Sealing Ability of Calcium Silicate-based Materials in the Repair of Furcal Perforations: A Laboratory Comparative Study

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ABSTRACT

Aim: To assess the sealing ability of two calcium silicate-based materials in the treatment of iatrogenic furcal perforations using a dye-penetration leakage model.

Materials and methods: Furcation perforations were performed using a size 12 round burr on the pulp chamber floor of 20 first mandibular molars. The teeth were then randomly divided into two groups, two additional molars served as negative controls. The defects were then filled with mineral trioxide aggregate (MTA) Angelus in the first group and Biodentine in the second group. Leakage at the repaired sites was then evaluated using the methylene blue dye penetration technique.

Results: Significant differences in microleakage were found between the two groups at 72 hours ($p < 0.001$). MTA Angelus had greater dye penetration than Biodentine with a statistically significant difference. Subsequently, the sealing ability of Biodentine was significantly better than MTA Angelus ($p < 0.001$). However, the mean values of leakage and inadequate adhesion were significantly different from the theoretical value for both the MTA Angelus ($p < 0.001$) and Biodentine ($p < 0.001$).

Conclusion: The current results suggested that Biodentine possesses higher sealing quality than MTA Angelus. Yet, both materials are not ideal and still need improvement to ensure perfect adhesion in case of furcal perforation.

Clinical significance: This article aims to compare the sealing ability of one dental repair material over another, after iatrogenically producing a furcal perforation. Leakage resistance and sealing ability are important factors in favoring the outcome of an endodontic treatment of a tooth that could otherwise be condemned for extraction.

Keywords: Biodentine, Calcium silicate material, Dye penetration, Furcal perforations, MTA Angelus, Sealing ability.

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INTRODUCTION

During the endodontic treatment, iatrogenic perforations can compromise the success of the primary root canal treatment.1 Whenever a furcal perforation is not immediately repaired, it will lead to bacterial ingress and complicated endodontic-periodontal lesions.2,3

Perforation repair materials span over a wide spectrum of options. This includes amalgam, zinc oxide eugenol, calcium hydroxide, resin composite, and glass ionomer, among others. However, none of them can restore normal and predictable architecture at the furcal level.4–7

In fact, the usage of inadequate repair materials can render the tooth vulnerable to leakage of periodontal substances leading ultimately to a failure of the endodontic treatment.8 Therefore, finding the repair material with the most favorable sealing properties is mandatory to ensure the longevity of the tooth.

Calcium silicate cements are indicated as furcal perforation repair materials because they can stimulate an inflammatory response at the injured site including fibroblasts, collagen fibers, and osteoclasts, among other immune agents that can lead to the formation of a newly mineralized tissue and the healing of the perforation.9

Mineral trioxide aggregate (MTA) is a calcium silicate-based material that has been extensively studied and proven to be bio-compatible since the early 1990s.10,11 MTA is composed of modified Portland cement with added bismuth oxide.12 Due to its high alkalinity, it has the ability to induce release of bioactive dentin matrix proteins13 and form newly mineralized tissue.14

Even though it offers excellent sealing ability, it suffers from some clinical disadvantages, namely its handling properties and long setting time.15–17

The MTA Angelus (MTA-Ang; Angelus, Londrina, PR, Brazil) was launched in 2002 and has several properties improved. It has been produced by removing dehydrated calcium sulfate from its composition to reduce the setting time to 10–15 minutes. It also

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contains a smaller amount of bismuth oxide, leading to reduced radiopacity.\textsuperscript{18} Biodentine (Septodont, Saint Maur des Foss, France) is also a calcium silicate cement, synthesized from pure raw materials contrary to the Portland cement in MTA, leading to the absence of trace elements.\textsuperscript{19} It is mixed with a water-based liquid containing calcium chloride that accelerates the setting reaction to a record of 9–12 minutes and hydrosoluble polycarboxylate-based polymer that reduces the water-to-cement ratio and maximizes its strength.\textsuperscript{20}

The objective of this study was to evaluate the sealing quality of MTA Angelus and Biodentine as repair materials for furcal perforations.

