*Section A-Research Paper*

# TIPS, TRICKS, TECHNIQUES OF "INTRA ORIFICE BARRIER"

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## Abstract

**Introduction:** Intra orifice barrier placed over the canal orifices has been recommended to ensure good coronal seal. This in vitro study compared the microbial leakage of *E. faecalis* bacteria through MTA, Biodentine, Cention –N and Ketac - Silver when used as intra orifice coronal barrier, in an in-vitro experimental set up and also to compare the preferred thickness between 2 mm and 4 mm of intra orifice coronal barrier.

**Materials and methods**: A total of 120 human extracted, single rooted teeth were selected. Decoronation done, access opening done, working length determined, and canals were cleaned and shaped with pro taper file and obturated with sealer and gutta percha using lateral condensation technique. Samples were divided into four experimental groups and two control groups. Approximately 2 mm and 4 mm of Gp was removed from the coronal orifice and restored with respective restorative materials. Teeth were attached in 1.5 ml of eppendorf tube and suspended in 30ml reagent bottle containing BHI broth which was used to check bacterial leakage. Tubes were incubated and checked for turbidity for 30 days. Data were analyzed using chi –squared test between the test groups.

**Results:** Significantly the least number of samples turned turbid in the MTA 4mm group followed by MTA 2 mm, Biodentine 4 mm, 2 mm, Cention - N 4 mm, 2 mm and Ketac – Silver 4 mm, 2 mm showed the maximum turbidity.

**Conclusion:** The MTA is a better intra orifice barrier, followed by Biodentine, Cention-N and Ketac –Silver.

**Keywords:** Decoronation, Coronal orifice, Eppendorf tube, BHI broth, Turbidity, Bacterial leakage, MTA, Biodentine, Cention N, Ketac Silver

**Introduction:** The ultimate goal of dental treatment is the preservation of tooth in a healthy state. Preservation of a diseased tooth by successful endodontic treatment and adequate CORONAL SEAL ensuring preservation of aesthetics and functions of the tooth is not only the main priority of the dentist but also has been the main preference for most patients. Basic root canal treatment has been shown to be a predictable endodontic procedure with a high rate of success but post treatment failures are also very much common.There are various instances of clinical cases where root canal treatment resulted in failure despite maintaining the highest standards[1]**.** The cause may be due to persistent intra and extra radicular infections. Over a period of time emphasis has been given only on maintaining hermetic apical seal, and maintaining adequate and effective coronal seal was given not much importance. Various studies have suggested that coronal leakage is much more likely a determinant of clinical success or failure than apical leakage. According to *Leonard J.E et al (1996)* coronal leakage provides a constant source of microorganisms and nutrients that initiate and maintain periradicular inflammation and may well be the largest cause of failure in endodontic treatment[2]**.**

Inadequate Coronal seal leading to microleakage has been considered as a detrimental factor related to endodontic failure and much importance is placed on the quality of coronal restoration. More over Coronal seal is further strengthened by the placement of intra orifice barriers. [Figure:1] This technique includes application of sealing plugs into the root canal orifice immediately after removal of coronal gutta-percha and sealer.

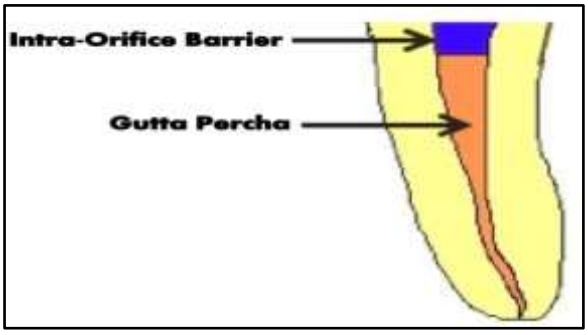



Fig 1

Various materials have been investigated as coronal sealants but they have shown various degrees of leakage. One of these materials, Mineral trioxide aggregate has been evaluated with the excellent physical properties, sealing ability, biocompatibility, bioactivity and clinical performance which has led to various applications of MTA in both surgical and nonsurgical cases. Its chemical formulation resembles that of Type 1 Portland cement and is a combination of dicalcium silicate, tricalcium silicate, tricalcium aluminate, and tetra calcium aluminoferrite and bismuth oxide. Similarly another material used in the present study is the latest bioactive calcium-silicate based material Biodentine.

