Original Research

Comparative Evaluation of Sealing Ability of Calcium Sulfate with Self-etch Adhesive, Mineral Trioxide Aggregate Plus, and Bone Cement as Furcal Perforation Repair Materials: An *In vitro* Dye Extraction Study

Abstract

Aim: The aim of this study was to determine the sealing ability of three different materials mineral trioxide aggregate (MTA) Plus, bone cement, and calcium sulfate with self-etch adhesive (SEA) for the repair of furcal perforation, using dye extraction method. **Materials and Methods:** Forty-eight extracted human permanent first and second molars were included and randomly divided into four groups: Group 1, n = 12, negative control, perforation not repaired with any material, Group 2, n = 12, perforation repair material used, MTA Plus, Group 3, n = 12, perforation repair material used, calcium sulfate with SEA, Group 4, n = 12, perforation repair material used, bone cement. The teeth were then coated with two coats of clear nail varnish immersed in methylene blue dye for 24 h, kept in 65% concentrated nitric acid for 3 days. Dye leakage was measured with the dye extraction method using a spectrophotometer at 550 nm. **Results:** The negative control showed the highest mean values of dye absorbance (1.45). Bone cement (0.94) came second. Calcium sulfate with SEA (0.58) and MTA Plus (0.32) had no significant difference in their dye absorbance values. **Conclusion:** Within the limitations of the study, MTA Plus showed the least microleakage followed by calcium sulfate with SEA which has shown promising results and can be used as an alternative followed by bone cement which showed the highest microleakage.

Keywords: *Bone cement, calcium sulfate, dye extraction, mineral trioxide aggregate Plus, self-etch adhesive*

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Background

Root canal treatment is a dental procedure in which the diseased or damaged pulp of a tooth is removed and cleaned, and the root canal spaces are filled with biologically inert materials and sealed. Thus while performing the treatment, procedural accidents are encountered that will affect the prognosis of the root canal treatment. One of these accidents is furcation perforation.[1] Perforations are unfortunate complications which account for at least 10% of endodontic failures in endodontic treatment.[2] The causes of perforations are resorptive defects, caries, and iatrogenic events that occur during and after endodontic treatment.[3] If perforations are not repaired, it will lead to bacterial ingress leading to complicated endodontic-periodontal lesions. An ideal perforation repair material should provide an adequate seal, be biocompatible, cost-effective, dimensionally stable, insoluble, radiopaque, and allow easy manipulation and placement.[4]

Mineral trioxide aggregate (MTA), which is a calcium silicate-based material, is currently the choice of material in perforation repair. The sealing ability of MTA has shown to be superior to amalgam, zinc oxide-eugenol, resin-modified glass ionomer cement (GIC), and resin materials. Furthermore, cytotoxicity of MTA has been found to be less than super EBA.[4] MTA used for the perforation sealing has almost all the characteristics required to become an ideal material for furcal perforation repair. Despite its many advantages, MTA has some drawbacks such as long setting time (around 24 h), less compressive and flexure strength, nonbonding to enamel and dentine, and discoloration of teeth.[5] Thus, an improved version of MTA, that is, MTA Plus (Prevest DenPro) has been used in this study, which is fast setting as claimed by the manufacturer.

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Received : 11-12-2016
Revised : 31-12-2017
Accepted : 23-02-2018
Published : 18-11-2019

Access this article online
Website: www.ijdr.in
DOI: 10.4103/ijdr.IJDR_788_16
Quick Response Code:

How to cite this article: Shah S, De R, Kishan KV, Ravinathanan M, Shah N, Solanki N. Comparative evaluation of sealing ability of calcium sulfate with self-etch adhesive, mineral trioxide aggregate plus, and bone cement as furcal perforation repair materials: An *in vitro* dye extraction study. Indian J Dent Res 2019;30:573-8.

