ORIGINAL RESEARCH

# Evaluation of Surface Microstructure and Compressive Strength of Mineral Trioxide Aggregate and Biodentine in the Existence and Absence of Oral Tissue Fluids

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## Abstract

**Background:** Calcium silicate cement like mineral trioxide aggregate (MTA) and Biodentine are known for their biocompatibility and are effectively used as retrograde filling materials. During their placement, the materials interact with oral tissue fluids like saliva and blood, so the aim of the present study was to assess the effect of distilled water, saline, and blood on the surface microstructure and compressive strength of MTA and Biodentine.

**Materials and methods:** In this experimental study, a total of 84 custom-made cylindrical molds were randomly allocated into two main cement groups ($n$ = 42) MTA and Biodentine. Each group was further subdivided into three subgroups ($n$ = 14) as per the testing conditions, that is, samples exposed to distilled water, saline, and fresh blood. Around 10 samples from each subgroup were tested for compressive strength using a universal testing machine (UTM), and the remaining four samples were used to examine the surface characteristics of MTA and Biodentine using a scanning electron microscope (SEM). One-way analysis of variance (ANOVA) and Tukey's *post hoc* tests were employed to calculate the mean compressive strength and standard deviation values.

**Results:** There was a significant difference in the compressive strength between MTA and Biodentine, especially in the presence of blood. During the SEM analysis, it was found that samples contaminated with blood or saline were devoid of acicular crystals in both groups. MTA group showed a more porous matrix with few hexagonal crystals than Biodentine.

**Conclusion:** Biodentine may be advantageous as a root-end filling or root repair material in the presence of blood.

**Keywords:** Biodentine, Blood contamination, Compressive strength, Mineral trioxide aggregate, Scanning electron microscope, Surface microstructure.

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## Introduction

Root-end filling materials are utilized in endodontics to efficiently seal the apical part of the tooth after surgical intervention. An effective root-end filling material and, thus, the endodontic treatment should ensure a fully sealed apex that is impermeable to fluids. The requirements for ideal retrograde filling materials are biocompatibility, should be nontoxic, noncarcinogenic, stable in the oral environment, radiopaque, antibacterial, easy to handle, adequate compressive strength, and should be able to form a good apical seal.[1]

Mineral trioxide aggregate (MTA) has been consistently used as a biomaterial since its inception in the year 1993 in dental literature and, in 1998, was accepted by the Food and Drug Administration for its use in repair of perforations, root-end retrograde filling, and root canal apical fillings.[2–4] However, it does have some drawbacks, including challenges in manipulating and compacting the cement within the root canal, as well as a lengthy setting time of approximately 3 hours.[5] In 2009,[6] Septodont introduced Biodentine, a calcium-silicate-based cement, as a "dentin substitute" created using MTA-based technology, which boasts of a reduced setting time and no tooth discoloration, thus overcoming the major drawbacks of MTA.

During the clinical application of these materials, they might get exposed to blood[7] and saliva, which may affect their properties. A study by Vanderweele et al. reported that contact with blood negatively affected the retention of MTA. Also, Nekoofar et al. revealed that contact with blood impacted the compressive strength of MTA.[8] There is a lack of sufficient literature comparing the effect of saline and fresh blood on the compressive strength as well as the surface microstructure of MTA and Biodentine. Therefore, the aim of this study was to evaluate the effect of oral tissue fluids on (1) the compressive strength of MTA and Biodentine and (2) the surface microstructure of MTA and Biodentine.

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**Conflict of interest:** None

## Materials and Methods

This study included 84 polytetrafluoroethylene (PTFE) cylindrical molds of size 2 × 4 mm were made. These molds were allotted as MTA and Biodentine ($n$ = 42, respectively).

Samples in each group were allocated to three subgroups (A, B, and C) to stimulate the clinical condition:

- Subgroup 1A: The MTA exposed to distilled water ($n$ = 14).
- Subgroup 1B: The MTA exposed to saline ($n$ = 14).
- Subgroup 1C: The MTA exposed to fresh blood ($n$ = 14).
- Subgroup 2A: Biodentine exposed to distilled water ($n$ = 14).
- Subgroup 2B: Biodentine exposed to saline ($n$ = 14).
- Subgroup 2C: Biodentine exposed to fresh blood ($n$ = 14).

