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# Comparative Evaluation of Antimicrobial Properties of a Novel Calcium Silicate Cement: an in Vitro Study

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**Abstract:**

Aim:

The main objective of this particular study was to assess the antimicrobial properties of Biodentine, MTA in comparison to a novel calcium silicate material.

Materials and Method:

The three materials used in this study were MTA, Biodentine and a new calcium silicate material. The method of assessing the antimicrobial properties of the various cements was through the agar diffusion method at 24 hours. Ten samples were taken in each group. Statistical analysis was conducted using Analysis of variance (ANOVA) and Tukey's post hoc test to compare the groups.

Results:

The mean and the standard deviation values were taken of the measured (in $mm^2$) inhibition zones formed by the tested calcium silicate-based cement- MTA, Biodentine and a new calcium silicate cement against S. mutans and E.

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faecalis within 24 h were recorded. It was found that the new calcium silicate material had a larger zone of inhibition compared to MTA and Biodentin (31 +/- 6.5) and (29 +/- 6) against Streptococcus mutans and Enterococcus faecalis respectively. The results showed a p-value>0.05, which means that the New calcium silicate material shows significantly better antibacterial properties when compared to the other two materials.

Conclusion:

The results of the study revealed that the largest inhibition zone was formed around the New calcium silicate material. This suggests that the new silicate material is more potent in its antibacterial properties when compared to MTA and Biodentine.

**Introduction:**

The main objective of pediatric dentistry is to preserve the primary teeth in their normal functional and anatomical position till the period of normal exfoliation and physiological eruption of the succedaneous tooth (Xiaoqiu 2008). This partakes a major role in maintaining the development of phonetics, speech and masticatory function in children(Vittoba Setty and Srinivasan 2016). The primary teeth help in maintaining the dental arch, and aesthetics and prevent space loss.

Caries affects children worldwide as it progresses faster in primary teeth which often involve the pulp, resulting in infection and pain(Mathur and Dhillon 2018). Current literature describes various treatment techniques to manage pulpal treatment. Pulpotomy is a vital pulp therapy wherein the infected coronal pulp is removed and the unaffected pulpal tissue is capped with a biocompatible material(Fuks and Peretz 2016). An ideal cement used in pulp capping procedures is required to have superior physiological and biological properties which include being non-resorbable, tight seal, biocompatible and antimicrobial action. An antibacterial pulp capping agent plays a role in avoiding failures in conservative endodontic treatment as it reduces the infection and inflammatory reaction of the radicular portion of the pulp(Atom et al. 2021).

Formocresol is the gold standard material used for pulpotomy which is derived from formaldehyde(Marghalani, Omar, and Chen 2014). This is characterized by tissue fixation and does not cause tissue repair. In spite of the frequent usage of formocresol, controversies involving the toxicity and carcinogenic potential of the material have led to a decline in its usage over the recent years(Lewis and Chestner 1981).

Ever since the discovery of MTA by Torabinejad in 1993 to Biodentine produced by Septodont in 2010, there has been an increase in the role of calcium silicate cement in dentistry. MTA comprises different oxides namely calcium, and silicon, along with oxides of ferrous, aluminium and magnesium. This is remotely similar to a refined version of portland cement which is readily available in stores and additionally mixed with Bismuth for radiopacity. The most advantageous property of MTA is that it can set in the presence of saliva(Subramanyam and Vasantharajan 2017). Therefore the setting of the cement remains unaffected in the presence of blood and saliva. There is a wide range of uses of MTA, for example, it is used in endodontic procedures(Cervino et al. 2020) like inducing an apical plug, in the closure of perforations of the root, and for direct pulp capping. In spite of the various advantages of MTA, some of its disadvantages(Islam, Chng, and Yap 2006) are due to its prolonged setting time, crown discolouration, difficult handling properties, the compressive and flexural strength of the material is weak and is advised to not use this material in the form of a restorative base. The material is comparatively costly as well.

In 2010, when Biodentine was first introduced, it was marketed as a new substitute for dentine(Kusum, Rakesh, and Richa 2015; Koubi et al. 2012), consisting of a powder in a capsule and liquid sealed in a tiny plastic bottle. The

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powder mainly consists of both tricalcium and dicalcium silicate which are the vital components of MTA and Portland cement. The liquid component consists of an accelerant which is calcium chloride and a hydrosoluble polymer which is polycarboxylate(Camilleri 2013). The liquid is added to the capsule which consists of the powder and is triturated for 30 seconds. This mixture can be used in the place of MTA as a pulpotomy agent and additionally used in cases of internal and external root resorption, apical plug formation, pulpotomies and in the filling of the cervical portion of the crowns. A new novel calcium cement has been prepared whose properties are similar to Biodentine and MTA. The objective of this particular study was to assess the antimicrobial properties of Biodentine, MTA in comparison to a novel calcium silicate material.