Bone cement is a potentially new repair material that has been investigated in dentistry recently as a root-end filling material. It has been in use in orthopedic surgery for the past 40 years. Bone cements have many characteristics for a variety of endodontic treatments. Those are, good strength and load bearing capacity, good handling and working properties. Bone cement has faster setting time (around 15min) and can tolerate moist environment very well. It also has good marginal adaptation as a root- end filling material with low cytotoxicity comparable to MTA.[6]

Calcium sulfate is easily available and cost-effective and used as internal matrix under various perforation repair materials such as MTA, biodentine, and light-cure GIC. It has proved to provide a good barrier against the extrusion of the repair materials. It has the ability to exclude epithelial tissue from the site of bone formation and has a rapid rate of resorption which coincides with the rate of new bone growth.[7]

Calcium sulfate has been always used under perforation repair materials, and very scarce literature is available of it being used as perforation repair material alone, or with a self-etch adhesive (SEA) as per various search engines such as PubMed, ScienceDirect, Medline dated July 1, 2016. Hence, this study was undertaken to check the sealing ability of calcium sulfate when used with a SEA-containing bis-methacrylamide dihydrogen phosphate (MDP) in comparison to MTA Plus and bone cement.

The null hypothesis is that there will be no difference in the sealing ability of MTA Plus, bone cement, and calcium sulfate with SEA when used as a furcal perforation repair material.

Procedure

Forty-eight numbers of extracted human permanent first and second molars extracted purely for periodontal reasons were selected. Carious teeth with fused roots, hypoplastic teeth, cracked teeth, teeth with existing restorations, and fractured teeth were excluded from the study. All the samples were divided into four groups; each specimen was cleaned of any tissue remnants on the roots and disinfected in 0.5% chloramine T for 15 days, scaled by periodontal scalers, and subsequently stored in 0.9% normal saline solution. Endodontic access cavity was prepared with Endo-access bur for initial entry followed by Endo-z bur for lateral extension and finishing of cavity walls, with the use of high-speed air turbine handpiece. Orifices of the canals were negotiated and temporary filling material will be placed on the orifice of each canal. Intentional perforation was created on the pulpal floor by using a high-speed long shank round bur no. 4 (1.4 mm × 1.4 mm) such that it was between the mesial and distal orifices. Specimens were placed in tubes containing cotton moistened with saline to simulate the clinical condition.

Forty-eight molars were divided into following groups.

Group 1: Negative control (n = 12) perforation done (no sealing).

Group 2: MTA Plus (positive control) (n = 12 each): one scoop of MTA Plus powder and one small drop of MTA Plus gel, 1 ampoule next to the powder was dispensed on a nonabsorbent pad. Mixing was done with a plastic spatula in circular motion until a putty consistency of the mix is obtained. The mix is carried to the perforation site with the help of endodontic plugger and adapted to the perforation site. A moist cotton pellet was placed over MTA Plus, and the cavity was sealed with cavit.

Group 3: Calcium sulfate (n = 12) was mixed with sterile water. The mixed putty was placed into the perforation and condensed with endodontic pluggers until a base is created under the perforation. After settings of CS, a layer of SEA was applied over it and light cured for 10 s.

Group 4: Bone cement (n = 12) powder and liquid was dispensed on a nonabsorbent paper pad in a 2:1 ratio and mixed in a circular motion with a plastic spatula until the mix is into the putty stage of setting. The mix is carried to the perforation site with endodontic plugger and adapted to the perforation site.

After sealing of perforation and setting of all materials, every molar was completely covered including cavity walls and pulpal floor by two successive layers of clear nail varnish except the area 1 mm approximately around the margin of the perforation. All specimens were allowed to set for 1 h and later kept in 100% humidity for 24 h. Molars were then placed in test tubes according to each group. Methylene blue dye was added in the test tubes, and molars are immersed for 24 h for checking the sealing ability by dye extraction method. Molars were placed under running tap water for 30 min to remove all residues of methylene blue. Molars were then placed in vials containing 1 ml of concentrated (65%) nitric acid for 3 days for the extraction of the dye. Centrifugation of vials was done at 5000 rpm for 5 min to separate debris from the extracted dye. The supernatant was transferred from each sample to a measuring cubet. Then, the sample absorbance was determined by an automatic microplate spectrophotometer at 550 nm. The values were evaluated, tabulated [Table 1], and subjected to statistical analysis, and comparison was made between all groups and difference in sealing ability was evaluated.

