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RESEARCH ARTICLE

COMPARATIVE EFFECTIVENESS OF DIFFERENT CLEANSING PROTOCOLS ON BONDING OF A SELF-ETCH ADHESIVE TO DENTINCONTAMINATED BY ROOT CANAL SEALER: AN IN-VITRO STUDY

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Abstract

Objectives: To compare the effectiveness of different sealer cleaning methods on bonding of a self-etch adhesive to root canal sealer-contaminated dentine.
Methods: Forty-nine extracted molars were divided into seven groups. Dentine surfaces were exposed and contaminated with a thin layer of epoxy resin-based sealer (AH Plus (AHP)) or zinc oxide eugenol-based sealer (Pulp Canal Sealer (PCS)). Three cleaning protocols were examined: dry cotton pellet, 70% ethanol-saturated cotton pellet and a surfactant-based cleaner (ZirClean (ZC), Bisco, US). Uncontaminated dentine served as control. The dentine surfaces were bonded with a two-step self-etch adhesive and restored with resin composite. Bond strength testing was performed using a micro-tensile approach.
Results: Tensile bond strength for uncontaminated dentine control was 46.4±7.3 MPa; bond strength decreased significantly for the dry cotton group (29.6±4.2 MPa for AHP, 24.7±4.7 MPa for PCS, $p<0.05$). Both ethanol and ZC restored bonding performance after cleaning, with no significant difference from the control. Significantly lower mTBS was observed for the ZOE/ethanol subgroup (38.9±5.1 MPa).
Conclusions: Zirclean decontaminates root canal sealer-smeared dentine surfaces effectively and restores the bonding performance of a self-etch adhesive to dentine.
Clinical significance: The surfactant-based ZirClean Cleaner, originally designed for cleaning zirconia surfaces prior to adhesive bonding, may be used as for cleaning dentine that has been contaminated with unset root canal sealers after root canal treatment to restore the bonding strength of a self-etch adhesive to sealer-contaminated dentine.

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Introduction:-

Creation of an impervious seal of the obturated canal space does not only rely solely on the use of a root filling material alone, irrespective of the obturation technique employed. The use of root canal sealers is indispensable for obturation of root canals; together with the root filling material, they form a "package deal" [1]. Smearing of exposed dentine in the endodontic access with sealer is inevitable during root canal obturation. Adhesive bonding of the intraradicular and intracoronal dentine around the coronal part of the canal space, canal orifice and pulpal floor is important for achieving coronal seal as well as for post retention. Sealer that is not removed prior to bonding may contaminate the associated dentine, resulting in inferior polymerisation of adhesive resin monomers, interfacial microleakage, coronal discolouration and bond deterioration [2-6]. The contribution of a good coronal seal cannot be over-emphasized in root canal treatment, particularly in cases with apical periodontitis [7, 8]. Thorough decontamination of a root canal sealer from the canal orifice is mandatory prior to the placement of acoronal resin composite restoration. Optimally cleaning of sealer-contaminated dentine requires an appreciation of the physical and chemical properties of root canal sealers. These properties vary extensively among zinc oxide-eugenol sealers, epoxy resin-based sealers and tricalcium silicate-based sealers.[9]