The powder mainly contains tricalcium and dicalcium silicate, the principal component of Portland cement, and also calcium carbonate and Oxide which acts as filler. Zirconium dioxide serves as contrast medium and radio opacifier agent and liquid consists of Calcium chloride in aqueous solution as an accelerator and water reducing Hydrosoluble polymer

which acts as a super plasticizer. The phenomenal feature of Biodentine is in its high mechanical properties with excellent biocompatibility as well as bioactive behaviour.

Cention N is an UDMA **(Urethane dimethacrylate)** based, self curing powder/liquid restorative with optional additional light-curing. The liquid part comprises of dimethacrylate and initiators, and the powder part contains various glass fillers, initiators and pigments [3].

Newer materials are emerging on to the market every day. Very few studies are available on Biodentine and Cention –N as they are newly introduced materials. Thus taking into account the importance of coronal seal and the role of intra orifice barrier in decreasing bacterial microleakage, the need for finding an affordable material of appropriate thickness and optimal properties for use as an intra orifice barrier is the need of the hour. Thus this in vitro study compared the microbial leakage of *E. faecalis* bacteria through MTA, Biodentine, Cention –N and Ketac -Silver when used as intra orifice coronal barrier, in an in-vitro experimental set up and also compared the preferred thickness between 2mm and 4mm of intra orifice coronal barrier.

**Materials and Method:** A total of 120 extracted, single rooted human teeth which were non –carious, free from cracks, anatomically normal with mature apex were selected. Teeth were mechanically cleaned with ultrasonic scaler and then stored in 10%formalin solution for 2 weeks. Teeth were rinsed properly and then kept in distilled water prior to the usage. The procedure for tooth preparation and obturation was standardized for all groups and performed by a single operator.

The crown portion in each tooth were decoronated at the cementoenamel junction (CEJ) using diamond disc attached in a high speed micromotor handpiece under a continuous air water spray coolant at a plane perpendicular to the long axis of the tooth. Root canal was debrided and patency of canal was determined with size 10k file. Working length was determined a 10 k file was inserted until it was visible at the apical foramen and 1 mm short of the file length was the established working length. The root canals were instrumented in crown-down technique manner using hand protaper files **(Dentsply, Maillefer, Ballaigues, Switzerland)** and Gates Glidden drill**(MANI, INC Japan)**to the working length upto Master apical file size with constant irrigation with 2.5% NaOCl and saline solution in between each instrumentation using a disposable syringe and 24 gauge needle. The canals were dried with absorbent paper points **(Dentsply Maillefer)** and obturated with gutta-percha and AH plus sealer using lateral condensation method. Excess gutta-percha was removed with GP cutter and Coronal GP was vertically condensed. Tooth samples were randomly divided into 6 experimental groups with 20 number of tooth samples equally divided in each group and control groups both positive and negative control group. The GP level was reduced using Gates Glidden drills **(MANI, INC Japan)** to a depth of 2mm from CEJ in half of the tooth samples ie 10 tooth samples in each group and 4mm from CEJ in other half of the samples ie the remaining 10 tooth samples in each group and in this way intra orifice space was created. [Fig 2a,2b,2c,2d] The different restorative materials used as intra orifice barriers in the present study were

- Cention-N (Ivoclar Vivadent, Liechtenstein)
- White MTA (Angelus, Brazil)
- Biodentine (Septodont, France)

- Ketac Silver (3M,Dental Products, Germany)

Different intra orifice restorative materials were mixed according to manufacturer's instructions and the material were transferred to the canal orifice of each respective tooth sample by plastic filling instrument and condensed with condensers. The excess material was removed from the coronal portion. For MTA group tooth samples the coronal potion was condensed with tip of the plastic filling instrument wrapped in moist cotton. No restorative materials were used as intra orifice barrier material in both positive and negative control group. The apical portion containing the condensed gutta-percha was removed using diamond disc. Each tooth sample was coated two layers of nail polish on the external surfaces of desired sized tooth samples. The negative control group tooth samples were fully coated with nail polish including over intra orifice barrier material containing coronal part. The apparatus model design used in this bacterial leakage study was modified from the Dual chamber technique described previously by Torabinejad et al. [4]

