

## A COMPARATIVE EVALUATION OF SEALING ABILITY OF ENDOSEQUENCE BC SEALER AND PROROOT MTA AS ROOT CANAL SEALER: AN EX VIVO STUDY

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### ABSTRACT

#### BACKGROUND

The most common method of obturation uses semisolid material such as gutta-percha in combination with a root canal sealer. The sealers must preferably stimulate the process of apical and periapical repair along with providing a hermetic seal.

#### MATERIALS AND METHODS

A total of 38 single rooted freshly extracted mandibular teeth were selected and divided into two groups with 15 samples each; Group A (obtured with EndoSequence BC sealer) and Group B (obtured with ProRoot MTA ) and 4 samples each were used for positive control and negative control groups. Sealing ability of the two sealers was evaluated using Microbial Leakage test.

#### RESULTS

The results of the study showed that 5 samples leaked in Group A (33.33%) and 6 samples leaked in Group B (40%). The results of this study suggest that the percentage of specimens without leakage was higher for Group A when compared with Group B. Moreover, early leakage was observed in samples with ProRoot MTA than with EndoSequence BC sealer. However, on applying Mann-Whitney test, the difference was found to be statistically insignificant. Hence, it can be inferred that 'EndoSequence BC Sealer' has comparable sealing ability as compared to 'ProRoot MTA'.

#### CONCLUSION

It may be concluded that 'EndoSequence BC Sealer' being antibacterial, hydrophilic, bioactive, biocompatible, aluminium-free nanoparticulate bioceramic sealer having superior handling characteristics may be recommended for clinical use. However, before any definite conclusion can be drawn, meticulous studies, a larger number of samples and clinical evaluation for longer duration should be encouraged to evaluate the efficacy of these sealers and potential for achieving the hermetic seal.

#### KEYWORDS

Bioceramics, Sealing Ability, Microbial Leakage Test.

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#### BACKGROUND

Endodontic sealers are necessary to seal the space between the dentinal walls and the obturating core interface. Moreover, sealers fill the voids and irregularities in the root canal, lateral and accessory canals, and spaces between core materials.

Recently, challenges in handling properties of sealers have now been met with the new 'EndoSequence bioceramic sealer' composed of zirconium oxide, calcium silicates, calcium phosphate monobasic and calcium hydroxide along with filler and thickening agents. Since the literature is deficient as regard the sealing ability of EndoSequence bioceramic sealer, the purpose of this study is to evaluate and compare the sealing ability of 'EndoSequence BC sealer (Brassler, Savannah, GA)' in comparison with white 'Pro-Root MTA (Dentsply, Tulsa Dental, Johnson City, USA)' when used as a root canal sealer in an ex vivo study.

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#### MATERIAL AND METHODS

##### Selection of Teeth

A total of 38 single rooted freshly extracted human mandibular posterior teeth were collected from the Oral Surgery Department, Punjab Government Dental College and Hospital, Amritsar. All collected teeth were stored in a sealed container with sterile saline and refrigerated prior to the study. Once the teeth were selected, calculus and soft tissue debris were removed from the root surface with hand scaling instruments. Following debridement of the root surface, teeth were immersed in 5.2% sodium hypochlorite for 30 minutes and mechanically debrided with a soft brush. Teeth were stored in 10% formalin at room temperature. Teeth were accessed and #10 K- type endodontic file was inserted into the root canal and advanced out of the apical foramen of all teeth. Initial radiographs were taken in the mesial-distal and buccolingual direction to confirm that type 1 root canal system was present. Prior to the study all teeth were decoronated at a fixed length i.e. 13 mm from the apex using a diamond disc.

##### Canal Instrumentation

Working length was determined by passing a #10 K-type endodontic file into the root canal until the file was just visible at the apical foramen, then subtracting 1 mm to

establish the file length measurement. Rotary ProTaper files (Dentsply Maillefer, Ballaigues, Switzerland) were used to prepare root canal, following crown-down technique and irrigated with 2.5% sodium hypochlorite (Asian Acrylates, Mumbai, India).

Instrumentation of all teeth was performed with a crown-down technique using the ProTaper rotary files. Glyde, (Dentsply Maillefer, Ballaigues, Switzerland) a lubricant to facilitate movement of rotary instruments in the canal was used between all files. Root canal irrigation was performed using 1 mL of 5.2% sodium hypochlorite between each file. A #10 K-type file (Dentsply Maillefer, Ballaigues, Switzerland) was used to maintain apical patency. The root canals were prepared to the working length with ProTaper instruments according to the manufacturer's instructions.