**Materials and method:**

The materials evaluated in this study were Biodentine (Septodont) , MTA (Angelus) and a new calcium silicate material. The microbial activity of ten samples in each cement was evaluated against Streptococcus mutans and Enterococcus Faecalis using three different concentrations -20, 50 and 100µl. Inoculation was done by brushing the culture media with the use of a sterile cotton swab. Wells measuring 4mm in diameter and 4mm in depth were prepared on plates with a copper puncher, and under aseptic conditions according to the manufacturer's instructions. These were then immediately filled with the freshly manipulated test materials. The plates were then incubated at 37°C in an incubator for 24hrs and evaluated post the test period. Zones of bacterial inhibition were assessed and photographed. The zones were measured in millimetres (mm), using a vernier calliper. The measurements were taken by measuring the distance between two points at the outer limit of the ring which was formed around the well. This test was performed ten times to confirm the homogeneity of the results. The results were expressed as the mean and standard deviation and data were analyzed statistically by one-way analysis of variance using SPSS software version 22.0 (SPSS Inc., ChicagoIII., USA).

**Results:**

The means and the standard deviation values of the largest diameter(in mm) of the zone of inhibition formed by the tested calcium silicate-based cements- MTA, Biodentine and a new calcium silicate cement against S. mutans and E. faecalis within 24 h were recorded. It was observed that the new calcium silicate material had a larger zone of inhibition (31 +/- 6.5) and (29 +/- 6) with respect to both the organisms in question as compared to MTA with inhibition of (10.6 +/- 1.15) and (9 +/- 0.5) and Biodentin (10+/- 1.73) and (10+/-1.5) against Streptococcus mutans and Enterococcus faecalis respectively. Statistical analysis was conducted using Analysis of variance (ANOVA) and Tukey's post hoc test was conducted for comparison of groups.The results showed a p-value>0.05, which means that the New calcium silicate material shows significantly better antibacterial properties when compared to the other two materials.

| Pathogen | Zone of Inhibition (Mean in $mm^2$) | | |
|---|---|---|---|
| | MTA | Biodentin | New Calcium Silicate Material |
| Streptococcus mutans | 10.6 +/- 1.15 | 10+/- 1.73 | 31+/-6.5 |
| Enterococcus Faecalis | 9 +/- 0.5 | 10+/-1.5 | 29 +/- 6 |

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**Discussion:**

The long term success of any vital pulp therapy is the maintenance of the pulp vitality which allows the tooth to remain in the oral cavity and withstand heavy masticatory forces(Vij et al. 2004). This study was conducted to evaluate the antibacterial activities of three calcium silicate-based cements- MTA, Biodentin and a new calcium silicate-based material. S. mutans and E. faecalis were included because of the action of S.mutans as one of the major causative agents for the pulpal lesion that initially occurs and its subsequent pulpal pathology(Zayed, Hassan, and Riad 2015). E.faecalis is commonly seen in cases of failed endodontic therapy(Rôças, Siqueira, and Santos 2004).

Many studies have been conducted evaluating the antibacterial effects of various calcium silicate cements and have shown contradicting results. Some studies showed that MTA was effective against E faecalis while other studies proved its limited antimicrobial activity(Morgental et al. 2011). The study conducted by Parirokh and Torabinejad et al found that there was antibacterial effect on facultative bacteria but no such effect was seen on strictly anaerobic bacteria with the usage of MTA(Parirokh and Torabinejad 2010), this shortcoming was overcome by adding Metronidazole to the new calcium silicate material which proved to have a wider range of action when compared to Biodentin and MTA(Müller 1983).

Apart from its antimicrobial action, MTA is a hydrophilic biocompatible cement that exhibits dentin like mechanical properties and can be utilized as a dentin substitute as it stimulates the formation of tertiary dentin which leads to a positive result on vital pulp cells(Jain, Gupta, and Agarwal 2018).

