

## A CONFOCAL LASER SCANNING MICROSCOPIC STUDY EVALUATING THE SEALING ABILITY OF MINERAL TRIOXIDE AGGREGATE, BIODENTINE AND A NEW PULP CAPPING AGENT- THERACAL

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### ABSTRACT

**Aims and Objectives:** Viability and health of the pulpal tissue after an exposure can be stimulated with biocompatible pulp capping materials. Mineral trioxide aggregate(MTA),though biocompatible with excellent sealing ability, has clinical disadvantages like poor handling properties and long setting time. New pulp capping agents- Biodentine and Theracal have proven to overcome MTA's shortcomings. This study aimed to comparatively evaluate the sealing ability of Mineral Trioxide Aggregate(Angelus), Biodentine (Septodont) and Theracal (Bisco) when used as pulp capping agents. **Materials and Methods:** Occlusal cavities were prepared in extracted human third molars. The cavities were divided into 3 groups containing 12 samples each. Pulp capping in samples of group I, II and III was done with MTA, Biodentine and Theracal respectively. All samples were kept in a damp environment for 24 hrs. In each tooth, the root was cut just below the cementum-enamel junction. An aqueous 0.25% solution of Rhodamine-B was put in the open pulp chambers of all the samples and placed upside-down in a damp environment. The dye was left for 3 hrs to permeate toward the interface. After being stained, all samples were rinsed with distilled water and sectioned vertically. A Confocal Laser Scanning Microscope was used to image the samples. **Results:** No significant difference was found in interfacial microleakage between MTA and Biodentine. Theracal exhibited less interfacial microleakage than the two. **Conclusion:** Biodentine and MTA exhibit an efficient and durable protection of pulp. Theracal exhibits better sealing ability as a pulp capping agent than MTA and Biodentine.

**Keywords:** MTA, Biodentine, Theracal

### INTRODUCTION

The fundamental component of operative and endodontic treatment is to maintain the vitality of pulp with the objective of permitting continued odontogenic development. A reasoned strong argument can be made that the ideal endodontic obturation material is the vital asymptomatic pulp itself.<sup>1</sup> A wide variety of materials are being introduced in the vital pulp therapy arena but their appreciation is still lost.

Calcium hydroxide has been considered the standard of care because of beneficial properties such as induction of mineralization, high pH, and low cytotoxicity.<sup>2-4</sup> However, some of the limitations reported include dissolution

over time, mechanically weak, and presence of tunnels in the dentin barrier.<sup>3-7</sup> In addition, the handling properties are less than ideal.<sup>8</sup>

Ever since its introduction as a root-end filling material by Torabinejad in 1993, the use of MTA has subsequently been expanded to other endodontic applications which include direct pulp capping, repair of root and furcation perforations, and root apexification procedures. MTA has been recognized as a bioactive material<sup>9</sup> that is hard tissue conductive,<sup>10</sup> hard tissue inductive, and biocompatible.<sup>11</sup> Several studies have demonstrated the excellent physico-chemical properties of MTA, including the high sealing ability and adaptation to

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the dentinal walls, high radiopacity and excellent tissue response. The high quality of the material-dentin interface which improves over time secures long-term clinical success and reduces the risk of marginal percolation. The main drawbacks of this class of materials so far have been slow setting kinetics and complicated handling, which rendered these technique sensitive procedures even more difficult and restricted their use to specialists.<sup>11</sup>

New materials have been introduced in the market as an alternative to MTA and these materials claim their effectiveness in maintaining the pulp vitality. BioDentine (Septodont, Saint Maur des Fosses, France) is part of a new approach seeking to simplify clinical procedures. Biodentine is composed of a highly purified tricalcium silicate powder that contains small proportions of dicalcium silicate, calcium carbonate, and a radiopaquer.<sup>12</sup> It is dispensed in a fixed powder:liquid proportion, providing a shorter setting time of 12 min (manufacturer's data sheets), compared with the 3 to 4 hrs of MTA (Torabinejad et al., 1995).<sup>13</sup> A modified composition, the addition of setting accelerators and softeners, and a new predosed capsule formulation for use in a mixing device, largely improved the physical properties of this material making it much more user-friendly (Wang et al. 2008, Wonkornchaowalit and Lertchirakarn 2011).<sup>14,15</sup>