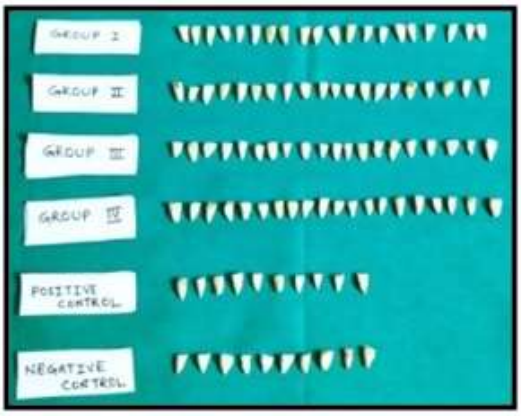



Fig 2a

Fig2b

Fig 2c

Fig 2d

**Microleakage Test:** For the bacterial leakage test, one test apparatus was fabricated for each specimen of tooth sample comprising of autoclavable TARSON made 30 ml narrow mouth screw capped bottle and 1.5 mm Eppendorf tube. A hole was made at the center of each screw-cap and the desired thickness of tooth segment was attached into the end part of eppendorf tube such that the tooth portion with the restorative material is immersed in BHI

broth. The eppendorf tube was slightly cut at the lower portion to fix the experimental tooth sample portion of desired width and thickness. The experimental tooth sample was fixed by Cyanoacrylate and a layer of flowable composite material 0.8 ml Artificial saliva and 0.2ml *E. faecalis (ATCC NO 2922)* inoculums was inoculated in the 1.5 mm eppendorf tube. Artificial saliva and bacterial inoculum was replaced with freshly prepared saliva and inoculums[4, 5, 6] after 3 days .

4 different restorative materials were used as study samples for checking sealing ability distributed equally in experimental tooth samples. The restorative materials were **KETAC SILVER, CENTION-N, MTA** and **BIODENTINE.** The restorative materials were checked for sealing ability in bacterial leakage model in anaerobic condition using AnerobicJar .The temperature was maintained at $37^0$C in incubator. Sealing ability was seen and judged on the basis of microleakage properties of the used different materials causing turbidity of the culture media depending on bacterial growth. Bacterial growth in the form of turbidity was checked in the BHI culture media in the adjoining portion of the tooth containing eppendorf tube in the lower portion of an autoclavable TARSON made 30 ml narrow mouth screw capped bottle. Bacterial samples were withdrawn in time interval of 24 hours (1day), $7^{th}$day, $15^{th}$ day, and $30^{th}$ day for carrying out Gram staining, culture plating on ***E. faecalis*** specific, selective media to confirm the sole presence of ***E faecalis*** without any contamination.[Fig 3a,3b,3c,3d,3e]

Fig 3a

Fig 3b

Fig 3c

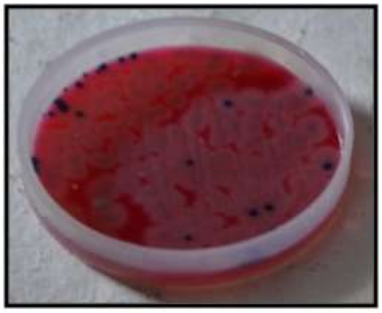


Fig 3d

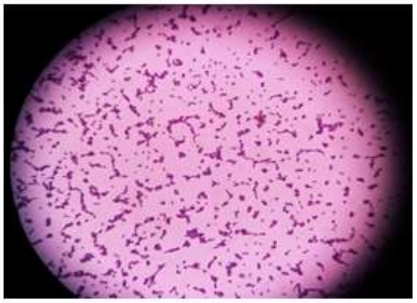


Fig 3e

**Results:** The number of samples that caused the turbidity of culture media was significantly lesser in MTA group (both 2mm, 4mm thickness), followed closely by Biodentine (2mm,4mm), Cention –N (2 mm, 4 mm) and the maximum number of tooth samples that turned the culture media turbid was the Ketac –Silver group(2 mm,4 mm). Student T TEST