Results

Statistical analysis was performed by using one-way analysis of variance comparing the means [Table 2]. A *post hoc* test [Table 3] was used for pair-wise comparison between the means when analysis of variance test was significant. The statistical significance level was set at $P \leq 0.05$.

The negative control showed the highest dye absorbance (1.45), whereas MTA Plus showed the least amount of dye absorbance (0.32), followed by calcium sulfate with SEA (0.58) and bone cement (0.9). However, there was no significant difference between dye absorbance of MTA Plus and calcium sulfate with SEA. There was statistically significant difference present between the dye absorbance values of bone cement and calcium sulfate with SEA, also between dye absorbance values of MTA Plus and bone cement. The graph below [Figure 1] compares the means of all the groups.

Table 1: Values obtained from spectrophotometer for all samples

Sample number	Group 1: Negative control	Group 2: MTA Plus	Group 3: Calcium sulfate with self-etch adhesive	Group 4: Bone cement
1	1.898	0.514	0.701	1.105
2	1.627	0.203	0.644	0.901
3	1.5	0.262	0.56	0.926
4	1.246	0.384	0.55	0.995
5	1.203	0.464	0.537	0.902
6	1.25	0.414	0.601	0.858
7	1.798	0.303	0.544	1.101
8	1.727	0.162	0.66	0.905
9	1.4	0.484	0.65	0.930
10	1.346	0.364	0.507	0.991
11	1.203	0.202	0.524	0.898
12	1.25	0.102	0.494	0.862

Table 2: Mean, Standard Deviation (SD) Values for Absorbance Units (AU) of four groups of materials

Materials	*n*	Mean	SD	SE	ANOVA *P*
Negative control	12	1.454	0.2746	0.1121	<0.001
MTA Plus	12	0.322	0.1594	0.0651	
Calcium sulfate + SEA	12	0.581	0.0765	0.0312	
Bone cement	12	0.948	0.0890	0.0364	

ANOVA=Analysis of variance, SEA=Self-etch adhesive, SD=Standard deviation, SE=Standard error

Table 3: Comparison between individual groups

Pair-wise comparison	Between the means	Mean difference (I–J)	SE	*P*
Negative control	MTA Plus	1.1325	0.0977	<0.001*
Negative control	Calcium sulfate + SEA	0.8730	0.0977	<0.001*
Negative control	Bone cement	0.5057	0.0977	0.0002*
MTA Plus	Calcium sulfate + SEA	−0.2595	0.0977	0.0668#
MTA Plus	Bone cement	−0.6268	0.0977	<0.001*
Calcium sulfate + SEA	Bone cement	−0.3673	0.0977	0.0062*

*High significant difference, #No significant difference. SE=Standard error, SEA=Self-etch adhesive

Discussion

Irrespective of the location or the etiology of furcation perforations, they interfere with the prognosis of the root canal-treated tooth. It is like a vicious circle of the seeping bacteria from the periapical tissues which releases harmful tissue-damaging toxins causing inflammation and infection which ultimately leads to the failure of RCT. Thus, it should be sealed as soon as possible.[4]

Mandibular and maxillary molars were used in this study to create perforation. As Kvinnsland *et al*. found that the first molars were the teeth most frequently perforated. It might be because that these teeth are also the most heavily restored teeth in the oral cavity.[8] Almost 73% of the cases reported in their study were of molars, of which maxillary molars were higher in number compared to mandibular molar.

In the current study, the sample was stored in 0.5% chloramine T until use. Because the solution provides disinfection as well as it prevents the dehydration of the extracted teeth. Human teeth are considered as a prospective source of bloodborne pathogens; therefore, it is essential to eliminate any organism capable of transmitting disease from extracted human teeth. Furthermore, chloramine T was opted in the current study as it has no adverse effect on the organic structure of dentin.[9]

Furcation perforations were induced by a #4 long shank carbide round (SS White) bur from pulpal floor to furcation area. This resulted in perforations of almost 1.4 mm in diameter. The prognosis of tooth with furcation diameter 1–1.5 mm is better than those having much bigger perforation diameter sizes.[10] Thus, the diameter of perforation done in this study is justified.