Theracal LC (Bisco Inc, Schamburg, IL, USA) is a new class of materials called Resin Modified Calcium Silicates (RMCS) that has been reported to stimulate apatite formation and the formation of secondary dentin.<sup>16</sup> Technically it is an alkaline calcium silicate based on the chemistry of MTA which has a lengthy history of hard tissue promoting response.<sup>17</sup> TheraCal contains approximately 45% wt mineral material (type III Portland cement), 10% wt radiopaque component, 5% wt hydrophilic thickening agent (fumed silica) and approximately 45% resin (Suh et al. 2008).<sup>18</sup> The resin consists of a hydrophobic component (comprising hydrophobic monomers) such as urethane dimethacrylate (UDMA), bisphenol A-glycidyl methacrylate (BisGMA), triethylene glycol dimethacrylate (TriEDMA or TEGDMA) and a hydrophilic component (containing hydrophilic monomers) such as hydroxyethyl methacrylate (HEMA) and polyethylene glycol dimethacrylate

(PEGDMA) (Suh et al. 2008). It is an optimal replacement to calcium hydroxide and MTA because of its optimal handling, ease of placement and enhanced biological properties.<sup>18</sup>

Sealing ability refers to the materials ability to resist micro leakage through the entire thickness of the material. Inadequate seal is a major cause of failure in any restorative procedure. It is common to examine leakage using methods like Fluid filtration, dye leakage, protein leakage or bacterial leakage. Confocal Laser Scanning Microscope has number of advantages like non destructive in nature, non dehydrated samples can be used, no specificity required for sectioning and most importantly gives three dimensional images.

The purpose of this in vitro study was to compare the sealing ability of two recently introduced calcium silicate based pulp capping agents, Biodentine and Theracal LC with the conventionally used one i.e, Mineral Trioxide Aggregate using Confocal Laser Scanning microscope.

## MATERIALS AND METHODS

36 human third molars were extracted after the appropriate informed consent was obtained from the patients. Occlusal cavities were prepared in these teeth by the use of a high-speed handpiece with No. 245 bur, and standardized to  $7 \times 4 \times 4$  mm, and a cavosurface angle of  $90^\circ$ . All the teeth were then cleaned for 5 minutes in an ultrasonic bath. These teeth were then divided into 3 groups containing 12 samples each.

In group 1, cavities were filled with the white MTA, mixed as per manufacturer's instructions. An aliquot of 0.34 g distilled water was added to 1 g MTA powder until it was saturated.

In group 2, cavities were filled with Biodentine, mixed as per manufacturer's instructions for 30 sec with an amalgamator.

In group 3, cavities were filled with Theracal LC. A thin layer of Theracal LC was placed at the base of the cavity. Light curing was done for 20 seconds each from the facial and the lingual surface of the teeth.

All samples were kept wet inside an incubator at  $37^\circ\text{C}$  for 24 hrs before being labeled and imaged. In each tooth, the root was cut just below the cementum-enamel junction by means of a water-cooled diamond disc.

**Imaging**

An aqueous 0.25% solution of Rhodamine-B was put in the open pulp chambers of all the samples. The samples were then placed upside-down in a damp environment. The dye was left for 3 hrs to permeate toward the interface.

Samples were then imaged by Confocal Laser Scanning Microscope (CLSM-A1R Model) with an 10x/1.4NA OI objective lens in conjunction with and 543-nm laser excitation and 640-nm emission filters for the Rhodamine-B.

The amount of dye penetration was measured in um using the ZEISS LSM IMAGE BROWSER

SOFTWARE (Figure 1). Statistical analysis was done using a one-way ANOVA test, independent samples t-test and Scheffers post hoc test using SPSS Version 16 for Windows.

**RESULTS**

All three materials, MTA, Biodentine and Theracal showed microleakage. Comparison of microleakage showed maximum peak values of 8.90 for MTA, 8.40 for Biodentine and 6.50 for Theracal.

The mean microleakage values, arranged in order, starting from the least to the maximum, were:

Group III: 6.50 ≤ Group II: 8.40 ≤ Group I: 8.90 (Table 2).