The recently introduced Biodentine over MTA is superior due to the setting time of the cement as well as its greater viscosity. The application of MTA during direct pulp capping necessitates two dental visits- placement of MTA in the first visit and the placement of a final restoration after the MTA has hardened in the second visit. Two visits may increase the risk of bacterial contamination. This disadvantage is overcome by using Biodentine in a single visit.

Regarding our results, all three materials showed bacterial inhibition. The new calcium silicate material showed the best results when compared to MTA and Biodentin against Streptococcus mutans and Enterococcus Faecalis which are commonly found in primary teeth(Tchaou et al. 1995). Calcium silicate cements are slowly being preferred over other materials. The new material contains metronidazole which acts against anaerobic bacteria. This can reduce the chances of flare-up and failure of pulpotomy procedures.

In this study, diffusion by agar plate method was the method for evaluating the antibacterial properties of the silicate materials. The process required fewer materials and was inexpensive. It evaluated both set and unset materials. The limitation of this method is that it is unable to distinguish whether the material is bactericidal or bacteriostatic. The test was done in a laboratory setting and does not stimulate the same conditions as in the oral cavity.

**Conclusion:**

The results of the study revealed that the largest inhibition zone formed around the New calcium silicate material. This suggests that the new silicate material is more potent in its antibacterial properties when compared to MTA and Biodentine.

**Conflict of interest:** The authors declare that they have no conflict of interest.

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Fig 1: Agar diffusion plates containing S. mutans and E.Faecalis with different concentrations of Biodentine.

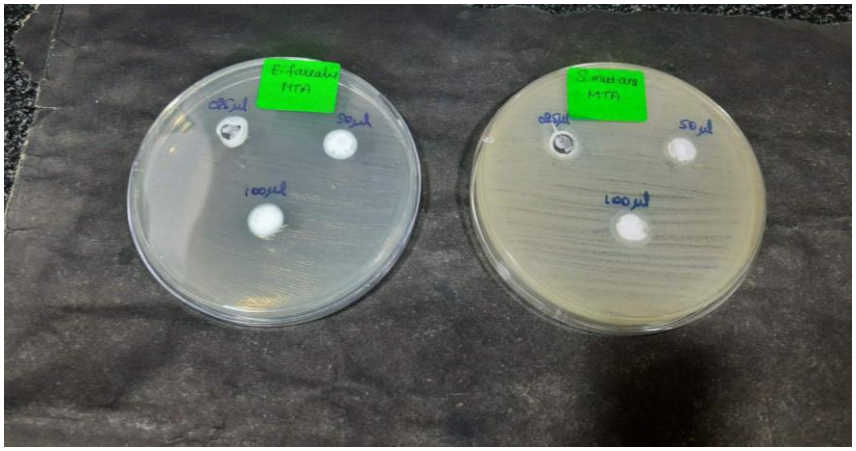

Fig2: Agar diffusion plates containing S. mutans and E.Faecalis with different concentrations of MTA.

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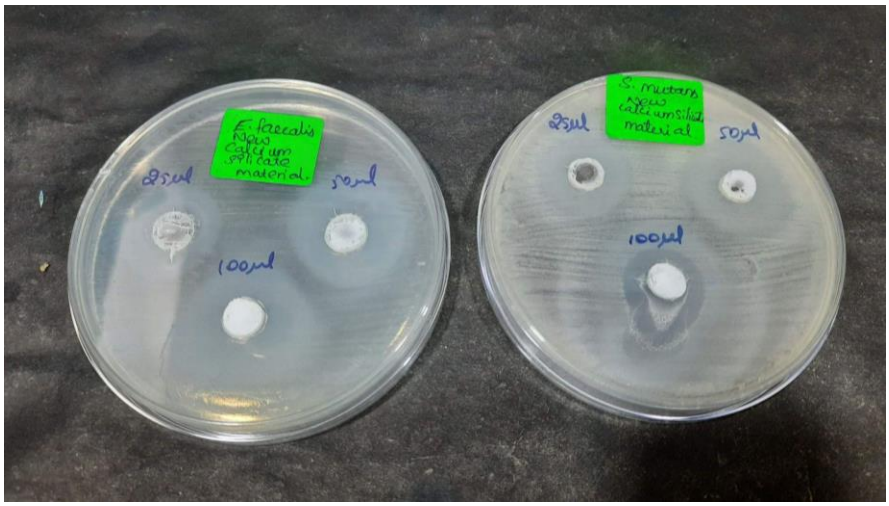


Fig 3: Agar diffusion plates containing S. mutans and E.Faecalis with different concentrations of a new calcium silicate cement .