented leakage, and the number of colony-forming units per milliliter of sample (CFU/ml) for each one.

Once gathered the information in the data collection instrument, a matrix was designed in Excel, in which it was primarily transcribed.

Data analysis technique

The information was entered into a database using SPSS statistical software version 21.0. The variables were processed and presented in tables.

Statistical analysis

Considering the number and type of variable, level of measurement in which the values of the variables are expressed, manner of choice or occurrence of the subjects or elements of the study, and the sample size, non-parametric tests were used. Fisher's Exact Test was used to verify the association between the presence or absence of leakage of the samples by type of bioceramic material used and the Mann-Whitney U test to verify the existence of a difference between the average of Colony-Forming Units by type of bioceramic material used.

To evaluate the statistical significance of the hypotheses of association and differences formulated, we used as Significance Level ($\alpha = 0.05$) and a 95% Confidence Level and, as a decision rule for rejecting the null hypothesis (H_0) formulated, when the p value associated with the contrast statistic resulted lower than the Significance Level set ($\alpha = 0.05$), that is, when $p < 0.05$.

Results

Of the total samples prepared for each type of bioceramic material, after 30 days of evaluation, 60% (9/15) of the tubes with MTA leaked, and 40% (6/15) of the BC RRM-fast set putty. 100% (5/5) of the controls filtered on the first day of evaluation, and of the negative controls, only 20% (1/5) of the sample leaked in the second week.

Table 1 shows the percentage of samples leaked for each material and the controls, according to the weeks of evaluation. Regarding the specimens from the MTA group, in weeks 1 and 2, 20% (3/15) leaked each week, and 6.7% (1/15) in weeks 3, 4, and 5, respectively (Table 1). Concerning the BC RRM-fast set putty samples, the following occurred 13.3% (2/15) leaked during the first week, and in weeks 2, 3, and 4 filtered 6.7% (1/15) each.

Table 1
Distribution of Leaked Samples, According to Bioceramic Material and Week of Evaluation.

Bioceramic material	Total	Leaked samples				
		Week 1	Week 2	Week 3	Week 4	Week 5
MTA	60.0% (9/15)	20.0% (3/15)	20.0% (3/15)	6.7% (1/15)	6.7% (1/15)	6.7% (1/15)
BC-RRM	40.0% (6/15)	13.3% (2/15)	6.7% (1/15)	6.7% (1/15)	6.7% (1/15)	6.7% (1/15)
C+	100.0% (5/5)	100.0% (5/5)	0.0% (0/5)	0.0% (0/5)	0.0% (0/5)	0.0% (0/5)
C-	20.0% (1/5)	0.0% (0/5)	0.0% (0/5)	0.0% (0/5)	0.0% (0/5)	0.0% (0/5)

Source: C+: positive control, C-: negative control.

Regarding identification and quantification of the microorganisms leaked in the systems, the negative controls showed no bacterial growth, while the positive control group showed 100% growth. As for the bioceramic materials, the presence of *E. faecalis* in the leaked broth was corroborated by microbiological culture and Gram staining, showing white colonies, rounded and with smooth edges in the blood agar; and purple bacteria, spherical-shaped and grouped in chains, after staining, classifying them as Gram-positive.

Quantification of the bacterial population for the MTA leaked samples showed an average of $1.70 \times 10^9 \pm 0.33 \times 10^9$ CFU, while the BC RRM-fast set putty average was $1.29 \times 10^9 \pm 0.33 \times 10^9$ CFU, as shown in Table 2. There was no statistically significant difference between the groups ($p= 0.101$).

Table 2

Leaked Samples and Average Colony-Forming Units of *Enterococcus faecalis* After 30 Days of Incubation, According to Bioceramic Material Used.

Sample Type Bioceramic Material	Leaked samples (%)	- Mean (Y) ±SD	Median (Me)	Range
Experimental:				
With MTA	9/15 (60.0)	$(1.70 \times 10^9) \pm 0.33 \times 10^9$	1.76×10^9	$1.35 - 2.04 \times 10^9$
With BC RRM	6/15 (40.0)	$(1.29 \times 10^9) \pm 0.38 \times 10^9$	1.08×10^9	$0.88 - 1.69 \times 10^9$
Control:				
Positive	5 (100.0)	0	0	0
Negative	1 (20.0)	0	0	0
Source: Own study				
Bioceramic Materials: MTA: Mineral Trioxide Aggregate				
BC RRM: EndoSequence Root Repair Material				

The percentage of leaked samples, according to the bioceramic material used, highlighted that MTA had a higher proportion of leakage than BC RRM. Regarding the control group, the positive had 100% (5/5) filtration, while the negative had only 10% (1/5) (Figure 2). There was no significant difference ($p= 1.000$) with the presence or absence of leakage of the samples by type of bioceramic material used, as shown in Table 3.