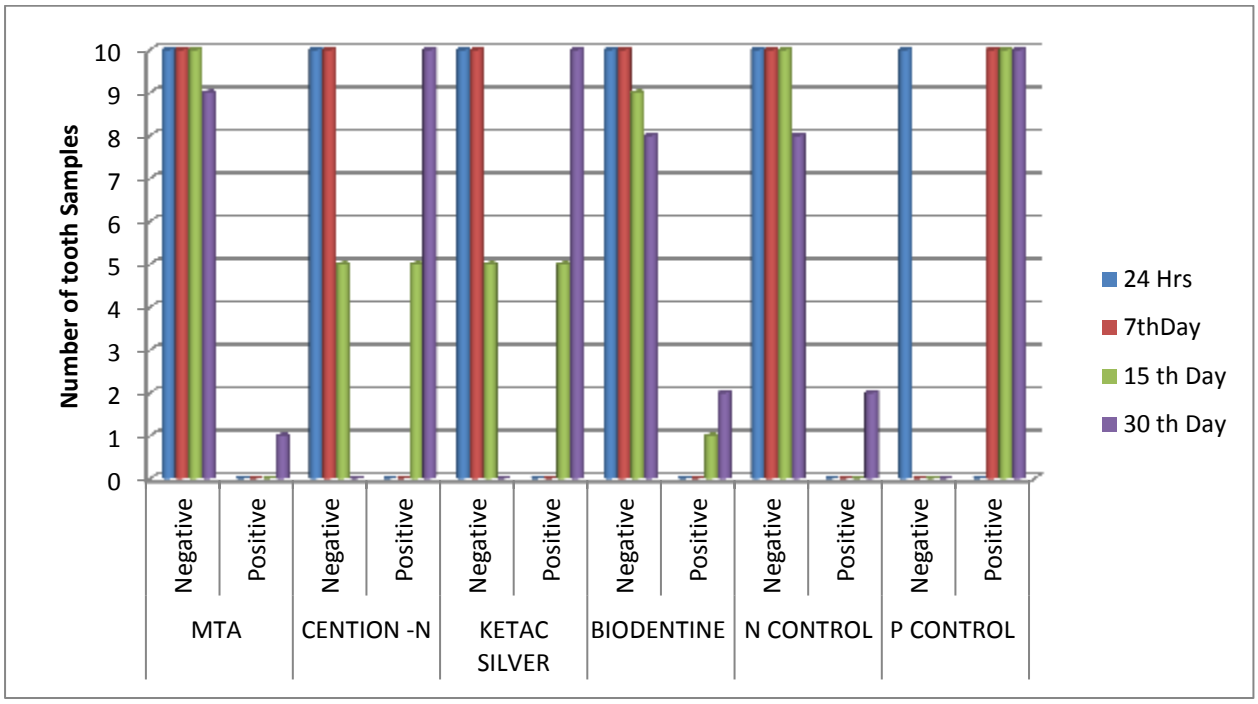
was/chi SQUARED TEST was applied and the probability value was found to be <0.001, indicating that the difference between the test groups and control groups is statistically significant. 4 mm thickness of each restorative material showed better results i.e. lesser leakage causing lesser turbidity than 2mm thickness of each material, 4mm thickness of intra orifice barrier is suitable.

**Discussion:** Success of endodontic treatment depends on both hermetic apical seal as well as good coronal seal. It has been reported in studies that 59.4% of endodontically treated teeth failed due to restorative reasons, 32% due to periodontal reasons and 8.6% due to endodontic reasons. It is clearly apparent that coronal seal is equivalent to that of apical seal and prevention of coronal micro leakage is critical to success[7]. In 1995 the study done by Ray and Trope was a landmark study which is conceived as a paradigm shift in endodontic treatment philosophy. Taking into consideration more than 1000 patients, Ray and Trope (1995) conducted a retrospective clinical study based on the radiographic assessment of patients with high incidence of apical pathology. In their study the absence of apical pathology (success percentage) was compared to the quality of coronal seal. According to their clinical study Ray and Trope ( 1995), concluded that the **quality of the coronal restoration** is significantly more important than **quality of the endodontic treatment** for periapical health[8]. In a similar type of study toRay and Trope (1995), L. Tronstad (2000) et al concluded that highest success rate was found in teeth with good endodontics and good restoration (81%) and in comparison to the teeth with good endodontics and poor restoration the success rate dropped to 71%[9]. Various studies have shown that conventional root filling materials such as gutta-percha and sealer provide minimal resistance to bacterial microleakage.