All teeth were painted with coat of nail polish to prevent dye penetration into lateral and accessory canals, especially in furcation area. Gutman, in his study on furcation region of permanent molars, had reported patency and the ability of fluid to pass through accessory canals in furcation.[11]

The dye used was methylene blue dye; it has got low molecular size (MW = 319.868 g/mol-1). The internal

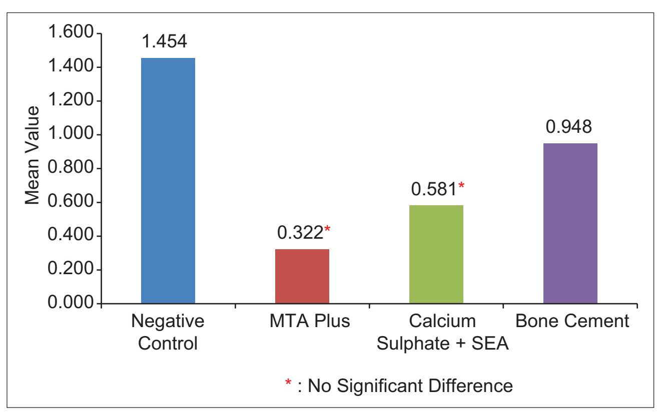

Figure 1: Bar Graph Comparing mean of all the Groups

diameter of the dentinal tubules (1–4 μm) is larger than the particle size of dye making it suitable for use in dye extraction technique. Preparation is simple and inexpensive, and the usage of complex laboratory equipment is not required for it. Furthermore, Tifeng Jiao *et al*. stated that it has got a high removal rate, which reaches above 95% within only 30 min.[12]

The dye penetration techniques have long been used in endodontics because of its ease of performance and difficulty of other available techniques. However, it has got several disadvantages such as the smaller molecular size of the dye molecules than bacteria, which do not measure the actual volume absorbed by the sample but merely measure the deepest point reached by the dye.[13,14] Actual depth of penetrated dye is very difficult to know as it depends on the random cutting of the tooth into two. Despite these drawbacks, Torabinejad *et al*.[13] stated that a material that is able to prevent the penetration of small molecules (dye) should be able to prevent larger substances such as bacteria and their by-products. Based on the explanation by Torabinejad, the dye extraction method seems to be a reliable technique, as it takes into account all absorbed dye by the samples. Furthermore, Camps and Pashley[14] reported that the dye extraction method had the same accuracy as the fluid filtration method and also saved much laboratory time.

Negative control samples in which perforations were not repaired had the highest dye absorbance of all groups (1.45) denoting the accuracy of the technique. MTA Plus demonstrated least amount of microleakage (0.322). MTA Plus was closely followed by calcium sulfate with SEA group (0.58). Although there was no significant difference present between the sealing ability of MTA Plus and calcium sulfate with SEA, bone cement showed highest values of microleakage (0.948) in the experimental groups.

MTA Plus (Prevest DenPro) showed the best sealing ability among all the groups, resulting from its hydrophilic properties and formations of an interfacial layer between the material and dentin.[15] The interfacial layer reduces the risk of marginal percolation and gives promising long-term clinical success. Kubo *et al*. found that the further hydration of MTA powder by moisture can result in an increase in the compressive strength and decrease leakage. This can be of significance as the environment in the furcation area is always moist further strengthening the MTA. Sakar *et al*. demonstrated that MTA has the ability to precipitate hydroxyapatite crystals in the presence of fluid which may be relevant in minimizing leakage thereafter.[16] Furthermore, the better performance of the new MTA Plus could be attributed to the finer particle size and the presence of an anti-washout gel which drastically increases the anti-washout resistance of MTA Plus.