Table 3
Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson's Chi-square	.040 ^a	1	.842		
Continuity correction ^b	.000	1	1.000		
Likelihood ratio	.039	1	.843		
Fisher's Exact Test				1.000	.600
Linear-by-linear association	.038	1	.843		
N of Valid cases	30				
a. Two cells (50.0%) have an expected frequency of less than 5. The minimum expected frequency is 1.80					
b. Computed only for a 2x2 table					

Discussion

In the past, *in vitro* microleakage was evaluated using dye penetration methodologies with various pigments, such as methylene blue and India ink, however, the reliability, reproducibility, and clinical relevance of these methods are questionable [17, 18, 19, 20]. Some researchers disagree with using such dyes because they have low molecular weights and, therefore, can penetrate sites where proteins and bacteria cannot [17, 18, 19, 20]. Ahlberg et al. [18] reported discrepancies in the filtration patterns of methylene blue and India ink in obturated teeth. They found a higher level and broader variation of methylene blue penetration than India ink in all experimental groups. Similarly, studies using radioisotope techniques, the type of isotope, the distance between the radiation source and the emulsion, and different exposure times can affect the results. Radioisotope tracers are smaller than bacteria and can distribute differently. Because of the lack of correlation between dye particles and isotopes, the efficacy of retrofilling materials can be better determined using a bacterial microleakage model [18, 19, 20, 21, 22, 23].

For the bacterial microleakage model of this experiment, *E. faecalis* was chosen because it is part of the regular biofilm in humans and is frequently found in mixed infections. Additionally, *E. faecalis* is one of the most isolated microbes in the root canal in secondary infections [9, 13, 24, 25]. The present study evaluated the sealing ability of bioceramic materials (MTA and BC RRM) to prevent microleakage of *E. faecalis* when used as a retrofilling material. Besides, it evaluated qualitatively and quantitatively the presence of the selected bacteria in the filtrate of the samples through serial dilutions.

Both bioceramic materials showed intermediate leakage percentages, with BC RRM showing the best results; however, there were no statistically significant differences. As for the bacterial count, the averages were within the range of 10^9 CFU, with no statistically significant differences between the materials. The leakage in one of the negative control samples may be due to the detachment of the nail varnish or wax from the root surface, a technical error that led to its contamination.

The results of this study agree with those reported by Nair et al., [9] with leakage in over 50% of the experimental specimens, without finding statistically significant differences between the study groups (BC-BCRR 66.7% and MTA 53.3%). However, the method used by these authors differs from our study, as they obturated the canals in their experimental samples with gutta-percha and resin-based sealer cement, removed the coronal 2 mm of the gutta-percha to create a reservoir for the bacteria. This method required leakage of the obturation materials before encountering the retrofilling material at the root-end. It could suggest that the barrier created by the gutta-percha and sealer cement has underestimated the amount of actual leakage associated with this experimental setup because Ricucci et al. [26] demonstrated that correctly filled teeth resist bacterial invasion in the middle and apical third of the canal when the coronal portion is exposed to caries and saliva for periods of 3 months.

In our work, the leakage through the retrofilling materials was evaluated at the root-end, which allowed for a more accurate method of testing only the leakage associated with those materials and not with additional variables, such as gutta-percha and cement sealant.

Likewise, Antunes et al. [10] show satisfactory results for BC RRM in which they report filtration in 28% of the samples, being statistically like the MTA group in which leaked 50% of the specimens. They conclude that MTA and BC RRM show similar sealing abilities. However, Hirschberg et al. [13] differ from these results, reporting 93% filtration for BC RRM, compared to 20% for samples filled with MTA, the authors assume that this difference is because of the setting properties of BC RRM, which may be sensitive to the presence or absence of moisture, possibly affecting its sealing and filtration ability. Nevertheless, this hypothesis is unsupported in the literature; additionally, in the present study, we used the new formula of BC RRM-fast set putty although its composition is the same as the previous one, its setting chemistry is faster, of only 20 minutes so, again, no direct comparison could be made.

MTA is widely used in endodontic surgery as a retrofilling material, and clinical research has shown a high prevalence of repair after using this material [27, 28, 29]. Chen et al. [30] conducted a study in canines, where compared the periradicular tissue repair following surgery using MTA and BC RRM as retrofilling materials, reporting that a low inflammatory response was observed in most periapical areas, regardless of the material. However, cementum-like tissue, periodontal ligament, and bone covered a significantly larger root-end surface area in the BC RRM group than in the MTA group. When evaluating periapical radiographs, the repair rate in the BC RRM and MTA groups was 92.6% and 75%, respectively. The difference was not statistically significant ($p = 0.073$). Though, on CBCT and micro-CT images, the BC RRM group showed significantly superior repair. BC RRM showed a proliferative effect on osteogenic and odontogenic cells and induced osteoblastic and cementoblastic differentiation in those cells.

Although no study has so far compared the two materials in terms of surgical outcomes in humans, a recent retrospective study showed that BC RRM was associated with high repair rates by clinical and radiographic evaluation over three years of patients who had undergone endodontic surgery [31]. Among other factors, the good clinical outcomes reported for these materials are potentially related to their sealing ability, as shown in the present study.