Contemporary studies were predominantly focused on the decontamination of epoxy resin-based sealer-smeared dentine because of the superior sealing ability and long-term success associated with this class of sealers.[10-15] Nevertheless, other studies have also investigated the decontamination of zinc oxide eugenol-based sealer-smeared dentine [16-18]. Different sealer cleaning methods have been proposed and examined in previous studies, including the use of dry cotton, sandblasting, ethanol, eucalyptol, isopropyl alcohol, acetone, Endosolv R (Septodont, Saint-Maur-des-Foss´es, France), amyl acetate, ethyl acetate, chloroform, orange oil, chlorhexidine as well as AH Plus Cleaner (comprising ethanol and butanol; Dentsply Sirona).[3, 10-18]Most of these cleaning methods involve the use of organic solvents. The major issues associated with the use of organic solvents are biocompatibility, storage difficulty and inadequate accessibility within the dental clinic. These issues have limited their use.[19] Ethanol is the most widely used method because of its accessibility, low cost and low toxicity.[20] However, several studies have reported less than optimal outcomes produced by ethanol cleaning of root canal sealers. This is particularly so when ethanol was used prior to the application of self-etch adhesives.[3, 10, 14] A new surfactant-based dental cleansing protocol has recently been introduced. The commercial version of the product, ZirClean Cleaner (Bisco dental, US), was originally designed to clean zirconia ceramic surfaces. The cleansing solution comprises a 10- methacryloloxydecyl dihydrogen phosphate (MDP) salt derived from the phosphate ester resin monomer, MDP and triethanolamine. When ZirCleanCleaner is applied to the surface of a tooth or a prosthetic restoration that is contaminated with saliva or plasma, the hydrophobic group of the MDP salt is attached to the contaminant. The MDP salt weakens the surface tension of the contaminant and enables it to be rinsed off with water. Unlike other commercially available zirconia cleansers, ZirClean Cleaner does not contain caustic agents such as NaOH or KOH. With a pH value of 4.51. Although ZirClean Cleaner may be used for intraoral cleaning of tooth structure, the effectiveness of this surfactant-based cleansing approach on removal of root canal sealers from sealer-contaminated dentine has never been investigated. Unlike saliva and plasma which are proteinaceous contaminants, non-water-based sealers contain fillers, essential oils, resins and catalysts. It is not known whether a surfactant-based cleaner recommended by its manufacturer for intraoral cleaning of saliva- or blood-contaminated tooth or prosthetic surfaces is equally adept at decontaminating dentine that is smeared by different types of root canal sealers Accordingly, the objective of the present study was to compare the effectiveness of different sealer removal methods and their influence on bonding with a two-step self-etch adhesive to sealer-contaminated dentine. The null hypothesis tested was that there is no difference in the cleaning effects of a surfactant-based cleaner, cleaning with ethanol and cleaning with a dry cotton pellet, on dentine contaminated with different types of root canal sealers.

Materials And Methods:-

Forty-nine recently extracted caries-free human third molars were collected based on the inclusion and exclusion criteria. The extracted teeth were stored in 0.9 % (w/v) NaCl containing 0.02 % sodium azide at 4◦C for no longer than one month. Thirty-five of the collected teeth were used for bond strength evaluation and 14 teeth for scanning electron microscopy (SEM). For teeth that were used for bond strength testing, coronal dentine was exposed by cutting off the coronal enamel perpendicular to the longitudinal axis of each tooth, using a slow-speed diamond saw (Isomet, Buehler Ltd, USA) with copious water irrigation. A stereoscopical microscope was used at 30x magnification to verify complete removal of enamel on the dentine surface. The flat dentine surface was polished with 180-grit silicon carbide paper to create a standardized substrate for subsequent bonding. For teeth that were used for SEM examination, the pulpal floor was exposed by cutting off the crown at the level of the cementoenamel junction without touching the floor dentine structure.

Two root canal sealers were used to simulate clinical sealer contamination. The first sealer was an epoxy resin-based sealer [AH Plus (AHP), Dentsply Sirona]. The second sealer was a zinc oxide eugenol-based sealer [Pulp Canal Sealer (PCS), Kerr Endodontics]. Each sealer was smeared separately on the dentine surface of a designated tooth and left undisturbed for 5 min. Three cleaning methods were employed to clean the sealer-contaminated dentine:

1. Dry cotton wipe with a size 2 cotton pellet. The dentine surface that was contaminated with a root canal sealer was hand-scrubbed with the dry cotton pellet until it was visibly clean.

2. Use of an ethanol cotton wipe with a size 2 cotton pellet that was saturated with 70% ethanol. The sealer-contaminated dentine surface was hand-scrubbed with the ethanol-saturated cotton pellet until it was visibly clean.
3. Use of a surfactant-based multi-purpose cleaner (ZirClean Cleaner). One drop of the purple-coloured cleaner was dispensed from the bottle without shaking, applied to the sealer-contaminated dentine with a microbrush and agitated for 10 sec. After application of the ZirClean Cleaner, the dentine surface was rinsed with deionized water for at least 10 s until the purple colour was no longer visible. The use of two sealers and three cleaning protocols resulted in 6 experimental groups. The last group of teeth were not smeared with any sealer; those uncontaminated dentine surfaces were not cleaned and were used as the control.