Preparation of the apical third was considered complete when a size 30 file was inserted without force up to the working length. Following final irrigation, the canals were dried with sterile, coarse paper points. To prevent dehydration, all roots were handled using water-moistened gauze during resection and instrumentation. All teeth were then sterilised by autoclaving (121<sup>o</sup> C and 15 lbs. pressure) after canal preparation.

Eight specimens were designated as controls: positive (n=4) and negative (n=4). The positive and negative controls ensured the apparatus model was working properly. The remaining teeth were randomly assigned to two test groups i.e. Group A and Group B with 15 samples each.

### Root Canal Obturation

Single cone obturation of gutta-percha was the technique used for obturation. Intra canal syringe tips were spirit (denaturated ethanol) swabbed. All instruments to be used were autoclaved. Obturation of the root canal was done with aseptic techniques.

### Group A

The teeth were obturated with gutta-percha cone and 'EndoSequence bioceramic sealer' using single cone obturation technique. This sealer is available in a pre-mixed form in a syringe. The manufacturers supply flexible intra canal tips with the same. The syringe cap was removed and intra canal tip was inserted onto it. The intra canal tip was inserted up till coronal third of the root and the material was dispensed. Then the root canal sealer was coated along the canal walls using hand K file #15. The master cone was then coated with the bioceramic sealer and fitted to the working length.

### Group B

The teeth were obturated with gutta-percha cone and MTA sealer using single cone obturation technique. MTA powder and liquid was mixed in a ratio of 3:1 on a non-absorbent paper mixing pad for 30-60 seconds. A hand K file #15 was used to coat the mixed sealer onto the canal walls. Then the master cone was coated with the sealer and fitted to length. Following obturation radiographs were taken in buccolingual and mesiodistal directions to ensure length and density of the

fill. All specimens in Group A and Group B were placed in sterile bags with wet gauze to maintain hydration and allow setting of the sealer for 1 week. Then the teeth were coated with two layers of nail varnish on their circumference barring the apical 2 mm and the coronal access.

The positive control (n=4) specimens were neither obturated nor coated with nail varnish. This allowed free communication of the bacteria in the upper chamber with the growth medium in the lower chamber. The negative control (n=4) specimens were obturated with ProRoot MTA/EndoSequence bioceramic sealer. The samples in this group were coated with nail varnish to seal the apical opening and the coronal access to prevent any leakage.

### Microbial Leakage Test

A microbial leakage apparatus was constructed using a two-chamber method. Natural human saliva was used in this study to determine microleakage.

Transparent bottles and Eppendorf vials (Eppendorf-Elkay, Shrewsbury, USA) were used for making the apparatus. The Eppendorf vials have a closed end and an open end with a hinged lid that provides a contamination-proof seal. The closed end of the Eppendorf vial was cut open with diamond disc such that the tooth sample could be suspended from it. The junction between the vial and tooth was sealed with cyanoacrylate glue and sticky wax.

Transparent bottles were used to suspend the Eppendorf vial along with the tooth sample. A #6 round bur mounted in a high speed hand piece was used to make a hole through the center of every cap. Each Eppendorf vial containing the tooth sample was placed into the fabricated hole in the cap of the transparent bottles, up to its lid, and secured using cyanoacrylate glue and sticky wax. All prepared samples were sealed in a pouch which were placed under ethylene trioxide steriliser for sterilisation and obtained after 10 hours cycle.

This sterilised system was recapped in the bottles containing sterile Trypticase soy broth (HiMedia Laboratories, Mumbai, India). Apical two mm of root structure was submerged into the broth without contacting the floor of the bottle. The upper (Coronal) chamber consisted of Eppendorf vial along with the tooth sample.

The lower chamber of the apparatus was created by the space between the root tip and floor of the bottle. The coronal chamber was filled with a mix of natural human saliva and Trypticase soy broth (ratio 3:1). Fresh broth (Medium) mixed with human saliva (Microorganisms) was added to the upper chamber every 7 days to ensure viable bacteria were present. Replenishing of the upper chamber was accomplished using sterile technique using disposable syringes every time. All experimental samples were placed in a 5.0% CO<sub>2</sub> incubator at 37<sup>o</sup>C and 100% humidity for a total of thirty days.