The null hypothesis was there is no significant difference in the sealing ability of a furcal perforation between MTA Angelus and Biodentine.

**Materials and Methods**

The study was done in the laboratory of Cranio-Facial Research, Oral Biology unit, at Saint Joseph University, Beirut. From a pool of 100 extracted mandibular molars, 22 first mandibular molars (n = 22) were selected for this study. Inclusion criteria consisted of teeth with intact coronal walls of at least 3 mm on three sides, well-developed unmerged roots, an intact pulp chamber, and teeth without any prior root canal preparation or obturation. The reasons of molar extractions were independent from this study. Each patient signed a written informed consent with requirements of anonymity, under an ethics-approved protocol (Tfemd/2020/34) by the ethical review committee of the Saint-Joseph University, Beirut, Lebanon.

**Preparation of the Perforations**

Teeth were kept in 5.25% sodium hypochlorite solution for 24 hours and then rinsed under tap water. The access cavities were made using a size 14 diamond burr (Dentsply Sirona, Ballaigues, Switzerland) under constant cooling water. The endo Z burr (Dentsply Sirona) was used to deroof entirely the pulp chamber and create divergent walls. Teeth were then rinsed with water and air-dried. Acid etching was applied at the canal orifices and at the apical end of each root with a 38% phosphoric acid gel (Elsodent, G-Etch, France) for 20 seconds. The Adapter Single Bond adhesive system (Tetric N-Bond, Dental Adhesive, Ivoclar Vivadent, Germany) was applied in two consecutive thin layers using a microbrush air blown before being light-cured for 20 seconds with blue light (1200 mW/cm\textsuperscript{2}, Bluephase20i, Ivoclar-Vivadent). The canal orifices and the apical end of each root were sealed with a fluid composite (SDI Wave, Restorative system A3, Australia) and hardend for 30 seconds under blue light. Later, the perforations were made in the center of the pulp floor using a round diamond bur size 12 (Dentsply Sirona) mounted on a slow-speed handpiece at 20,000 rpm under coolant water; the perforation diameter being equal to the diameter of the burr equivalent to 4 mm.\textsuperscript{21} To simulate the periodontal environment, the teeth were then soaked into a wet sponge, up to their cervical section.

**Repair of the Perforations**

Prepared teeth were then randomly divided into two groups, A and B (n = 10), treated with MTA Angelus and Biodentine, respectively, under an operating microscope (x16) (Zeiss Extaro 300, Oberkochen, Germany). A single dose of MTA powder with a small drop of liquid was gradually mixed until the desired putty consistency was obtained (Angelus Indústria de Produtos Odontológicos S/A). It was then placed using a MAP System-Endo (Produits Dentaires) and condensed using a Machtooth plunger number 3 (Dentsply Sirona).

As for the Biodentine, the capsule was gently tapped on a hard surface to loosen the powder following the manufacturer’s instructions (Biodentine Scientific File Septodont). Five drops of liquid were poured into the capsule that was placed in an amalgamator for 30 seconds. The Biodentine was condensed at the perforation site in a similar manner to the MTA.

Subsequently, a cotton pellet moistened with a saline solution was kept in the pulp chamber and the teeth were kept in an incubator at 37°C for 72 hours. The repair materials were then examined using a scratch test with a probe number 23.