Five teeth from each group were used for bond strength testing. After sealer removal using the designated protocol, each dentine surface was bonded with a two-step self-etch adhesive (Clearfil Protect Bond) according to the manufacturer's instructions. Briefly, the primer component of the two-step self-etch adhesive was liberally applied to a dentine surface and left untouched for 20 sec. After priming the dentine surface, the volatile ingredients of the primer were evaporated using moisture-free air. The bond component of the adhesive system was then applied to the primer-conditioned dentine, gently blow-dried and light-cured for 10 s using a light-emitting diode light-curing unit (Ivoclar bluephase, India) with an output intensity of 1100 mW/cm2. A resin composite core build-up was placed over each bonded dentine surface, using a hybrid resin composite (Clearfil Majesty ES-2) in 2 mm-thick increments. Each increment was light-cured individually for 40 s each. After storage in water at 37◦C for 24 h, each tooth with core build-up was sectioned longitudinally into 0.9 mm thick slabs. Two adjacent central slabs were selected and further sectioned into 0.9 mm X 0.9 mm beams using the Isomet saw with water cooling. The two longest beams adjacent to the pulp space from each slab were used for testing, yielding20 beams for each group. Each beam was secured in a testing jig and stressed to failure under tension using a universal testing machine (HEICO, New Delhi, India) at a crosshead speed of 1 mm/min. Premature failure was recorded as null bond strength and included in the statistical analysis. Failure modes were examined using a stereoscopical microscope at 30x magnification. Failure modes were classified as adhesive failure, mixed failure and cohesive failure in resin composite or dentine.

Scanning electron microscopy was performed using a JSM SEM (Kyowa Getner, Japan) to observe the cleanliness of the pulpal dentine surface with or without sealer contamination andafter different cleaning protocols. Two teeth from each group were used for examination. The exposed pulpal floor was immersed in 6% NaOCl for 10 min to remove organic remnants within the pulp chamber and to expose the dentinal tubules. Each specimen was air-dried instead of dehydrated with an ascending ethanol series to avoid dissolution of the remnant sealer with ethanol. The desiccated specimens were sputter-coated with gold/palladium. Three spots on each dentine surface were randomly selected for SEM examination. The specimens were examined with high vacuum mode at 15 KeV, using secondary electron imaging. Images were acquired for each spot at a magnification of 2000x.

Statistical analysis

Bond strength analyses were performed for data derived from groups of the same root canal sealer and the control group. No attempt was made to compare results acquired from the two sealers. For each sealer, data derived from the four subgroups (control; sealer; sealer – ethanol; sealer –ZirClean Cleaner) were tested using the Shapiro-Wilk test and modified Levene test, respectively, prior to the use of parametric statistical methods. If those assumptions were satisfied, the data sets were analysed with one-factor analysis of variance and Holmpairwise multiple comparisons. If those assumptions were violated, the data were nonlinearly transformed to satisfy those assumptions prior to the use of the aforementioned parametric statistical procedures. All analyses were performed using SPSS v20 (IBM Corp, NY, USA). Statistical significance for both analyses were pre-determined at $\alpha = 0.05$.

