ORIGINAL RESEARCH

Quantitative Microbial Leakage Evaluation of Restorative Materials with/without Antibacterial Primer as an Intracoronal Barrier: An *Ex Vivo* Study

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Abstract

Aim: Aim of this research was to assess the microbial leakage of restorative materials with/without antibacterial primer as an intracoronal barrier.

Materials and methods: Fifty-five extracted single-rooted teeth were included in this study. The canals were cleaned, shaped, and obturated with gutta-percha and AH plus sealer at the established working length. After removing 2 mm of coronal gutta-percha, the teeth were incubated for 24 hours. The teeth were divided into groups according to the materials used as intracoronary orifice barriers as follows:

- Group I: Clearfil Protect Bond/Clearfil AP-X
- Group II: Xeno IV/Clearfil AP-X
- Group III: Chemflex (glass ionomer)
- Group IV: Positive control (no barrier)
- Group V: Negative control (no barrier and inoculated with sterile broth)

Sterile 2 chambers bacterial technique was used to assess the microleakage and *Enterococcus faecalis* was considered as a microbial marker. The percentage of samples leaked, the time taken for leakage, and the number of colony-forming units (CFUs) in the leaked samples were calculated and analyzed statistically.

Results: There was no statistically significant difference found in bacterial penetration among the three investigated materials after 120 days of use as an intracoronal orifice barrier. This study can also infer that the leaked sample from the Clearfil Protect Bond showed the least mean number of CFUs (43 CFUs) followed by Xeno IV (61 CFUs) and glass ionomer cement (GIC) (63 CFUs).

Conclusion: This study concluded that all three experimental antibacterial primers performed better as intracoronal barrier. However, Clearfil Protect Bond with an antibacterial primer showed promising results as an intracoronal orifice barrier in reducing the number of bacterial leakages.

Clinical significance: The significance of intracoronal orifice barriers in the success of endodontic treatment depends on the ability of the materials to prevent microleakage. This helps clinicians to provide successful antibacterial therapy against endodontic anaerobes.

Keywords: Antibacterial, Clearfil, Intracoronal orifice barrier, Microleakage, Primer.

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Introduction

The success of root canal therapy depends on thorough cleaning and shaping of the root canal system followed by its three-dimensional obturation and to achieve a fluid impermeable seal at both its apical and coronal ends. Failures in endodontic treatment are still not uncommon with coronal leakage being one of the factors responsible for it.[1] Without a sufficient coronal seal, the prognosis is still uncertain because the microbiota may be able to see obturated root canals, which could slow healing and cause infection in the periradicular, supporting osseous structure, or periodontal ligament.[2]

Intraorifice barrier is an efficient alternative method to mitigate coronal leakage in endodontically treated teeth. This procedure includes placing additional material into the canal orifices immediately after removal of the coronal portion of gutta-percha and sealer.[3] According to Schwartz and Fransman, orifice barriers placed below the permanent restoration allow a second line of defense mechanism against the bacterial leakage.[4] Characteristics that qualify a restorative material as an ideal intracoronal barrier include ease and speed of placement, sealing efficacy, high bond strength, and antibacterial efficacy. Commonly used permanent filling materials have been considered as intracoronal barriers to prevent coronal microleakage. Recently glass ionomer, resin-

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modified glass ionomer, mineral trioxide aggregate (MTA), and flowable composites have been proven effective in this regard.[5,6]

Dentin bonding agents are widely used in restorative dentistry to improve the bond of materials to teeth thereby preventing microleakage under restorations. In addition, bonding systems that have intrinsic antibacterial properties would be effective as an intracoronal orifice barrier. Self-etch adhesives containing the resin monomer 12-methacryloyloxy dodecyl-pyridinium bromide (MDPB) stands out among the antibacterial agents used in adhesive adhesives. The key advantage of MDPBs is the ability to copolymerize with other resin monomers trapped inside the polymer matrix, ensuring its safety and long-term antibacterial activity.[7] It also does not leach into the media. This property also ensures a high restoration survival rate, as MDPB, unlike soluble antibacterial agents, has no negative impact on the adhesive materials' physical and mechanical qualities. Its successful antibacterial activity against the endodontic anaerobes makes it an ideal choice for it to be used as an orifice barrier.[8]

Hence, this present study was designed to evaluate the effectiveness of an adhesive system containing antibacterial components along with composite as an intracoronal orifice barrier and compare it with other commonly used materials such as glass ionomer, an adhesive system without any antibacterial component.