A positive incidence of leakage visualised by the naked eye was determined by turbidity of the growth medium in the lower chamber. The micro leakage experiment was conducted for 30 days and the medium in the lower chamber was examined daily for turbidity changes. Turbidity time was recorded for each sample and the differences between the groups were compared using the Mann-Whitney test.

**RESULTS**

Group	No. of Samples	No.(%) of Samples Showing Leakage	No.(%) of Samples Showing no Leakage
Group A (GP+ES)	15	5 (33.33%)	10 (66.67%)
Group B (GP+ProRoot MTA)	15	6 (40%)	9 (60.00%)
Positive control group	4	4 (100%)	0 (0%)
Negative control group	4	0 (0%)	4 (100%)

**Table I. (Ex vivo) Percentage of Leakage in each Group**

In Group A, 5 out of 15 (33.33%) samples showed bacterial leakage, however, no leakage was found in 10 samples over a span of 30 days.

In Group B, 6 out of 15 (40.00%) samples showed bacterial leakage, however, no leakage was found in 9 samples over a span of 30 days.

The positive control group showed bacterial leakage in all 4 samples (100%) whereas the negative control group did not show bacterial leakage in any of the group (0%) signifying therefore, that the ex vivo study models worked adequately

Group	No. of Samples	Day on which Leakage Occurred	Mean±SD	Median
Group A (GP+ES)	15	7, 10, 15, 23, 29	16.80±9.121	15
Group B (GP+ProRoot MTA)	15	7, 8, 14, 15, 20, 22	14.33±6.09	14.5
Positive control group	4	1, 1, 2, 2	1.50±0.58	1.5
Negative control group	4	none	-	-

**Table II. (Ex vivo) Time in days on which Leakage Occurred**

Comparison	Mean Rank	P value
Group A	15.00	0.710 <sup>NS</sup>
Group B	16.00	

**Table III. Comparison for Leakage using Mann-Whitney test**

As per the results emerging from the table above, the difference for mean rank between the two groups is non-significant showing that the two sealers have comparable sealing ability.(p>0.05)

**DISCUSSION**

The present ex vivo study was conducted on 38 extracted a single rooted mandibular premolars. The Centres for Disease Control and Prevention (CDC) recommends that extracted teeth to be used for study purpose must be cleaned and

disinfected before use by scrubbing to remove debris and immersion in sodium hypochlorite.<sup>1</sup>

Ruddle CJ (2002)<sup>2</sup> has shown that with the use of rotating Ni-Ti instruments, the risk of instrument separation increases. To minimise the risk of separation, torque controlled endodontic motor was used (X-Smart; Dentsply Maillefer, Ballaigues, Switzerland) with the rotary files at torque values specific for each file as recommended by the manufacturers. In addition a viscous chelator, Glyde, was used with every instrument to minimize force on the instrument. The presence of residual smear layer after chemomechanical preparation is thought to be responsible for leakage between the root canal walls and the filling material. In one study, Baumgartner JC and Mader CL (1987)<sup>3</sup> reported an effective smear layer removal with the use of NaOCl and EDTA as irrigation solutions during root canal instrumentation. Based on this information, 5.25% NaOCl and 17% EDTA (Glyde) was used to remove the smear layer. Finally, the canals were irrigated with normal saline to avoid the prolonged effects of the EDTA and NaOCl solutions.

Single-cone technique is simple, less time-consuming and provides an effective apical seal. Yilmaz Z et al (2009)<sup>4</sup> compared the apical leakage in root canals filled with the LC and SC (Single-Cone) techniques and reported no difference between the two techniques. Therefore, in our study, we used single cones corresponding to the Pro Taper rotary files for obturation. In addition, it maintained experimental consistency amongst the groups.

MTA being a bioactive material, has the ability to create an ideal environment for healing, by forming an apatite like layer on its surface on coming in contact with physiological fluids. Sarkar NK et al (2005)<sup>5</sup> suggested that the biocompatibility, sealing ability and dentinogenic activity of MTA result from the physicochemical reactions between MTA and tissue fluids during the formation of HA. Linear setting expansion values of 0.08% have been reported for white ProRoot MTA that enhances its sealing ability.