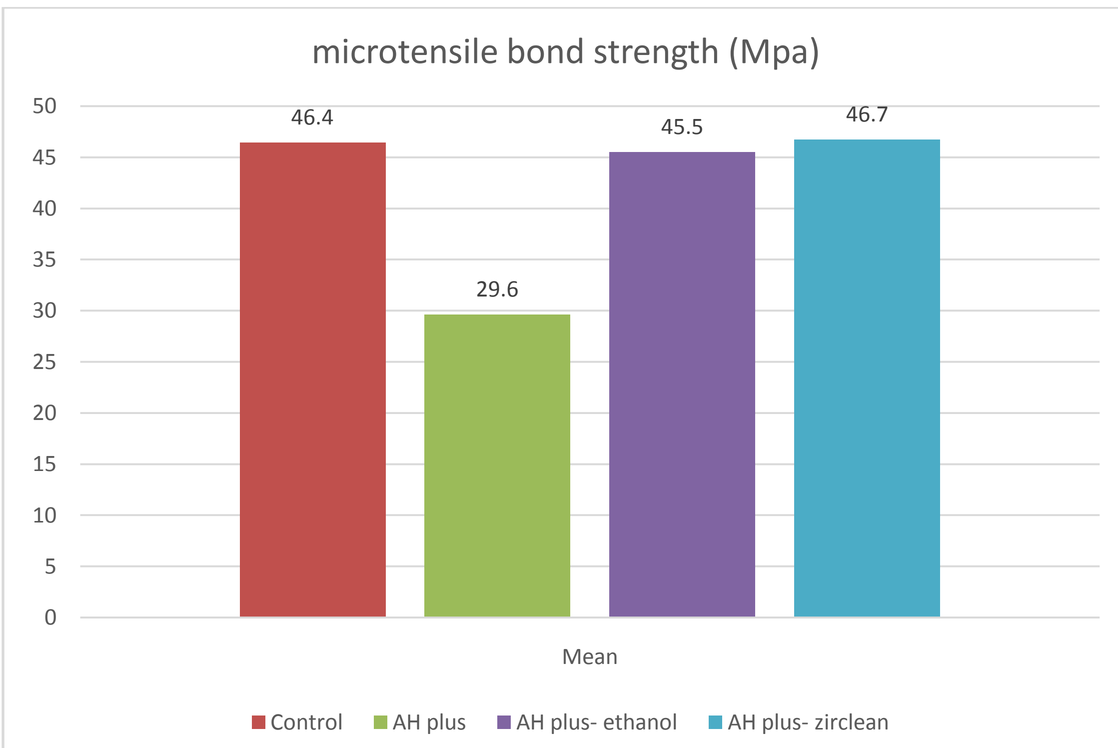
Results:-

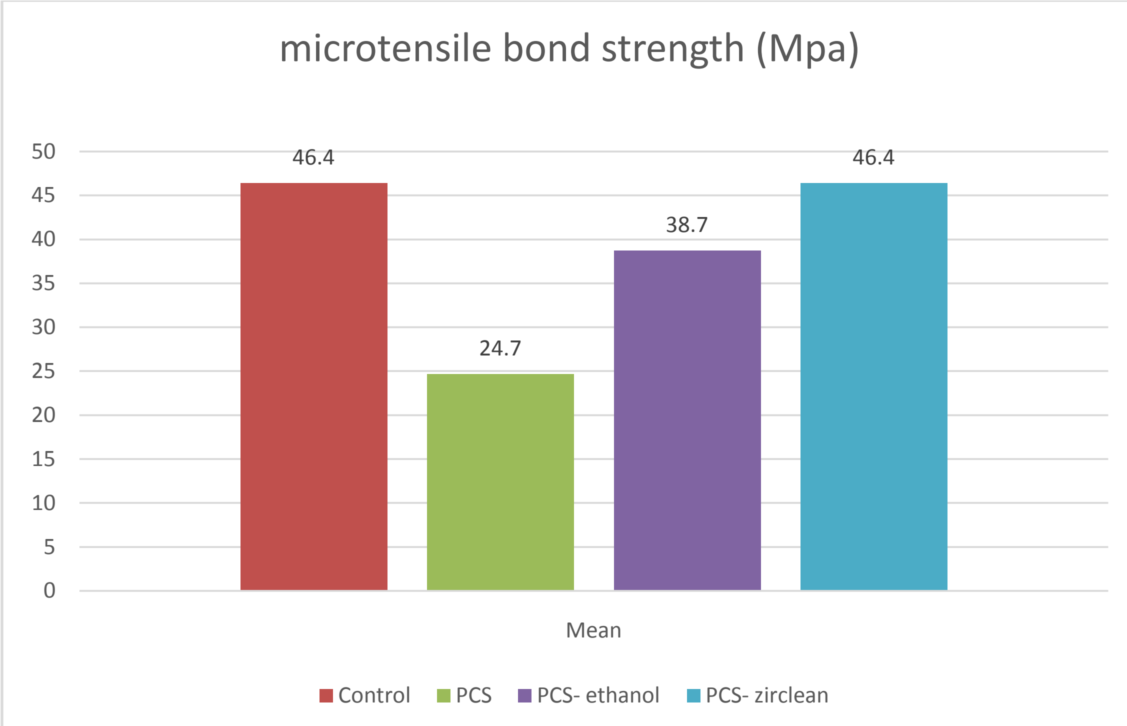
Tensile bond strength results are summarised in Fig. 1A for groups associated with the AHP sealer and Fig. 1B for groups associated with the PCS sealer. Tensile bond strength for the self-etch adhesive to uncontaminated dentine was 46.4 ± 7.3 MPa. Wiping the sealer-contaminated dentine with dry cotton pellets significantly reduced bond strength: 29.6 ± 4.2 MPa for AHP-contaminated dentine ($p < 0.05$) and 24.7 ± 4.8 MPa for PCS-contaminated dentine ($p < 0.05$). For the AHP sealer, both ethanol (45.5 ± 5.8 MPa) and ZirClean Cleaner (46.7 ± 6.5 MPa) restored bond strength to its original level, with no significant difference between the two cleaning methods ($p > 0.05$). For the PCS sealer, bond strength to dentine was in the order: uncontaminatedcontrol = ZirClean Cleaner (46.4 ± 6.3 MPa) >ethanol (38.7 ±4 .8 MPa) >dry cotton-wiped PCS-contaminated dentine ($p < 0.05$).