In subgroups 1A and 1B, ProRoot MTA was mixed following the manufacturer's instructions, that is a 3:1 powder/liquid ratio with supplied sterile water. The material was mixed on a glass slab with a stainless steel spatula to a putty-like texture. Mixing was done for about one minute to ensure all the powder particles were hydrated. MTA was placed into the PTFE molds under minimal pressure using a mixing spatula.

In subgroups 2A and 2B, Biodentine was mixed in accordance with the manufacturer's instructions in an amalgamator (DB-338 Amalgamator; Foshan COXO Medical Instrument Co., Ltd) and was condensed into the molds.

In subgroups 1C and 2C, all the cylindrical molds of test groups were filled with freshly procured human blood using a syringe before the placement of MTA or Biodentine, respectively. Excess blood was aspirated using a syringe.

Around 2 mL Eppendorf tubes were then used to keep the undersurface of specimens in contact with distilled water, saline, or fresh blood during storage, according to the groups.

A fully saturated, humid environment was created by covering the molds in the Eppendorf tubes with a moist cotton pellet, avoiding contact with the filled material. Following 4 days of incubation at 37°C, the samples were then polished with 1200-grit fine-grain sandpaper. The samples were then removed from the PTFE matrix by cutting the molds using a disposable surgical blade. Following removal, a visual inspection of all the samples was done to ensure the absence of voids, and the samples were subjected to universal testing followed by SEM analysis.

### Compressive Strength Test

Ten samples from each group were tested in the universal testing machine (UTM) for compressive strength (PSI Sales Private Limited Serve series 50 kN) at a crosshead plate speed of 1 mm/minute till the specimen was fractured.

### Scanning Electron Microscope

Four samples from each of the six groups were analyzed with SEM (Hitachi S- 340N, Japan). Images were then evaluated qualitatively to describe the surface characteristics of specimens.

### Statistical Analysis

One-way analysis of variance (ANOVA) and Tukey's *post hoc* tests were applied to the data obtained.

## Results

### Compressive Strength

After 4 days of incubation, samples were tested for compressive strength (MPa). Among the six groups, Biodentine exposed to distilled water (subgroup 2A) showed the highest compressive strength (Table 1), followed by Biodentine exposed to fresh blood (subgroup 2C), MTA exposed to water (subgroup 1A), MTA exposed to saline (subgroup 1B), Biodentine exposed to saline (subgroup 2B), and MTA exposed to fresh blood (subgroup 1C).

### Scanning Electron Microscope

In the group of MTA exposed to saline (subgroup 1B), variable distinct crystalline formations were seen. These included angular and laminar crystals along with needle-like crystalline structures characteristic of ettringite, including barbed ball-like clusters and masses of long-span structures that interconnect with other crystals (Fig. 1).

The MTA exposed to blood showed a microstructure that consisted of a spongy matrix with several microscopic channels and fewer hexagonal crystals partly covered by an amorphous gellaceous structure. This group had more bead-like constituents, rather than the angular structures seen in MTA exposed to saline (Fig. 2).

Several microscopic pathways and a few rounded six-sided crystals partly covered by a gellaceous layer are evident in samples of Biodentine exposed to saline, and acicular structures can be seen (Fig. 3). In blood-exposed Biodentine, the formation of numerous crystalline plates and hexagonal crystals can be seen. Another

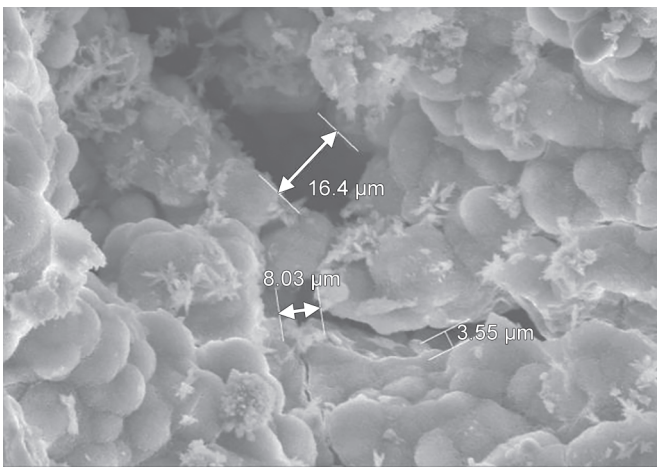


**Fig. 1:** Mineral trioxide aggregate (MTA) in saline

**Table 1:** Mean ± standard deviation of compressive strength (MPa) of MTA and Biodentine among six different experimental groups