Camilleri *et al*. determined that the crystalline particles in MTA Plus were smaller (50% of the particles finer than the 1 μm) than those present in ProRoot MTA although the chemical composition was found to be similar. Smaller particle size is important for physical properties as it will increase the surface available for hydration and cause greater early strength as well as ease of handling.[17] There are not many studies published comparing the new MTA Plus. Formosa *et al*.[18] were the first to investigate the chemical and physical properties of the novel MTA Plus. The anti-washout gel changed the rheology and properties of the material; it had a far more viscous and rubbery consistency and is most dough like. This consistency also improves the handling characteristics of the material; It did not affect the radiopacity of resultant material and an increase in compressive strength is observed.[19]

Calcium sulfate has always been used under perforation repair materials as internal matrix but never used as perforation sealing material alone. In the present study, the experimental protocol of using calcium sulfate with SEA (Tetric N Ceram, Ivoclar) was successful; this promising performance of CS + SEA can be attributed to the 10-methacryloxydecyl dihydrogen phosphate (10-MDP) present in the SEA. It forms strong ionic bonds with calcium from tooth and calcium sulfate. 10-MDP, an acidic functional monomer used in SEAs, will chemically bond to calcium resulting in stable bonds providing micromechanical interlocking as well.[20] Besides the strong chemical affinity to calcium, a specific property of 10-MDP is that it builds a particular nano-sized structure that improves bond stability: the self-assembled so-called "nano-layering".

Bone cement (CMW, DePuy, Johnson and Johnson, England) is common in the practice of orthopedic surgery, especially in artificial hip replacement. The material is packaged as a powder (polymethylmethacrylate polymer, methyl methacrylate-styrene copolymer, and barium sulfate) and a liquid (methyl methacrylate monomer) that are mixed at the time of application.[21] The increased microleakage in case of bone cement can be attributed due to its polymerization shrinkage and subsequent porosities at the dentin cement interface as the same was demonstrated by Gilbert *et al*. in their study.[21] The bone cement is said to exhibit low cytotoxicity, and the bone cement powder was found to be nontoxic in nature. It exhibits excellent biocompatibility thus allowing for tissue reattachment. In addition, bone cement tolerates a moist environment very well.[22] Furthermore, it has got good compressive strength. All these attributes make bone cement a very promising material, but more research should be carried out to minimize the polymerization shrinkage for improving the sealing ability.

The results of our study are in accordance with the studies of Saraswathi *et al*.[15] and Gilbert *et al*., which demonstrated that MTA Plus performed better in comparison to conventional MTA and biodentine as root-end filling material whereas Gilbert *et al*.[21] demonstrated the role of polymerization shrinkage at the dentin cement interface in case of bone cement. The results of this study are not in accordance with the studies of Chordiya *et al*. and Holt et al., which stated that bone cement gave comparable results to that of the MTA.[23,24] Solanki NP in a systematic review has stated that both MTA and biodentine are analogous in terms of biocompatibility with potential to provide positive environment for the cell, showing cell proliferation and osteogenic capability.[25]

All the materials used in this *in vitro* study were tested under similar conditions, and therefore, the material showing the least leakage, that is, MTA Plus is the material of choice for clinical applications.

Thus from the above discussion, it can be concluded that the null hypothesis for this study has been rejected.

Conclusion

Within the limitations of the study:

1. The new MTA Plus showed the least amount of microleakage followed by Calcium sulfate with SEA which has shown promising results and can be used as a very cost-effective alternative
2. As there are very few studies done to evaluate such sealing protocol, more studies and research should be carried out on calcium sulfate and SEA to further evaluate and improve the material.

Clinical significance

- MTA as a repair material has superior sealing ability, but it is not readily available and is expensive
- As sealing of perforation should be done immediately, calcium sulfate + SEA (easily available and cost-effective) can be used for short-term perforation sealing when MTA is not available, as the sealing ability is comparable to that of MTA.

Limitations

- Being an *in vitro* study, it is difficult to evaluate which material would have performed better in clinical conditions; hence, more *in vivo* trials should be carried out to select the best material for sealing furcation perforations, for both long term and short term.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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