ANOVA was applied to test for any possibility of equality of mean among the categories. Level of significance was set at 0.05. There was no statistically significant difference in microleakage between the samples of Group I and Group II. Results of the test indicated that Group III exhibited statistically significant difference in microleakage when compared to Group I and Group II.

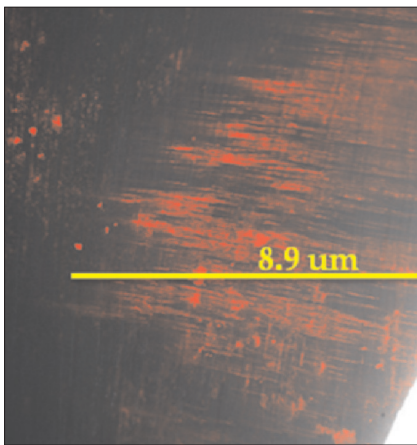
Table 1

ANOVA LEAKAGE					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	33.765	2	16.883	286.807	<.001**
Within Groups	1.943	33	.059		
Total	35.708	35			

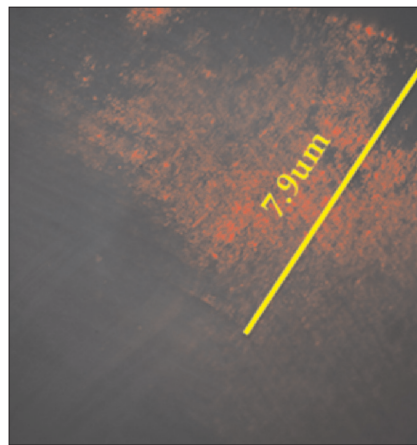
**Oneway**

Table 2

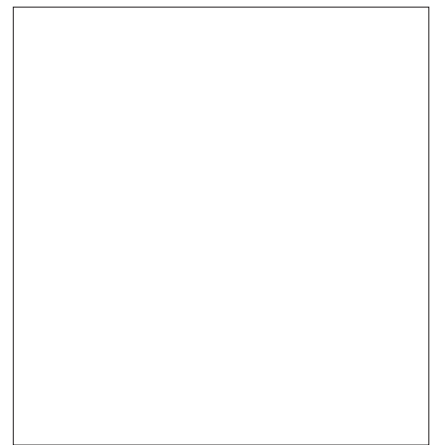
DESCRIPTIVES LEAKAGE								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
MTA	12	8.4500	.30000	.08660	8.2594	8.6406	8.10	8.90
BIODENTINE	12	8.0500	.23932	.06908	7.8979	8.2021	7.80	8.40
THERACAL	12	6.2250	.17123	.04943	6.1162	6.3338	6.10	6.50
Total	36	7.5750	1.01006	.16834	7.2332	7.9168	6.10	8.90



MTA



BIODENTINE



THERACAL

Figure 1

## DISCUSSION

The primary aim of a pulp capping material is to induce a specific hard tissue formation by pulp cells that seal the exposure site and ultimately contribute to continued pulp vitality (Schröder 1985). A liner must act as a barrier to protect the dental pulpal complex and induce the formation of new dentine bridge or dentine-like bridge between the pulp and restorative material.

MTA has now almost replaced the gold standard of pulp capping agents i.e calcium hydroxide. However, even MTA has certain drawbacks such as difficulty in handling and very long setting time which might contribute to leakage, surface disintegration, loss of marginal adaptation and continuity of the material.<sup>11</sup>

In this study, two new materials that might potentially provide the necessary properties of an ideal pulp capping agent, Biodentine and Theracal LC, were compared with MTA with regard to its sealing ability using Rhodamine B fluorescent dye and Confocal Laser Scanning microscope.

Many techniques including dyes (India ink, methylene blue), chemical tracers (silver nitrate, silver chloride), radioactive isotopes, neutron activation analysis, scanning electron microscopy and electrical conductivity have been recommended to test the sealing properties of restorative materials both in vivo and in vitro.