| | *Compressive strength (MPa)* | | | |
|---|---|---|---|---|
| *Group* | *Distilled water* | *Saline* | *Fresh blood* | *F-value* |
| MTA | 67.40 ± 3.950 | 64.10 ± 5.910 | 44.20 ± 3.615 | 74.239** |
| Biodentine | 81.60 ± 5.082 | 53.80 ± 5.116 | 75.90 ± 7.490 | 59.840** |

**, $p$-value ≤ 0.01, means significant difference; MPa, megapascals; MTA, mineral trioxide aggregate

finding of small acicular crystals dispersed between well-formed polygonal and formless crystals in some areas is also evident (Fig. 4).

In the MTA specimens exposed to water (Fig. 5), sheet-like crystals with defined ends implanted in an irregular crystalline matrix containing microchannels were seen. Needle-like crystals were noticed in groups of barbed ball formations, along with large spanning forms spread across the irregular matrix. In the group of Biodentine exposed to water (Fig. 6), needle-shaped crystals and cubic crystals were seen.

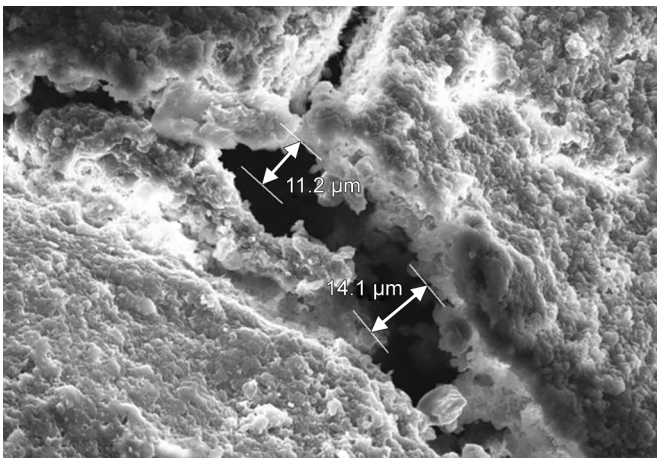


**Fig. 2:** Mineral trioxide aggregate (MTA) in fresh blood

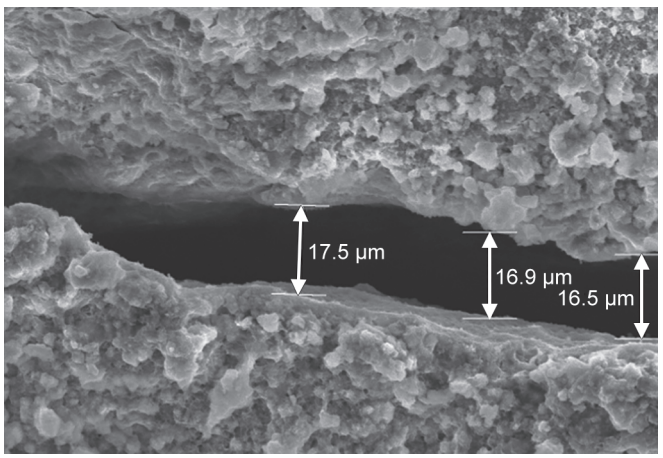


**Fig. 3:** Biodentine in saline

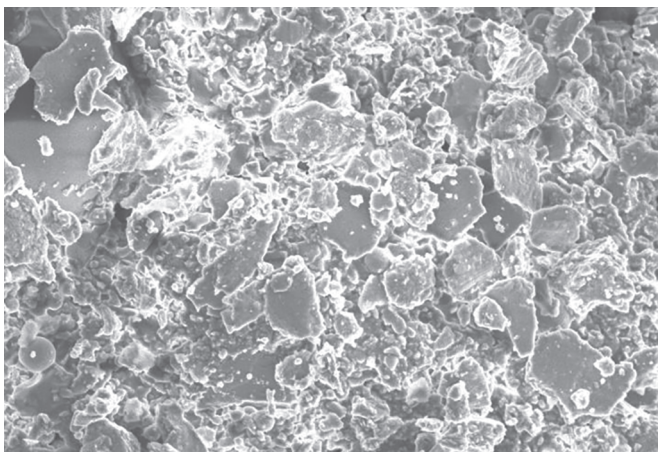

**Fig. 4:** Biodentine in fresh blood

## Discussion

Current calcium silicate cements like MTA have displayed immense potential as retrograde filling material due to their biocompatibility, but the properties of these cements have been affected by their site of placement as they constantly interact with tissues and oral fluids. As the setting reaction proceeds, more and more hydrophilic powder comes in contact with water and forms calcium hydroxide ($CaOH_2$) and calcium silicate hydrate ($3CaO{\cdot}SiO_2$, $2CaO{\cdot}SiO_2$) in the initial phase.