**Post Build-up and Restoration**

The access cavities were filled with resin composite (Tetric N-Ceram A2, Ivoclar Vivadent, Germany), following the same bonding procedures mentioned earlier. A rubber ice cube tray was used as a support, where the teeth were dipped into the methylene blue dye.\textsuperscript{22} The molars were first covered with two layers of nail varnish and then held by their cervical parts with a wax sheet to prevent total immersion into the dye solution. Hence, only the roots of the molars were immersed. This method was adopted to isolate the roots against the penetrating dye as well as to color-code and differentiate the molars of the two groups, which were then dried for 24 hours.\textsuperscript{23} The roots were afterwards immersed in a 1% methylene blue dye for 72 hours up to their cervical sections. Then, they were rinsed for 10 minutes under running water.\textsuperscript{24,25} Using a thin marker (0.7 mm), two straight lines were drawn from the center of the furcal perforation, on the buccal and lingual side of each tooth. Their junction at the occlusal level formed a landmark, precisely locating the median line used to cut the tooth into two halves. To stabilize the molars during sectioning, clear acrylic blocks were created. The acrylic resin was poured into a plastic tray that contained the molars, held by the apex by a layer of silicone (Honigum DMG, Germany). Each molar unit was delineated by a wax mold to allow the resin acrylic to flow in a box shape.\textsuperscript{26}

Finally, the teeth were sectioned vertically using a microtome (Exakt300, Exakt Technologies, Inc., Norderstedt, Germany). Both halves were included for analysis. A specific code was assigned to each block in order to organize the statistical values.

Two additional molars were selected as negative controls, in which no furcal perforation was made, only a resin composite build-up followed by methylene blue dye penetration, sectioning, and a photograph.

**Image Processing and Parameters Quantification**

The penetration of the dye was examined for both groups. Each slice was digitally photographed using a compound microscope (Olympus CX41, Olympus, Tokyo, Japan) at a magnification of x100 with a built-in digital camera without any sample preparation (Figs 1 to 3).

The ImageJ Software [Java-based image processing program developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin)] was used to analyze the images and perform the measurements by altering the contrast of the image for greater accuracy.

A calibration between the magnification of the image and the actual value of the tooth was necessary to enable the use of the measurement software accurately. It was therefore necessary...
to calibrate the pixel value seen on the image to its respective millimeter value as measured on the tooth, and this standardization was done as follows:

First, a fixed reference on the tooth was chosen. Since the visualization of the filling material was the clearest, a measurement of its vertical median bisecting line was made using a millimeter scale. Second, the exact same measurement was replicated on the software in pixels. Subsequently, a proportional ratio of the values was made between the actual height of the material (in mm) and the ImageJ measurement system (in pixels).

Dye percolation was then converted using the following formula:

$$ \text{Dye percolation (mm)} = \text{Dye percolation (pixel)} \times \text{Magnification value} $$

$$ \text{Magnification value} = \frac{\text{True filling material length (mm)}}{\text{Filling material length on image (pixel)}} $$

Four measurements characterized each tooth: mesiobuccal (MB), mesiolingual (ML), distobuccal (DB), distolingual (DL). The same series of imaging calibration and conversion was applied for all the 22 teeth.

**Statistical Analysis**

The statistical software SPSS (version 25.0) was used to statistically analyze the data obtained. The significance level corresponds to $p$ value $\leq 0.05$, to derive the differences in leakage and seal strength in both filling biomaterials within the dental walls (mm). The mean and standard deviation were used to describe this quantitative variable. Intragroup and intergroup correlation coefficients were calculated to assess the reproducibility of the measurements between the same examiner and two different others. Shapiro–Wilk tests, Student tests and one-sample $t$-tests were used to analyze the measurements.

**Results**

**Reproducibility of Measurements of a Random Variable**

The same examiner evaluated the measurements in two different stages of eight randomly chosen teeth treated with MTA Angelus and Biodentine, four teeth of each group. The intraclass correlation coefficient (ICC) was calculated to assess intraoperator reproducibility. The CCI value was greater than 0.95 indicating excellent reproducibility.

Two different examiners evaluated the same measurements at a random set of eight teeth treated with MTA Angelus and Biodentine, four teeth of each group. The ICC was calculated to assess interoperator reproducibility. The ICC value was greater than 0.95, indicating perfect reproducibility.