Materials and Methods

This study was conducted in the department of conservative dentistry and endodontics, PMNM Dental College and Hospital, Bagalkot, India. Fifty-five human single-rooted single-canal teeth which were extracted for orthodontic reasons were collected and cleaned using ultrasonic scalers. Using a water-cooled diamond disc, the coronal portion of these teeth was sectioned at the CEJ, which was parallel to the long axis of the roots, to standardize the length of all specimens (15–18 mm). All of the teeth were cleaned, shaped, and obturated by a single operator. Also, RC-Prep (Medical Products Laboratories, Inc.) and 5.25% NaOCl (Vishal Dentocare Pvt Ltd., India) were used in between each instrument, and the working length was visually determined by subtracting 1 mm from the length of a 10-size K-file (Dentsply, Maillefer, and Ballaigues) at the apex. The employment of Gates Glidden burs Nos. 2, 3, and 4 for coronal preflaring (Mani, Inc., Japan). Up to No. 25, root canals were cleaned and shaped utilizing the K-Files step-back technique. After the root canal had been instrumented, it was cleaned with 2 mL of 17% ethylenediaminetetra-acetic acid (EDTA) (Dent Wash, Prime Dental products), followed by a final rinse of 0.2% w/v chlorhexidine (ICPA Health Products Ltd., India). Drying the Canal involved using sterile paper points. After apical gauging, the specimens were obturated using the cold lateral compaction method with size 25, 2% Taper GP points (Dentsply, Maillefer, and Ballaigues), and AH-plus sealer (Dentsply Detrey of MbH Germany). Following that, teeth were incubated for 24 hours at 37°C with 100% humidity for the dental sealer to set. Gutta-percha point was removed to a depth of 3.5 mm from the orifice using a No. 5 Gates Glidden bur (Mani Inc., Japan)

At this stage, teeth specimens were divided into three groups of 15 specimens each (n = 15), namely, groups I, II, and III. Then, 10 specimens were randomly placed in two control groups (n = 5 specimens/control group) as follows:

- Group I: Comprised n = 15 specimens; a 3-mm intracoronal barrier of Clearfil Protect Bond (Kuraray Medical, Inc., Kurashiki, Okayama, Japan)/Clearfil AP-X (Kuraray Noritake Dental, Tokyo, Japan) (a self-etching primer adhesive system containing 12MDPB and light-cure composite resin).
- Group II: Comprised n = 15 specimens; a 3-mm intracoronal barrier of Xeno IV (Dentsply/Caulk, Milford, USA) (a self-etching adhesive system without an antibacterial component and light-cure composite resin).
- Group III: Comprised n = 15 specimens; a 3-mm intracoronal barrier of Chemflex Dentsply Detrey, Konstanz, Germany) (a high-strength GIC).
- Group IV: Comprised n = 5; positive control.
- Group V: Comprised n = 5; negative control.

Two layers of nail polish were coated on the root surfaces, with the exception of the apical 2 mm and the coronal surface to prevent the leakage from any other area along the root surface.

Bacterial Microleakage Analysis

The two-chamber model discussed in Pisano et al.[9] was utilized to evaluate the microleakage. Figure 1, the coronal 2 mm of each tooth was linked to a polyvinyl tube (12 mm × 50 mm). Pre-sterilized scintillation vials' plastic lids were produced with holes, and the tubes were inserted through the holes (Borosil, India). A layer of PVC adhesive (M seal, Pidilite, India) and two layers of cyanoacrylate (Fewikwik, Pidilite, India) adhesive were used to affix the tube/tooth interface and the cap/tube interface in order to create a fluid-tight seal. The root assembly and all scintillation vials were sterilized in a hot air oven. Sterilized brain heart infusion broth (LW F027, Hi Media) was added to the vials after sterilization and poured in until it completely encircled the apical 3 mm of each root specimen. To ensure full sterilization, the specimens were then sealed in the vials and kept there for 24 hours at 37°C. A culture of the *E. faecalis* (ATCC29212) strain was incubated at 37°C overnight in brain heart infusion broth, yielding an absorbance (A660) of 0.35 (108 CFU/mL). Every 7 days, a fresh 100 µL of *E. faecalis* suspension was added to the tube leading into each root canal. To guard against environmental contamination, each vial was individually covered with sterile modelling wax (Vishal Dento Care Pvt Ltd., India) on

Fig. 1: Chamber model used in this study

Source of support: Nil
Conflict of interest: None

top. As positive controls, five teeth without coronal restorations were similarly injected with *E. faecalis* as the experimental groups were. Five obturated teeth were not given coronal restorations and were injected with sterile BHI broth to ensure that no contamination took place during the study period (negative control). The broth at the bottom of each vial was checked daily for turbidity, a sign of bacterial development, and all experimental groups were incubated at 37°C. This study was conducted for 120 days. The presence of *E. faecalis* was then verified and quantified by culturing the turbid broth. After 120 days, serial 10-fold dilutions of the turbid broth from the leaked sample were made up to 1:10^5) in physiological saline solution. From the serial dilutions, 0.1 mL was transferred, streaked, and plated on blood agar medium plates. The plates were then incubated in an aerobic chamber for 24 hours at 37°C. When bacterial growth was detected, colony count was performed and tabulated. Colonies of bacteria were counted using the classic bacterial counting method and the results were given as the number of CFUs (Figs 2 to 4).