The setting reaction proceeds as follows:

$$3CaO{\cdot}SiO_2 + H_2O \rightarrow CaO{\cdot}2SiO_2{\cdot}3H_2O + Ca(OH)_2$$
$$2CaO{\cdot}SiO_2 + H_2O \rightarrow 3CaO{\cdot}2SiO_2{\cdot}3H_2O + Ca(OH)_2$$

Following this reaction, a spongy lattice structure called "silica gel" is formed. $Ca^{2+}$ and $OH^-$ ions combine and convert into $Ca(OH)_2$. Ettringite (or sulphoaluminate calcium) with high sulfate content ($6CaO{\cdot}Al_2O_3{\cdot}3SO_3{\cdot}32H_2O$) is formed when hydrated tricalcium aluminate ($3CaAl_2O_4$) reacts with calcium sulfate ($CaSO_4$).

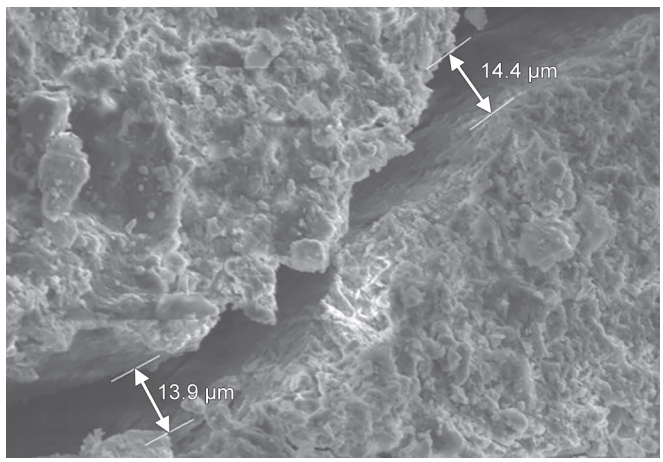


**Fig. 5:** Mineral trioxide aggregate (MTA) in distilled water

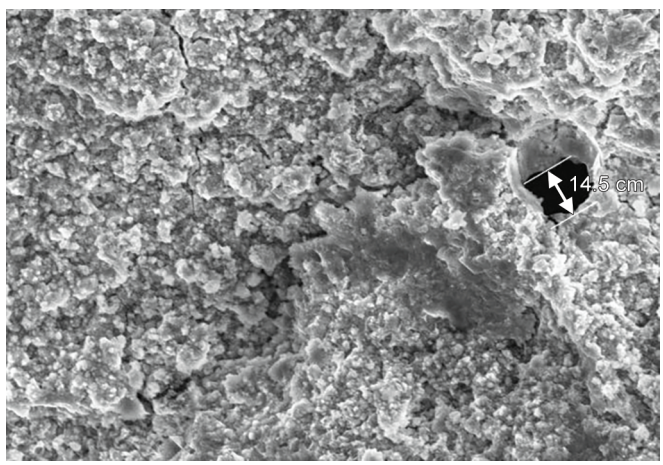


**Fig. 6:** Biodentine in distilled water

$3CaO{\cdot}Al_2O_3{\cdot}6H_2O + H_2O + CaSO_4{\cdot}2H_2O \rightarrow 6CaO{\cdot}Al_2O_3{\cdot}3SO_3{\cdot}32H_2O$

This proceeds till the consumption of all sulfate ions in the formation of Ettringite, and once they are depleted, they get converted to low-sulfate sulphoaluminate (monosulfate). The ettringite forms a breakdown when it contacts the surface of the cement molecule and a hydrated silica coating is formed.[9]

In the majority of their applications, MTA and Biodentine slurry inadvertently come into contact with blood or might become mixed with blood during application. The shorter setting time of Biodentine is an improvement compared to MTA because of the presence of a catalyst leading to prompt setting, enhancing its manipulation and stability properties. This is an upgrade over MTA since a delayed setting time (Torabinejad et al.) predisposes to a greater risk of material loss and interface alteration.