MTA has been extensively researched in many *in vivo* and *in vitro* studies over the past 20 years[9, 10, 11, 12, 13, 17, 25, 27, 28, 29]. When comparing MTA with traditional retrograde filling materials, it has higher properties in terms of sealing ability, biocompatibility, and periradicular tissue regeneration [11, 12, 17]. Although MTA research has shown excellent chemical, physical, and biological properties, all these benefits can be null if the material is not properly placed nor well adapted to the root-end preparation. The extended setting time of the original MTA formulation (2 h 45 min) and poor handling characteristics are a clinical concern, as due to its gritty and brittle consistency, it is often difficult to condense in retrograde preparations.

An advantage of EndoSequence BC RRM-fast set putty, based on clinical experience, are its handling properties, besides the biological and physicochemical properties already mentioned; in this new presentation, a 20min fast-setting chemistry was added, which allows washing of the surgical site without the risk of losing the material, as occurs with MTA [8, 32, 33].

The development of dental materials is fundamental to improving clinical outcomes in dentistry, and their integration into clinical practice can open new treatment horizons that were difficult to implement in the past.

Conclusions

The results of our study showed that BC RRM-fast set putty was as good as MTA in resisting bacterial microleakage during the observation time. It also exhibited superior handling characteristics. It was noticeably easier to place in root-end retropreparations, and we should not underestimate this advantage in endodontic surgery. Based on the findings of this study, BC RRM-fast set putty is a superior alternative to MTA for use as a retrograde filling material.

Declarations

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Authors' contributions

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures



Figure 1

The complete samples set maintained at 37°C, during the 30-day observation period.

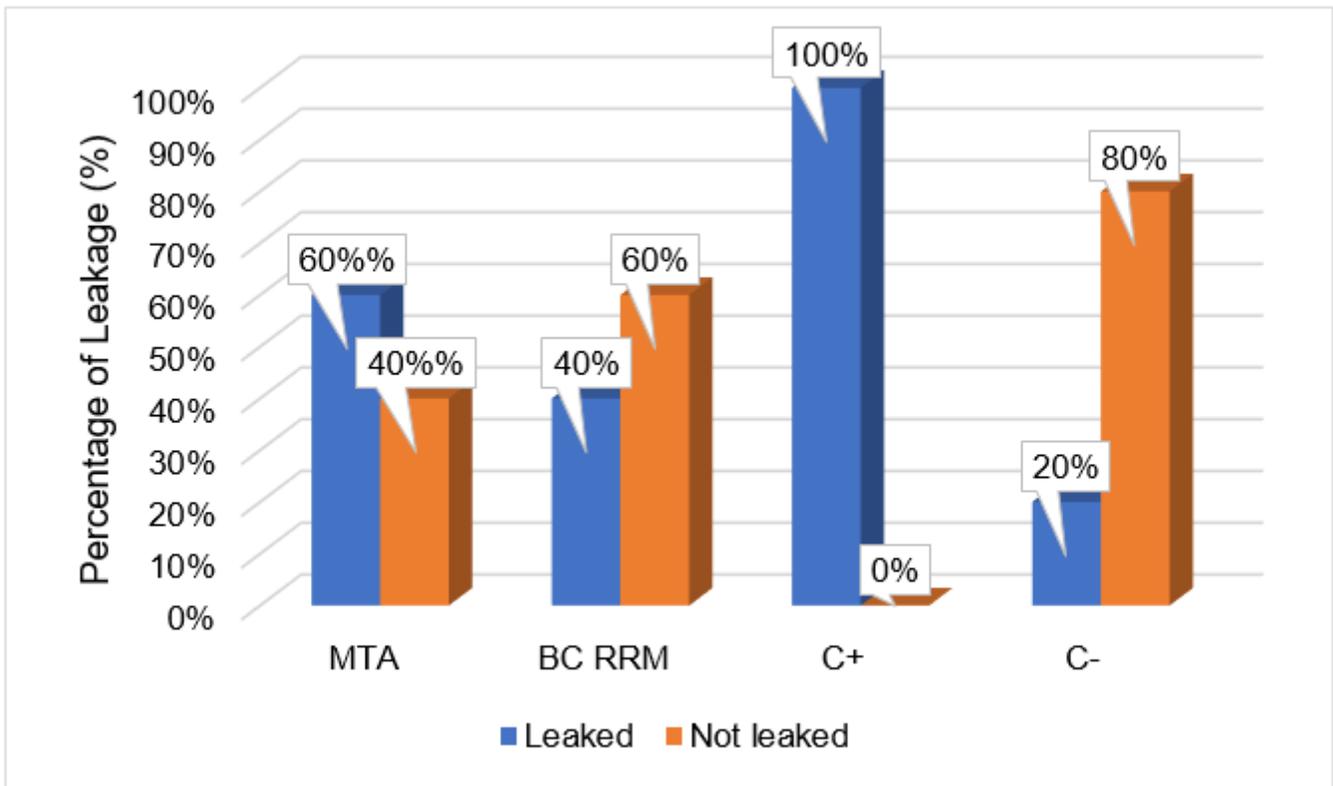


Figure 2

Percentage of leakage of the bioceramic materials used in the study