Failure mode results are shown in Table 1. Most of the failures were adhesive or mixed failure. Scanning electron microscopy images of sealer-contaminated pulpal floor dentine surfaces after cleaning with the three protocols are shown in Fig. 2 for the AHP sealer and Fig. 3 for the PCS sealer. In the uncontaminated control, the dentine surfaces were devoid of a smear layer and all dentinal tubules were rendered patent after the pulpal floor was treated with NaOCl (Figs. 2A and 3A). After dry cotton wiping, the dentine surface was still covered with sealer. Filler particles in the AHP sealer were highly granular (Fig. 2B) while those in the PCS sealer were finer (Fig. 3B). Wiping a sealer-contaminated dentine surface with ethanol reduced the sealer to a very thin smear. However, the dentinal tubules were barely noticeable (Figs. 2C and 3C). Cleaning sealer-contaminated dentine with the ZirClean Cleaner removed more sealer from the dentine surface. Dentinal tubules were visible but most of the tubule orifices were still filled with sealer (Figs. 2D and 3D).

Table 1: Analysis of failure mode (n = 20).

Failure mode	**Dentin**	**AH plus No cleaning**	**AH plus- Ethanol**	**AH plus-zirclean**	**PCS No cleaning**	**PCS-Ethanol**	**PCS-zirclean**
Adhesive	7	13	2	14	14	4	5
Mixed	13	7	11	4	5	14	12
Cohesive	0	0	7	2	1	2	3