In this study, to closely mimic the clinical situation where blood becomes mixed with MTA and Biodentine, the effect of contamination with the freshly procured human blood on compressive strength and microlevel surface properties of MTA and Biodentine was investigated. As highlighted by the results of this study, these fluids negatively impact the compressive strength of MTA.

Microscopically, blood-contaminated groups presented with an altered crystalline appearance. Evaluation of the SEM images brought forth an overall lack of needle-like crystals in samples exposed to freshly procured human blood when compared to distilled water, where needle-like crystals were noticed in the cluster of barbed ball formation. The deficiency of needle-like crystals in blood-contaminated groups can be interpreted as the inhibition of the hydration process due to the lowered moisture concentration in these groups. These acicular crystals, in turn, contributed to the highest compressive strength in samples exposed to distilled water. For the progression of hydration[10] and the formation of crystalline phases, the formation of microchannels and interconnected pore networks play a crucial role. SEM images of the samples exposed to water indicate the presence of microchannels in the setting cement, allowing for a greater volume of water molecules to be incorporated, resulting in enhanced hydration.

Ismail et al. stated that the closely packed web of needle-shaped crystals radiating from the cement particles was responsible for the bond between molecules of hydrated Portland cement.[11] Stutzman, in 2004, conducted an SEM and X-ray microanalysis study on the microstructure of hydrated MTA and found that the interlocking crystalline phase was composed of $3CaAl_2O_4$ and/or tetracalcium aluminoferrite.[12] Therefore, in our research, the lowered compressive strength values of the MTA contaminated with fresh human blood can be attributed to the lack of interconnecting needle-like crystals.

The absence of interlocking needle-shaped crystals following MTA exposure to lowered pH conditions has also been reported by various studies,[13–15] which might replicate the environment of infected oral tissues that have a more acidic pH than normal.[16] In the present study, the effect of distilled water and saline was also studied as the presence of sodium chloride (in saline) allows the faster setting of calcium silicate-based cement than distilled water.[17] Elnaghy concluded in his study that Biodentine, when compared with white mineral trioxide aggregate (WMTA), is appropriate for clinical use during exposure to a more acidic solution.[18] Though both the tested materials are tricalcium-based cement, the shorter setting time of Biodentine favors its use for apical surgeries.[19]

Kayahan et al.,[20] reported that acidic environments affected various physical properties of Biodentine, but they were not affected by exposure to blood. This may be explained by the presence of accelerators—calcium chloride and calcium carbonate, which hasten the setting process and the presence of a greater volume of amorphous phase in its hydrated form, thus making this cement less susceptible to external conditions. The compressive strength of Biodentine improves with time over several hours.[21] As supported by Camilleri et al., who stated that Biodentine is denser and less porous than MTA, which explains its decreased fluid uptake.[22] Generally, the lower porosity results in better mechanical properties.[23] Hence, in this study, the denser Biodentine showed higher compressive strength.

To the best of our knowledge from the available literature, this is the first study evaluating the effect of oral tissue fluids on the compressive strength as well as the surface microstructure of both MTA and Biodentine.

### Limitation

A small sample size for determining the physical properties is one of the major limitations of this study. Although an attempt was made to recreate the oral scenario, it was not done under well-controlled conditions. More clinical studies are required to determine the effect of tissue fluids in the oral cavity on the compressive strength and surface microstructure of these materials.

## Conclusion

Within the restrictions of this study, it was seen that Biodentine had greater compressive strength values in comparison with MTA in the blood-contaminated group. At the microscopic level, blood contamination of MTA resulted in a deficiency of acicular crystals, which can explain the reduction in compressive strength. Hence, Biodentine may be more advantageous in procedures where there is excessive bleeding.

## Orcid

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