The purpose of this study was to evaluate and compare the sealing ability of different restorative materials of appropriate thickness used as an intra orifice barrier after obturation of root canal system through bacteriological study model apparatus design. In the present study the materials being used are MTA, Ketac-Silver, and the newer materials like Biodentine and Cention-N. In the present study 120 extracted human single rooted teeth were endodontically treated and 2mm, and 4mm of intra coronal GP was removed from the cement enamel junction and replaced with the experimental restorative material. In the present study the materials being used are MTA, Ketac-Silver, and the newer materials like Biodentine and Cention-N. Several techniques have been attempted to assess marginal leakage such as dye leakage, ink, radiolabeled ions, bacterial leakage markers, air under pressure, and variations in temperature, human saliva, protein complex, and fluid filtration to analyze the sealing ability of root canal filling, retrograde filling materials and intra orifice barriers. In the present study microleakage was assessed by a bacterial marker .The culture medium turning turbid was the indication of root canal contamination. For the present study ***E. faecalis*** **(ATCC NO29212)** was chosen for assessment of bacterial microleakage since it is the dominant strain responsible for chronic apical periodontitis secondary to a failed endodontic treatment as described by various researchers and it has also been found in studies that. E. faecalis is the most often retrieved pathogen in asymptomatic endodontic infections because of its capacity to invade dentinal tubules, compete with other microorganisms and resist nutritional privation. It is a facultative anaerobic Gram-positive coccus that may present either isolated, in pairs or in chains[10,11]**.** The results of the present study showed that all the

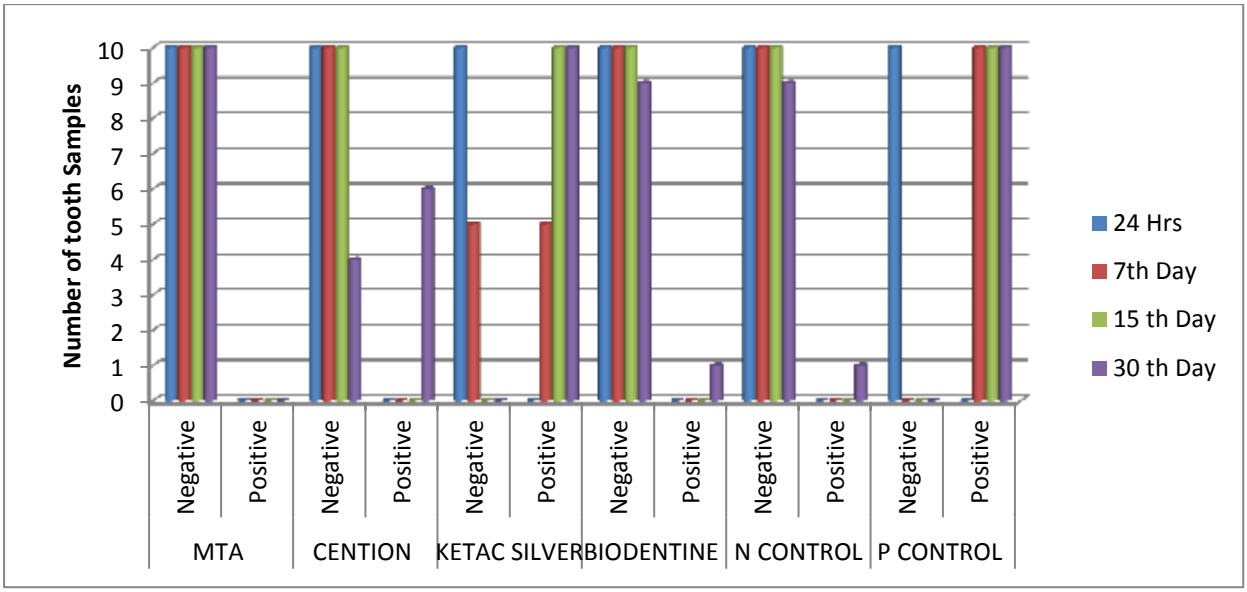
samples in the positive control group, where no coronal intra orifice barrier was placed, turned turbid demonstrating bacterial growth. This can be considered as a potential for bacterial leakage and is in accordance with studies conducted by *Saunders et al (1994) and Carman and Wallace (1994),* who showed that GP and sealer do not provide an adequate and effective barrier to coronal leakage, and therefore, a material with a better sealing ability is required to be placed[12,13]. Negative control groups showed no microleakage

| 2 MM | MTA | | CENTION | | CERMET | | BIODENTINE | | NEGATIVE CONTROL | | POSITIVE CONTROL | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GROUP** | **Negative** | **Positive** | **Negative** | **Positive** | **Negative** | **Positive** | **Negative** | **Positive** | **Negative** | **Positive** | **Negative** | **Positive** |
| **24 Hrs** | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 |
| **7thDay** | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 0 | 10 |
| **15 th Day** | 10 | 0 | 5 | 5 | 5 | 5 | 9 | 1 | 10 | 0 | 0 | 10 |
| **30 th Day** | 9 | 1 | 0 | 10 | 0 | 10 | 8 | 2 | 8 | 2 | 0 | 10 |
| **TOTAL** | 39 | 1 | 25 | 15 | 25 | 15 | 37 | 3 | 38 | 2 | 10 | 30 |
| **Chi-square value** | 3.0769 | | 29.3333 | | 29.3333 | | 3.9640 | | 6.3158 | | 40.0000 | |
| **p-value** | **0.3799** | | <0.0001 | | <0.0001 | | 0.2654 | | 0.0972 | | <0.0001 | |