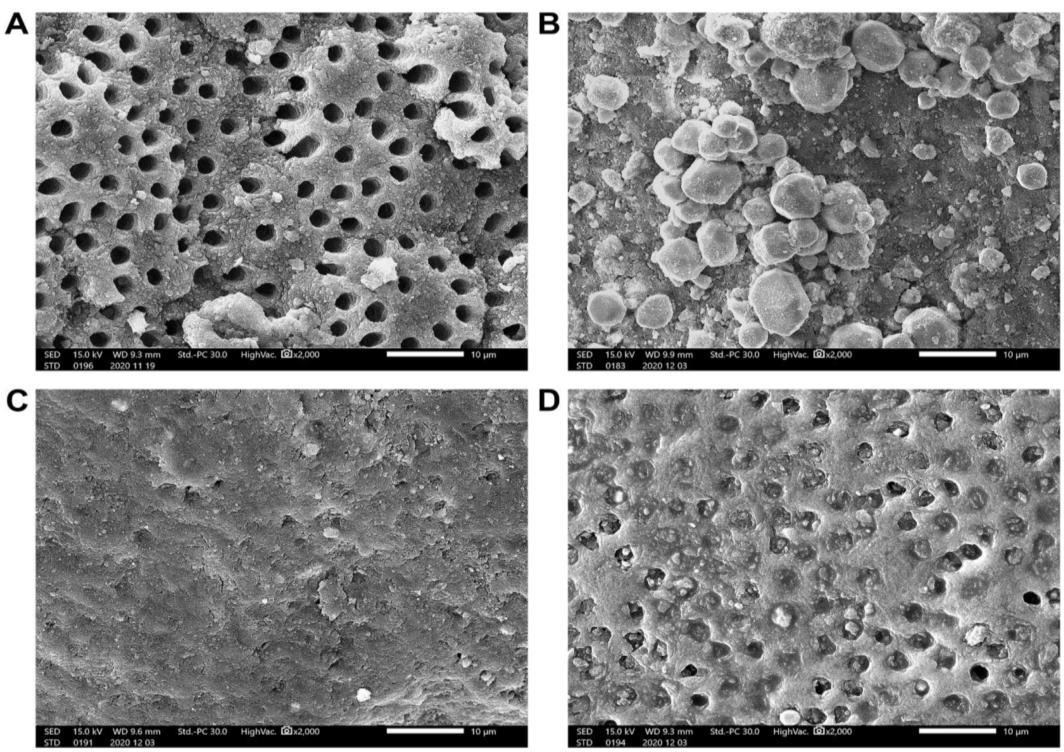


Fig. 2:- Representative scanning electron microscopy images of pulpal floor dentine of third molars after AH Plus sealer-contaminated dentine was cleaned with different protocols. (**A**) Uncontaminated dentine control - pulpal floor dentine after irrigation with 6.15% NaOCl for 10 min revealed clean surface with open dentinal tubules. (**B**) AH Plus-contaminated pulpal floor dentine that was wiped with a dry cotton pellet. A thick layer of nodular sealer particles remained and obscured the dentine surface. (**C**) AH Plus-contaminated pulpal floor dentine that was wiped with a cotton pellet saturated with ethanol. A thin smear of sealer was visible and the underlying dentine structure was hardly recognisable. (**D**) AH Plus-contaminated pulpal floor dentine that was cleaned with Zirclean Cleaner

using microbrush agitation for 10 s. Dentine structure could be identified with small amount of sealer remaining on dentine surface and within the dentinal tubules.

Discussion:-

In the context of present in vitro study, the surfactant-based cleaner, originally designed for decontamination of zirconia surfaces, achieved equal or better results than ethanol or dry cotton when it was used for cleaning dentine contaminated with an epoxy resin-based sealer or a zinc oxide eugenol-based sealer. The ZirClean Cleaner was capable of restoring the bonding potential of a two-step self-etch adhesive to sealer-contaminated dentine. Hence, the null hypothesis that "there is no difference in the cleaning effects of a surfactant-based cleaner, cleaning with ethanol and cleaning with a dry cotton pellet, on dentine contaminated with different types of root canal sealers" has to be rejected. The surfactant-based cleaner provides an alternative method to other organic solvents for cleaning remnant root canal sealers from sealer contaminated coronal access and the pulpal floor. It is pertinent to point out that none of the cleaning methods employed in the present study was capable of completely removing sealer from contaminated dentine surface due to the limited application time and the micro-morphology of the dentine surface. For the zinc oxide eugenol-based sealer, bond strength to dentine was higher after cleaning with the ZirClean Cleaner, compared with ethanol cleaning. This may be attributed to the infusion of the eugenol component of the sealer into the dentine. In a clinical scenario, a root canal sealer may infiltrate deep into the dentine tubules because of the heat and pressure produced by warm vertical compaction [28]. This is supported by the results of the present study in which residual root canal sealers were identified from all SEM images after cleaning. This warrants the need for the investigation of new sealer cleaning methods.