**Normality of Distribution of Quantitative Variables**

This study showed that the distribution of the quantitative variable at the level of each group is not significantly different from a normal distribution ($p > 0.050$); the use of parametric statistical tests was justified. There was a significant difference in total measurements in all sides between the MTA Angelus ($p = 0.526$) and the Biodentine ($p = 0.1777$) (Table 1).

**Comparison of the Sealing Measure between the Two Biomaterials**

The mean and standard deviation of space between the biomaterials and dental walls in the MB, ML, DB, DL, and average measurement regions were illustrated in the following table (Table 2). Mean measurements of the methylene blue leakage were significantly different between the MTA Angelus and Biodentine ($p < 0.001$); dye penetration measurements were higher for the MTA group compared to the Biodentine, in all calculated regions ($p < 0.001$). On average, leakage on the buccal surfaces of the perforations showed higher leakage compared to the lingual surfaces in both materials. Total measurements of dye penetration between tooth surface and restorative material showed significantly greater values in the MTA Angelus compared to Biodentine ($p < 0.001$) (Table 2).
In addition, the mean values of leakage and inadequate adhesion were significantly different from the theoretical value 0 for the MTA Angelus ($p < 0.001$) and Biodentine ($p < 0.001$).

**DISCUSSION**

MTA Angelus and Biodentine showed a very high sealing level ($p > 0.050$). However, there was a significant difference between them ($p < 0.001$); the null hypothesis was therefore rejected. Moreover, the current results showed greater penetration of the dye at the level of MTA Angelus than Biodentine indicating that the sealing capacity of Biodentine is significantly better than that of MTA Angelus ($p < 0.001$). Yet, the measurements were still far from the theoretical value for both materials, indicating dye leakage, which can ultimately lead to treatment failure.

Any perforation of the pulp floor would cause an imbalance of the dental structure. The degree of tissue response to perforations treated with various materials depends on several factors such as severity of initial damage to the periodontal tissue, sealing ability, cytotoxicity of repair materials, bacterial contamination, time elapsed before the defect is repaired, size, and location of perforations.

In the present study, the roots of the molars were immersed into the methylene dye solution up to the cementoenamel junction, and this was to rule out the possibility of leakage of the dye from the coronal portions of the teeth. The emphasis was to investigate migration of the dye, uniquely from the outward entourage of the molar, inwards, towards the pulp chamber and solely at the furcal level. Total immersion of the molars in the dye solution, on the other hand, would put in question the seal strength of the coronal restoration, and therefore another path of leakage would alter the results examined. This was supported by Patel et al. who demonstrated that microinfiltration was more probable with MTA from the pulp chamber outward into the molar entourage.

Different leakage models have been described in studies to assess the ability of materials to seal furcation perforations including fluid infiltration, dye penetration, bacterial leakage models, dye extraction, air pressure method, radioisotope method, an electrochemical method, metal solution tracers, and reverse diffusion. Camps and Pashley showed similar results with dye extraction and fluid infiltration while saving the laboratory time with the former method using 40 teeth. Kaya et al. also concluded that the volumetric determination of dye penetration was same as dye extraction but because of simplified procedure dye extraction may be preferred for further studies. Bacterial leakage studies, though more reliable as compared with the dye studies, do not simulate the conditions of the oral cavity and require long periods of observation time. De-Deus et al. evaluated the sensitivity and seal ability of dye extraction and bacterial leakage techniques. They concluded that both techniques have low sensitivity to detect differences between the filling techniques and these differences might have been too small to detect. The use of a dye