Statistical Analysis

Microleakage data (number of samples leaked, time taken for leakage and number of CFUs in the leaked samples) was then analyzed with the Chi-squared (χ^2) test, Kruskal–Wallis test, and Mann–Whitney *U* test with predetermined $p < 0.05$ using statistical package for social sciences software (SPSS, IBM) and the results were tabulated.

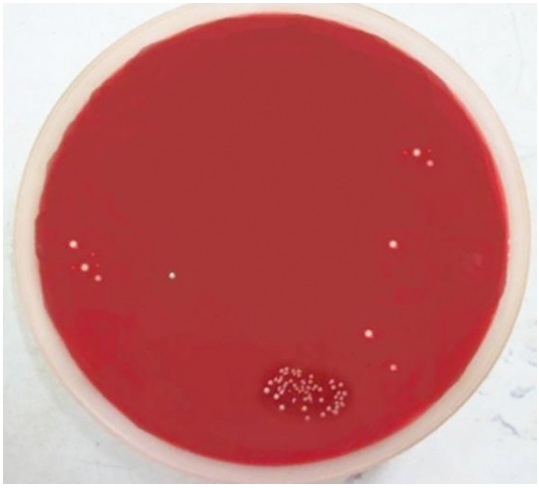

Fig. 2: Colony-forming units of Clearfil Protect Bond

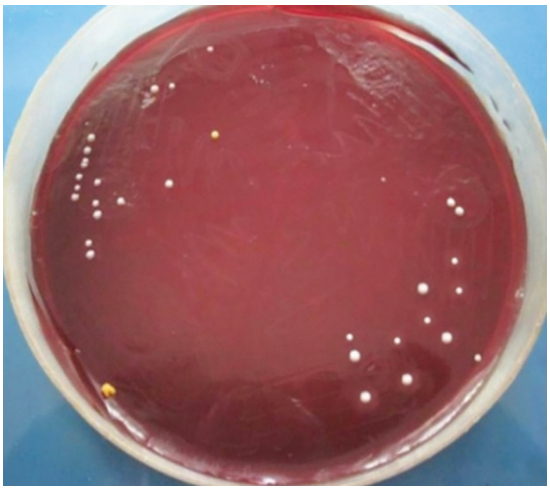

Fig. 3: Colony-forming units of Xeno IV

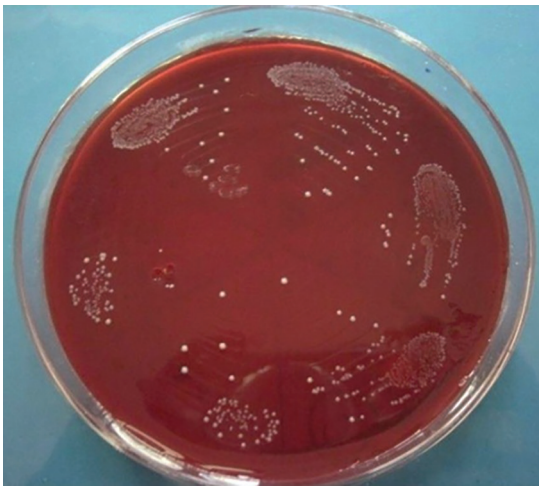

Fig. 4: Colony-forming units of GIC

Table 1: Percentage distribution of microleakage among three different groups

Groups	*Not leaked*	*%*	*Leaked*	*%*	*Total*
Group I	9	60.00	6	40.00	15
Group II	7	46.67	8	53.33	15
Group III	10	66.67	5	33.33	15
Total	26	57.8	19	42.2	45

$\chi^2 = 1.247$; $df = 2$; $p = 0.536$

Results

Table 1 represents the percentage of leaked samples among the three experimental materials. Here, the percentage of leaked samples in the glass ionomer group was the least (33%) followed by Clearfil Protect Bond (40%) and lastly Xeno IV (53%).

The Chi-squared test was further utilized to compare the percentages of leaked samples among the three experimental groups with a predetermined $p = 0.05$. The Chi-squared test indicated that there was no statistically significant difference in the percentage of samples leaked ($p > 0.05$).