For the evaluation of the interface between human dentin and MTA/Biodentine/Theracal, we used confocal reflection to observe interface micropermeability as it offers a number of advantages over other techniques which include:<sup>20,21,22</sup>

- Non destructive examination of the samples
- Non- dehydrated samples can be used: Drying of samples, which is indispensable for conventional SEM or TEM, is not necessary with CLSM, leading to a decreased risk of shrinking or other drying artifacts
- No specific sectioning technique required: This decreases the possibility of artifacts produced during the preparation of the specimens by dehydration and sputter-coating procedure for SEM evaluation
- Rapid sampling is also available
- Provides three dimensional images

In this study, representative CLSM fluorescence images for the samples show a dye-infiltrated layer positioned just beneath the interface. Figure 1 shows the distribution of Rhodamine-B permeating from the pulp and diffusing into the cement, but barely into the intertubular dentin.

The results of the present study showed that Biodentine exhibits good sealing ability which is comparable to that of MTA. However, Theracal LC exhibits sealing ability which is significantly better than both Biodentine and MTA.

The maximum peak values of microleakage exhibited by Biodentine is 8.40 which is comparable to the maximum peak values for MTA i.e 8.90.

Biodentine™ has been shown to be biocompatible, i.e. it does not damage pulpal cells in vitro or in vivo, and is capable of stimulating tertiary dentin formation. Hard tissue formation is seen both after indirect and direct capping with Biodentine.<sup>23-25</sup> Used for pulp capping, the material offers some benefits versus calcium hydroxide as well as MTA: It is stronger mechanically, less soluble and produces tighter seals.<sup>26</sup> This qualifies it for avoiding three major drawbacks of calcium hydroxide, i.e. material resorption, mechanical instability and the resultant failure of preventing microleakages.<sup>27</sup> At the entrance of the dentine tubules, some mineral re-crystallisation occurs, creating mineral tags. This induces micromechanical anchorage of Biodentine™. This process continues with time, improving the sealing.

Dejou evaluated the micro leakage resistance of Biodentine™ in comparison with one of the best sealing systems, resin modified glass ionomers (Fuji II LC, GC Corp.). Biodentine™ exhibited better leakage resistance both to enamel and to dentine compared to Fuji IILC.<sup>23</sup>

Compared to Mineral Trioxide Aggregate, Biodentine™ handles easily and needs much less time for setting.

Both MTA and Biodentine contain the apatite-stimulating calcium silicates that have been shown over many years to provide improved benefits of dentin stimulation and pulpal protection as compared to traditional calcium hydroxide and RMGI alone. Unfortunately, the cost per use, the slow setting qualities, and solubility hinder the use of these materials in everyday direct restorative dentistry.



TheraCal™ LC is dentistry's first radiopaque, HEMA-free, light-curable flowable resin containing “apatite stimulating” calcium silicates. It is the first of a new class of internal flowable materials to serve in protecting and stimulating pulpal repair and will likely be described by the profession as a light-curable resin-modified calcium silicate.<sup>28</sup>

In the present study, there was significantly less microleakage in Theracal LC when compared with Biodentine and MTA. The maximum peak values of microleakage for Theracal LC was 6.50 which is significantly less as compared to MTA i.e 8.90. Research supports that calcium silicates stimulate the formation of hydroxyapatite (HA) onto the surface of the supplying material and provides a biologic seal.

TheraCal LC's hydrophilic resin formula is unique. It is permeable to dentinal fluid but relatively insoluble to resist dissolution. TheraCal LC may act or resemble a scaffold for dentin formation. Dentinal fluids are readily absorbed within it resulting in the release of calcium and hydroxide ions. Immediately one of the tooth's responses is to form apatite (HA) to the undersurface of TheraCal LC potentiating the natural sealing ability of the product.<sup>11,16,29-32</sup>

Theracal LC has been approved as “apatite stimulating” by the US Food and Drug Administration and is an interactive flowable resin that provides the early high alkalinity, pH 10 to 11, required for pulpal healing but reverts back to a neutral pH after several days.<sup>31</sup> It is self-sealing, which aids in antimicrobial activity with initial bonds to dentin to resist accidental air-drying removal. This high calcium release has been shown to be critical for the stimulation of apatite formation and secondary dentin bridge formation while providing a mechanical seal of the pulp without adhesive.<sup>16</sup>

## CONCLUSION

Recent modifications in the calcium silicate based technology i.e, Biodentine and Theracal exhibit good sealing ability to dentin when compared to conventionally used Mineral Trioxide Aggregate. Biodentine and Theracal are interesting and promising products, which have the potential of making major contributions to maintaining pulp vitality in patients judiciously selected for direct pulp capping.

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