### Table 1: Normality of the distribution of the quantitative variable at the level of the different groups

| Measurements (mm) | Groups       | Shapiro-Wilk | Statistics | dof  | p value |
|-------------------|--------------|--------------|------------|------|---------|
| MB side           | MTA Angelus  | 0.933        | 10         | 0.482|
|                   | Biodentine   | 0.892        | 10         | 0.177|
| ML side           | MTA Angelus  | 0.887        | 10         | 0.155|
|                   | Biodentine   | 0.869        | 10         | 0.097|
| DB side           | MTA Angelus  | 0.958        | 10         | 0.766|
|                   | Biodentine   | 0.851        | 10         | 0.059|
| DL side           | MTA Angelus  | 0.954        | 10         | 0.712|
|                   | Biodentine   | 0.855        | 10         | 0.067|
| Total measurement | MTA Angelus  | 0.938        | 10         | 0.526|
|                   | Biodentine   | 0.892        | 10         | 0.177|

Dof, degree of freedom

### Table 2: Comparison of leakage measurements (mm) between MTA Angelus and Biodentine

| Colorant leakage measurements (mm) | Groups       | N  | Mean     | Standard deviation | p value   |
|-----------------------------------|--------------|----|----------|--------------------|-----------|
| MB side                           | MTA Angelus  | 10 | 0.436    | 0.1533             | $<0.001$  |
|                                   | Biodentine   | 10 | 0.115    | 0.0521             |           |
| ML side                           | MTA Angelus  | 10 | 0.485    | 0.1578             | $<0.001$  |
|                                   | Biodentine   | 10 | 0.095    | 0.0709             |           |
| DB side                           | MTA Angelus  | 10 | 0.458    | 0.1378             | $<0.001$  |
|                                   | Biodentine   | 10 | 0.112    | 0.0649             |           |
| DL side                           | MTA Angelus  | 10 | 0.464    | 0.1548             | $<0.001$  |
|                                   | Biodentine   | 10 | 0.116    | 0.0508             |           |
| Total measurement                 | MTA Angelus  | 10 | 0.461    | 0.1457             | $<0.001$  |
|                                   | Biodentine   | 10 | 0.109    | 0.0483             |           |
is still considered the simplest and most cost-effective formula for detecting microleaks.\textsuperscript{31} This technique has been recommended to test the sealing properties of restorative materials in both in vivo\textsuperscript{38,39} and in vitro studies.\textsuperscript{40,41} The 1% blue methylene dye was therefore used in our study since it allows an easy quantitative measurement of the penetration area of the dye through linear measurement means.\textsuperscript{42}

High-precision cutting technology, offered by the microtome, can create cuts without damaging the composition, surface, and structure of the samples with low operator risk of injury.\textsuperscript{33} During sectioning with microtome, no more than 0.25 mm is lost of the cut material, with no deformation, compression, or stress during the process.\textsuperscript{43} Jeevani et al.\textsuperscript{44} used in their study the UV spectrophotometric analysis eliminating the chance of any loss of material.\textsuperscript{45-47}

In this study, the mean values of leakage due to inadequate adhesion were significantly different from the theoretical value 0 for the MTA Angelus (p < 0.001) and Biodentine (p < 0.001). Despite favorable results with Biodentine, none of these materials was able to totally restore natural tooth architecture. Similarly, Samuel et al.\textsuperscript{48} have well elaborated the importance of preserving natural dental architecture in endodontic treatment success, due to the incapability of materials in totally solving the floor perforation.

The reduction in hermeticity at the joint of the MTA Angelus and Biodentine perforation obtained in this study may be related to the fact that, despite efforts to properly apply materials in the perforation cavities, the application was not done with the right pressure leading to a low sealing of the products.\textsuperscript{59}

As examined by other studies, seal strength is an important characteristic that warrants being reportedly investigated to ensure dental longevity.\textsuperscript{50} Many authors reported similar hermeticity results at perforation sites due to the high sealing capacity of Biodentine and MTA Angelus.\textsuperscript{51,52} Unfortunately, the number of studies reporting MTA hermeticity is much higher compared to those evaluating the sealing strength of Biodentine.\textsuperscript{43,53,54} Despite all studies that have supported the use of MTA in the repair of perforations, Sinkar et al.\textsuperscript{55} reported that Biodentine has a better seal strength and less leakage than MTA. As suggested by this study, the seal of Biodentine warrants further research investigation to appraise its seal superiority.