Since literature reviews provide a very promising picture for MTA, we chose it as a sealer for one experimental group (Group B). However, some drawbacks for MTA include discoloration potential, presence of toxic elements in material composition, high cost, porosity of set cement, an absence of a known solvent for this material, difficulty of its removal after curing, long setting time and its handling characteristics. The resultant mix after mixing of powder to water is difficult to manipulate. The mix is loose and sandy and does not adhere easily to instruments. Its working time is 4-5 minutes during which the mix constantly becomes dry due to evaporation of water. This might lead to void formation and less adaptability of MTA to root canal walls.

In order to combat all the drawbacks of MTA, for Group A, another calcium silicate based bioceramic material 'EndoSequence BC sealer' (Also known as iRoot SP) was selected based on the manufacturer's claim of superior handling characteristics. Zhang H et al (2009)<sup>6</sup> reported that ES is a sealer chemically based on bio-aggregate having physical properties similar to white MTA. The purpose of BC sealer is to improve the convenience and delivery method of an excellent root canal sealer. It is a hydraulic premixed sealer that eliminates the potential of heterogenous consistency during on-site mixing.

BC sealer has nano-sized particles that facilitates easy flow into dentinal tubules, lateral canals and webs which help in achieving excellent adhesion to the canal dentinal walls and more importantly forms a chemical bond with dentin. Further, it utilises the water inherent in dentinal tubules to drive the hydration reaction of the material, thereby shortening the setting time. The setting time of BC sealer is anywhere between 72 hours to achieve initial set and 240 hours to achieve final set. However, some studies state that MTA may take up to 4 weeks to fully cure. The setting expansion of ES has been reported to be 0.20 percent.

The obturations in this study were done with the respective sealers following which the teeth were radiographed to evaluate length and density of the fill. Following obturation, the roots were stored in sterile gauze moistened with sterile saline to maintain hydration, ensure the complete setting of sealers for 1 week, decrease the final setting time and not to affect microhardness of sealer. Similarly, Nair U et al (2011)<sup>7</sup> stored the specimens in humidior for 7 days to allow setting of the sealers. Loushine BA et al (2011)<sup>8</sup> revealed that with the increase in amount of water during the setting of EndoSequence BC sealer, there was an increase in initial setting time (180 hours), decrease in microhardness of the set cement and formation of a more porous matrix. Therefore, the samples in the present study were stored in moistened gauze for 7 days.

Several methodologies in vitro are used to estimate sealing quality, generally by measuring microleakage that allows the tracer agent to penetrate the filled canal. Commonly used tracers are dyes, radioisotopes, bacteria and their products, such as endotoxins. However, the reliability, reproducibility and clinical relevance of these materials is questionable. Some researchers disagree with the use of such dyes because they have low molecular weights and consequently, can penetrate into sites where protein and bacteria cannot (Barthel CR et al, 1999).<sup>9</sup> In addition, dye penetration lacks uniformity around the margins of the filling (Camps J and Pashley D, 2003).<sup>10</sup>

Bacteria most closely approximate what happens clinically in terms of leakage. Timpavat S et al (2001)<sup>11</sup> suggested the use of bacteria to assess leakage because it is considered to be of greater clinical and biological relevance than the dye penetration method. In addition, Goldman M et al (1989)<sup>12</sup> have pointed out that bacteria performed better than dye penetration for leakage of hydrophilic materials and that dyes could give a false positive reading if their molecules were small enough.

There are factors such as ionic charge, pH, temperature changes and the ability of viable microbes to change their shape and size and to move actively, duplicate or grow and this may play a role in the root canal which cannot be represented by an aqueous dye solution. Therefore, bacterial leakage study was chosen to assess the sealing ability of 'EndoSequence BC sealer' and 'ProRoot MTA'.

The root samples in Group A and Group B were coated with nail varnish except the apical 1-2 mm to seal off the other portals of entry.

Though various broths have been used in the literature, Trypticase soy broth was preferred because this is a highly nutritious medium used for cultivation of a wide variety of organisms. Trypticase soy broth is composed of pancreatic digest of casein (17.0 g/L), papaic digest of soybean meal (3.0

g/L), sodium chloride (5.0 g/L), dextrose (2.5 g/L) and dibasic potassium phosphate (2.5 g/L). The combination of pancreatic digest and soybean meal makes the medium nutritious by providing amino acids and long chain peptides for the growth of microorganisms. Dextrose and dibasic potassium serve as the carbohydrate source and the buffer, respectively in the medium. Sodium chloride maintains the osmotic balance of the medium.