| 4 MM | MTA | | CENTION | | CERMET | | BIODENTINE | | NEGATIVE CONTROL | | POSITIVE CONTROL | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GROUP** | **Negative** | **Positive** | **Negative** | **Positive** | **Negative** | **Positive** | **Negative** | **Positive** | **Negative** | **Positive** | **Negative** | **Positive** |
| **24 Hrs** | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 |
| **7thDay** | 10 | 0 | 10 | 0 | 5 | 5 | 10 | 0 | 10 | 0 | 0 | 10 |
| **15 th Day** | 10 | 0 | 10 | 0 | 0 | 10 | 10 | 0 | 10 | 0 | 0 | 10 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **30 th Day** | 10 | 0 | 4 | 6 | 0 | 10 | 9 | 1 | 9 | 1 | 0 | 10 |
| **TOTAL** | 40 | 0 | 34 | 6 | 15 | 25 | 39 | 1 | 39 | 1 | 10 | 30 |
| **Chi-square value** | NA | | 21.1765 | | 29.3333 | | 3.0769 | | 3.0769 | | 40.0000 | |
| **p-value** | **NA** | | <0.0001 | | <0.0001 | | 0.3799 | | 0.3799 | | <0.0001 | |




**Comparative Analysis of the Possible Reasons Behind the Results**

**Possible key factors behind Group MTA (white) showing lesser bacterial leakage.**

1. Its Superior sealing ability has been attributed to its hydrophilic nature and expansion when it sets in moist environment.[14]
2. Superior marginal adaptation of MTA accounts for its ability to resist leakage.[15]
3. Superior sealing ability and excellent adhesion of MTA is explained by the mechanism of its spontaneous formation of an adherent hydroxyapatite formation in the MTA –DENTIN interfacial region along with interactions with dentin like intra-fibrillar apatite deposition. The mechanical seal is due to formation of apatite crystal transforms into a chemical bonding through diffusion control reaction between apatite layer and dentin. This physiochemical reaction results in a biologic seal between dentin and MTA contributing to its superior sealing ability and marginal adaptation.[15,16]
4. Spontaneous hydroxyapatite formation, intrafibrillar apatite deposition, chemical bond with MTA and dentinal walls[17]

**Possible key factors behind Group ketac-Silver, Group Cention-N, and Group Biodentine showing comparatively more bacterial leakage then Group MTA**

1. Cention N, newer "**ALKASITE**" material is close to compomer or ormocer, subgroup of Composite material, presence of alkaline fillers[3,18]

2. **Polymerisation shrinkage** and inadequate **adhesion** to dentin may be the reason for imperfect sealing.
3. Time dependent intimate interaction of Biodentine with dentine and mean inter diameter gap relation with dentine is still under process of research and development.
4. **Marginal integrity and inter-molecular interaction of Cention –N, Biodentine with dentin** is questionable Research is still going on regarding these materials[18.]

Although 4 mm thickness MTA meets up all the criteria's put forth by researchers as material of choice as intra orifice barrier but if there is a need of removal of the orifice barrier material in retreatment cases the removal of the material is difficult.
Considering the limitations of bacterial leakage studies, it can be concluded that further research and clinical trials using larger sample size and well controlled in vivo studies through other techniques need to be done to correlate the results.

**Conclusion:** Various studies have shown that appropriate and adequate Coronal seal is one of the major parameters of ensuring successful endodontic treatment outcome. In this context Intraorifice barrier strengthens the coronal seal by acting as second line of defence. Coronal restoration plus intra orifice barrier layer of different biocompatible restorative materials respectively has changed the dimension of adequate and strong coronal seal altogether. Various biocompatible restorative materials like, MTA, Biodentine, Cention N, Ketac silver, GIC, composite, etc can be used as Intra orifice barrier and among which 2 mm, 4 mm MTA serve as the full proof and time-tested better Intra orifice barrier.
Last but not the least Intra orifice barriers significantly reduces the leakage from coronal portion and adds up in maintaining a three dimensional, fluid tight seal.

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