Many properties must be considered when opting for a furcal perforation repair material such as the handling, the biocompatibility, and the repercussion of an overfilling.\textsuperscript{49} MTA Angelus is manually mixed and this may be viewed as a weak point compared to Biodentine, which comes in a predosed capsule.\textsuperscript{44} However, an amalgamator is essential to achieve a good consistency of Biodentine material. Concerning the setting time, both biomaterials are viewed similar, since there is no significant difference in the setting duration of one compared to the other. An interesting property of calcium silicate cements is that an overfilling beyond the limits of the perforation and into the periodontal region would not cause any adverse effects.\textsuperscript{17} This is due to their bioactive nature when in direct contact with human tissues.\textsuperscript{41} An important advantage in the clinical applications is their bonding to resin materials in the final restoration, creating an adhesive interface that is able to spread stress relatively evenly over the entire area of the bond without compromising their sealing ability.\textsuperscript{51}

Concerning the detection on the radiographs, Biodentine is less distinctive compared to MTA Angelus that contains bismuth oxide causing a radio-opacity higher than the dentine.\textsuperscript{56} Biodentine has a radio-opacity very similar to the dentine, making it harder to delineate its borders with respect to the dental walls.\textsuperscript{57} In addition, Bhavya et al.\textsuperscript{58} attributed the contact of bismuth-containing substances with NaOCl to a coronal discoloration of the tooth. Camilleri\textsuperscript{59} reported that contact of MTA and other bismuth-containing materials with NaOCl produces a change to a darker color because the oxide is converted to bismuth metal in contact with sodium hypochlorite and oxygen is lost. Beatty and Svec\textsuperscript{60} proved that Biodentine caused a perceptible color change in bovine teeth in comparison with other cements, while Marconyak et al.\textsuperscript{61} showed that this material showed significantly less discoloration compared with white ProRoot MTA, MTA Angelus, and ProRoot MTA. Shah and Banga\textsuperscript{62} also showed discolorization when Biodentine was immersed in 2% chlorhexidine.

Manufacturers claim a reduced setting time for MTA Angelus (10–15 minutes) and Biodentine (9–12 minutes). Studies showed that the hydraulic nature of calcium silicate cements requires in fact sufficient moisture for 72 hours for ideal setting to minimize the displacement during restorative procedures and provide low solubility and desirable sealing ability. For this reason, in this study all samples were covered with a wet cotton pellet and remained untouched for 72 hours.\textsuperscript{53}

Finally, it is essential to mention that a greater awareness of the anatomy of the lower molars seems important to avoid furcal involvements.\textsuperscript{1} More so, clinical trainings should focus more on how to avoid the occurrence of furcal punctures and the best protocols to properly manage their incidents.\textsuperscript{54} Most importantly, the best practice should involve the least damage to the periodontal system and preventing the worsening of tooth’s prognosis after its pulp floor injury.\textsuperscript{63}

The limitations of this study lie in its laboratory design and the reduced sample size. Less frequent extractions of lower molars are being performed before all other salvaging treatments have been attempted, including an endodontic treatment, which was an exclusion criterion from the selected sample of this study.

**Conclusion**

Based on the results of this study, it can be concluded that Biodentine showed better seal strength and is the biomaterial of choice for the treatment of furcal perforations, compared to MTA Angelus. Although Biodentine and MTA Angelus both exhibit favorable characteristics, they can significantly improve the prognosis of teeth with weakness due to perforations. Direct extrapolation of the results to the clinical situation should only be undertaken with caution, as more in vivo investigations should confirm our findings.

**Acknowledgment**

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**Ethical Approval**

This article does not contain any studies with human participants or animals performed by any of the authors. The use of human teeth was approved by the institution’s ethical committee.
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Informed Consent
All patients signed a formal consent allowing the extraction and use of the extracted teeth for experimental purpose as part of a routine procedure at the Saint Joseph School.

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