Pulpal floor dentine was used for examination of dentineultra structure. The dentine examined in the control corresponded clinically to dentine that has been irrigated with ethylenediamine tetra-acetic acid and NaOCl. The smear layer was removed completely and the dentine tubule orifices were rendered patent for sealer penetration.[21]A flat dentine surface was created due to the requirements of bond strength testing. Cutting dentine to create a flat bonding surface produces a smear layer that may impede sealer penetration into the dentinal tubules. Nevertheless, such a scenario is still clinically relevant because a sealer may be smeared against the root canal wall when post space preparation is performed immediately after root canal treatment. A two-step self-etch adhesive was used in the present study. Self-etch adhesives generally demineralise dentine less aggressively than phosphoric acid etching and generate less discrepancy between etching depth and resin monomer infiltration depth when compared with etch-and-rinse bonding systems.[22]The non-rinsing nature of this adhesive category creates a potential problem when the adhesive is applied to sealer-contaminated dentine. That is, all remaining contaminants have to be incorporated into the hybrid layer.[3] Early studies that utilized etch-and-rinse adhesives for bonding after sealer cleaning failed to identify any difference between dry cotton sealer cleaning and ethanol sealer cleaning [11, 12]. This may be attributed to the additional cleaning effect of phosphoric acid etching on the dentine surface [23]. Removing sealers from dentine is comparable to surface cleaning/ decontamination that occurs with other facets of cleaning (e.g., hair, textiles). From an industrial cleaning perspective, decontamination of a surface involves the interfacial processes of desorption, dispersion and counteraction against electrostatic charges.[29] Factors that influence these processes include chemistry, time, mechanical action, temperature and water drainage.[30]Although the leeway for alteration of temperature and application time is relative limited in dental applications, the chemistry of a cleaner may be improved to facilitate those processes. In addition, mechanical action such as brush agitation may be incorporated to enhance the outcome of decontamination. There are three mechanisms associated with the cleaning of sealers from sealer-contaminated dentine: physical dissolution, mechanical removal and surface modification followed by removal. The most widely used method is physical dissolution with an organic solvent. The drawbacks associated with the use of organic solvents for decontamination are dependent on the specific solvent employed. Ethanol is a weak organic solvent because of its highly polar nature.[24] Acetone, in comparison, is less polar, volatile and inflammable. Mechanical removal such as dry cotton wiping is not effective due to the hydrophobic and sticky nature of most sealers. Sandblasting has been adopted in several studies and demonstrated optimal results [11, 13]. However, sandblasting may pose occupational safety issues when used chairside without an appropriate sand removal mechanism.[25] Based on these rational isations, the properties of an optimal root canal sealer cleaner include good cleaning capability, biocompatibility, low cost, availability and ease of removal. An optimal cleaner for root canal sealers should not damage the dentine structure, influence subsequent bonding procedures and resin polymerisation, or possess dentine staining potential. Based on the information provided by the manufacturer, the surfactant used in ZirClean Cleaner is an MDP salt. A purple colour indicator is incorporated into the cleaner to ensure that the surfactant is completely rinsed off. The manufacturer of ZirClean Cleaner recommends that its use for decontaminating blood- or saliva-contaminated enamel or dentine, zirconia, composites and fibre-posts prior to adhesive bonding. The results of the present study indicate that the cleaner is also effective for removal of sealers from contaminated dentine. The cleaning efficacy may be attributed to the action of surfactant as well as the brushing motion. The pH of the commercial cleaner is around 4-5. The potential interaction of the MDP salt with dentine in an acidic environment is unknown. Further investigations may be performed to see if the MDP surfactant present in ZirClean Cleaner is capable of decontaminating other prosthodontic surfaces such as titanium implants that have been contaminated with oil or saliva.

Bond strength testing and morphological evaluation of cleanliness are the major methods employed for evaluating cleaning effectiveness in previous studies. Grading of cleanliness or counting open tubules in SEM images are only semi-quantitative and is highly subjective.[14, 18] One study mixed stain with sealer to help indicate the amount of sealer left on a dentine surface and examined the decontamination outcome using a light microscope.[13] Such a method is incapable of determining whether a thin smear of sealer still remains on the dentine surface. It is prudent to mention that the more recently available tricalcium silicate-based sealers are not investigated in the present work. Presumably, these sealers are hydraulic sealers and can be easily rinsed off with water prior to setting. To date, less information is available for self-etch adhesives. Thus, it is meaningful to examine the effect of tricalcium silicate-based sealer or cement contamination on bonding of self-etch adhesives to dentine.

Conclusions:-

Based on the limitations of this study, Zirclean decontaminates root canal sealer-smeared dentine surfaces effectively and restores the bonding performance of a self-etch adhesive to dentine.

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