Table 2 shows the comparison of four groups with respect to days taken for microleakage by Kruskal–Wallis analysis of variance (ANOVA) test. The mean number of days taken for microleakage was the least for the positive control group (23 days). Clearfil Protect Bond took the longest time to exhibit microleakage (77 days).

Table 3 represents the pair-wise comparison of four groups with respect to days taken for microleakage by Mann–Whitney *U* test. No statistically significant difference was obtained when the three experimental materials were compared among themselves with $p > 0.05$. No significant difference in the number of days taken for microleakage among groups I–III. The mean number of days taken for microleakage among the groups in decreasing order group I > group II > group III > group IV.

Table 4 represents comparison of four groups with respect to CFUs by Kruskal–Wallis ANOVA test. It showed a statistically significant difference when four groups were compared among themselves. This study can also infer that the leaked sample from

Table 2: Comparison of four groups with respect to days taken for microleakage by Kruskal–Wallis ANOVA test

Groups	*Means*	*Standard deviation (SD)*	*Media (n)*	*Sum of ranks*	*H-value*	*p-value*
Group I	77.1667	12.8595	81.50	104.5000	12.4891	0.0059*
Group II	68.0000	14.3626	72.00	108.5000		
Group III	69.8000	9.2033	68.00	72.0000		
Group IV (positive control)	23.6000	5.6833	22.00	15.0000		

*Significant when $p < 0.05$

Table 3: Pair-wise comparison of four groups with respect to days taken for microleakage by Mann–Whitney *U* test

Groups	*Means*	*SD*	*Median*	*Sum of ranks*	*U-value*	*Z-value*	*p-value*
Group I	77.1667	12.8595	81.5000	55.5000	13.5000	−1.3555	0.1753
Group II	68.0000	14.3626	72.0000	49.5000			
Group I	77.1667	12.8595	81.5000	40.0000	11.0000	−0.7303	0.4652
Group III	69.8000	9.2033	68.0000	26.0000			
Group II	68.0000	14.3626	72.0000	55.0000	19.0000	−0.1464	0.8836
Group III	69.8000	9.2033	68.0000	36.0000			
Group I	77.1667	12.8595	81.5000	51.0000	0.0000	−2.739	0.006*
Group IV	23.6000	5.6833	22.0000	15.0000			
Group II	68.0000	14.3626	72.0000	76.0000	0.0000	−2.932	0.003*
Group IV	23.6000	5.6833	22.0000	15.0000			
Group III	69.8000	9.2033	68.0000	40.0000	0.0000	−2.611	0.009*
Group IV	23.6000	5.6833	22.0000	15.0000			

*Significant when $p < 0.05$

Table 4: Comparison of four groups with respect to CFUs by Kruskal–Wallis ANOVA test

Groups	*Means*	*SD*	*Median*	*Sum of ranks*	*H-value*	*p-value*
Group I	43.3333	15.8451	36.5000	39.5000	14.1087	0.0028*
Group II	61.3750	12.8834	93.5000	61.5000		
Group III	63.4000	14.4326	60.0000	69.0000		
Group IV (positive control)	138.4000	32.9970	110.0000	131.0000		

*Significant when $p < 0.05$

the Clearfil Protect Bond showed the least mean number of CFUs (43 CFUs) followed by Xeno IV (61 CFUs) and GIC (63 CFUs).

It is indicated that Clearfil Protect Bond performed significantly better than Xeno IV and the control group. Furthermore, even Xeno IV and GIC showed better performance than the positive controls.

Discussion

Despite the fact that all restorative materials exhibit some degree of leakage, their usage as intracoronal barriers is successful in minimizing microleakage.[9,10] Permanent restorative materials have been proved fruitful in this regard. Some of the common restorative materials used as intraorifice barriers are glass ionomer, composite resin, MTA, and amalgam.

Hence, the main purpose of this research was to assess the sealing ability of a restorative resin material with an antibacterial primer (Clearfil Protect Bond/Clearfil AP-X) when placed as an intraorifice barrier to prevent the coronal microleakage and compare it with the restorative materials like glass ionomer (Chemflex) and a resin restorative material without any antibacterial component (Xeno IV/Clearfil AP-X) using a bacterial leakage model.