For inoculation of the test specimens, many different strains of bacteria have been used; however, in our study, natural human saliva and a mix of the broth was used. Natural human saliva has some advantages over bacterial cultures. It overwhelms several different bacterial species, high bacterial density and bacterial products, enzymes, proteins and other elements not provided by the culture media. Hence, the use of saliva leakage test is advantageous to some degree because it closely approximates the real clinical situation.

Saliva was collected from a healthy donor and stored in a sterilised glass tube mixed with the broth. The broth fulfilled the nutritional requirements of the bacteria in the saliva. Moreover, the broth with microorganisms (saliva) in the upper chamber was changed every 7 days to ensure that viable bacteria are present for the entire test period of 30 days. The system was stored in an incubator at 37°C and 100% humidity. The lower chamber of the apparatus was examined daily for any change in opacity (turbidity) of the broth. The day on which turbidity appeared in different specimens, the turbidity time was recorded for each sample, and the specimen was discarded.

The results of the present study indicated that the broth turbidity was found in 5 roots of Group A (33.33%) and 6 roots of Group B (40%) at the end of 30 days.

Out of the 5 root samples in Group A, one sample showed turbidity after 7 days, one after 10 days, one after 15 days, one after 23 days and one after 29 days. Out of the 6 samples in Group B, one sample showed turbidity after 7 days, one after 8 days, one after 14 days, one after 15 days, one after 20 days and one after 22 days. All of the roots (100%) in positive control group showed broth turbidity within 48 hours. Roots in the negative control group did not show broth turbidity during the entire observation period, signifying that the ex vivo models worked adequately.

The results of our study suggest that the percentage of specimens without leakage was higher for Group A (EndoSequence BC sealer) when compared with Group B (ProRoot MTA). Moreover, quicker turbidity was observed in samples with ProRoot MTA than with EndoSequence BC sealer (ES). This may be due to the better sealing ability of ES in comparison with MTA. The resistance of both the sealers to the penetration of the bacteria was better when compared to the positive control group in which no sealer was used.

However, on statistical evaluation by applying non-parametric Mann-Whitney test, it was found that the difference between the two groups is statistically insignificant which implies that the sealers have comparable sealing ability.

The findings of the present study are in concurrence with the results of Nair U et al (2011) who compared the sealing ability of EndoSequence and ProRoot MTA using bacterial leakage model and found no significant difference among the groups.

However, the results of current study do not agree with Hirschberg CS et al (2013)<sup>13</sup> who found that the samples in the ES group leaked significantly more than the samples in the MTA group. A direct comparison cannot be made between the two studies because of the difference in methodology. The authors in their study suspended the samples with root end fillings without canal obturations directly in vials for bacterial leakage test and placed them in incubator with the coronal end of tooth sample uncovered. This method allowed leakage by directly encountering the root-end filling material.

In addition, before suspension the samples were stored in moistened gauze for 48 hours whereas Loushine BA et al (2011)<sup>8</sup> demonstrated that the initial setting time of EndoSequence BC sealer is 72 hours. As the initial setting of ES might not have occurred properly after storage in 100% humidity for 48 hours, consequently more ES samples leaked compared to MTA.

Zhang W et al (2009)<sup>14</sup> investigated the sealing ability of iRoot SP and observed that it was equivalent to AH Plus sealer. The iRoot SP is also known as the EndoSequence BC sealer, therefore superior qualities and handling ability of ES make it an innovative novel root canal sealer.

Al Anezi AZ et al (2010)<sup>15</sup> was the first one to evaluate the cytotoxicity of the two materials and showed that the two materials showed similar cell viability. Moreover, cytotoxicity of EndoSequence was similar to MTA in both freshly mixed as well as set conditions. Similar results were reported by Ciasca M et al (2012)<sup>16</sup> and Damas BA et al (2011).<sup>17</sup> In a comparative study done by Guven EP et al (2013)<sup>18</sup> both MTA and EndoSequence have been shown to have 'Apatite-forming ability'. In addition, both the sealers show setting expansion that enhances their seal.

## CONCLUSION

EndoSequence BC sealer, based on aluminium-free calcium silicate nanoparticulate composition, is a convenient, insoluble, radiopaque, biocompatible, osseoconductive, ready-to-use injectable hydraulic cement and may be recommended as a potential root canal sealer for endodontic treatment. However, before any definite conclusion can be drawn clinical evaluation for a longer duration with larger number of samples should be done to evaluate efficiency of the material.

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