Although the use of dyes, radioisotopes, fluid filtration, bacteria, and endotoxin penetration techniques have been used to evaluate the seal of intraorifice barriers, the bacterial leakage model has been advocated as a more clinically relevant model.[11]

Enterococcus faecalis is used as a biological marker as it is a persistent organism that plays a major role in the etiology of persistent periradicular lesions after root canal treatment.[12] The results obtained from the control groups validated the experimental model used. The quick and consistent penetration of *E. faecalis* in the positive controls confirmed the inability of gutta-percha and sealer alone to prevent bacterial ingress that is in agreement with the previous studies.[13] The negative controls confirmed the reliability of the experimental apparatus that permitted only one pathway for the bacterial migration into the broth by penetration along the filled root canal.

Despite the fact that there were no statistically significant differences between the three experimental groups, the glass ionomer (Chemflex) group had fewer contaminated samples than the other groups. The results obtained are in agreement with study where Celik et al.[10] indicated that when compared to dentin-bonding agent/flowable composite, glass ionomer leaks

much less. The reason may be due to the formation of an ionic bond between the hydroxyapatite of the dentin with the GIC and the fluoride release property of the same that would have prevented the entry of bacteria

Contemporary self-etch adhesives systems can be categorized as mild, moderate, and aggressive depending on the acid dissociation constants of the acidic resin monomers used and the concentration of monomers present in the adhesives. The bonding effectiveness of self-etch adhesives has been attributed to their ability to demineralize and infiltrate the dentine surface simultaneously to the same depth, thereby theoretically preventing incomplete penetration of the adhesive into the exposed collagen network. This might be the reason which would have been responsible for prevention of leakage in almost 60% of the samples in self-etch adhesive system groups (groups I and II).[14]

The usage of NaOCl irreversibly alters the physical characteristics of the dentin by causing the damage of the organic matrix, mainly the collagen fibrils. Santos et al.[15] demonstrated that the usage of NaOCl has a negative impact on the bond strength to pulp chamber dentin when a self-etching adhesive technique is employed. As an oxidizing agent, NaOCl has also been demonstrated to cause some dentin matrix constituents to oxidize, which may prevent resins from interfacially polymerizing.

Hayashi et al.[16] reported that demineralization and deproteinization, two morphological alterations caused by EDTA, occur in radicular dentin. These modifications could make it more difficult for the resin glue to adhere firmly to the demineralized radicular dentin. This could also be a factor in the leaking seen in the recovered samples with self-etch primer/adhesive and Clearfil AP-X.

Leakage associated with the Xeno IV (single step self-etch adhesive) may be due to the absence of HEMA, this adhesive is more prone to the occurrence of the so-called phenomenon of phase separation.[17] Another concern about the acidic monomers of the self-etch adhesives is that they are gradually buffered by the mineral content of the substrate. At this stage, such weakened monomers especially of Xeno IV (pH = 2.3) are only able to partially etch dentine. As a consequence, zones of partially demineralized but non-infiltrated dentine may be formed beneath the hybrid layer, defeating the conventional wisdom that such adhesives do not exhibit discrepancies between the depth of demineralization and the depth of resin infiltration.[14]

The time taken for the samples to exhibit the bacterial microleakage is more in the Clearfil Protect Bond/Clearfil AP-X group and even the CFU is lesser when compared to the other groups. A possible explanation might be due to the presence of an antibacterial component 12 – MDPB in the primer of Clearfil Protect Bond. The antibacterial monomer 12 – MDPB is synthesized by combining quaternary ammonium compound with a polymerizable group that was developed to provide resin-based materials with antibacterial effects.[18] Quaternary ammonium compounds interact electrostatically with bacterial membranes, which are negatively charged, and exert their effects through membrane damage, resulting in the leakage of intracellular components.[19] Therefore, MDPB is expected to be able to show rapid antibacterial effects. The advantage of MDPB is its capacity to copolymerize with other resin monomers and being immobilized within the polymer matrix after light curing, which confers safety and prolonged antibacterial action to this agent, as it does not leach to the medium.[20]

Although experimental studies cannot exactly reproduce clinical conditions, and the relationship of *in vitro* leakage measurements to the *in vivo* situation has not yet been established, the most reasonable way of testing the efficacy of coronal restoration is extrapolation of the data obtained from *in vitro* studies to clinical conditions and long-term clinical evaluation of the results.

The outcomes of this *in vitro* investigation show that no statistically significant difference ($p > 0.05$) exists between the time taken for leakage and the amount of bacterial penetration with Clearfil Protect Bond/Clearfil AP-X, and Xeno IV/Clearfil AP-X, Chemflex as intracoronal barriers by 120 days.

Conclusion

The present study concluded that all the three experimental antibacterial primers performed better as intracoronal barrier. However, Clearfil Protect Bond with an antibacterial primer showed promising results as an intracoronal orifice barrier in reducing the number of bacterial leakages.

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