Effect of 10% Triphala as final irrigant on pushout bond strength of Bioroot RCS to root canal dentin: *An Invitro study*

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Introduction

The key to successful endodontic treatment is complete removal of pathogens, thorough cleaning and shaping of the root canal, and proper sealing to prevent infection spread. A study shows 35% of the surfaces inside canal of the root (especially isthmi, canal fins, and cul-de-sacs) stay devoid of any instrumentation, emphasizing the fact that debridement of root canal is highly dependent on thorough irrigation. [1] Irrigants used during chemomechanical preparation flush these loose debris out, act as an antimicrobial agent, & dissolve the inorganic & organic component of smear layer leading to improved adaption between sealer to the dentinal walls. [2] EDTA is a polyamino-carboxylic acid which binds to metals via 4 carboxylate & 2 amine groups. [3] It is routinely used in the field of endodontics as a root canal irrigant & chelating agent. It was Nysgaard-Ostby who pioneered the use of EDTA as a chelating agent in Endodontics. It can chelate & remove calcium ions from dentin & dentinal debris. 17% EDTA is often considered to be the gold standard among chelators, and has displayed superior bond strength with BioRootTM RCS to radicular dentin when used in conjunction with 3% green tea extract over 5.25% NaOCl and 2% Chlorhexidine. [4] But despite its numerous advantages, EDTA can lead to inhibition of the macrophage function, causing an inflammatory response in periapical region, and may cause cytotoxicity. It leads to softening of dentin and causes a sharp decrease in the dentin microhardness. It also causes erosion of radicular dentin, which is deleterious to the POBS of any sealer to radicular dentin. [5] Thus, the search for more biocompatible irrigants than EDTA, which has less severe impact on periapical tissues continues.

Triphala has been utilised as an age-old remedy in Indian Ayurvedic system. Terminalia bellerica, Terminalia chebula & Emblica officinalis are the constituents present in Triphala. It has been shown to have long term substantivity, less cytotoxic, and lacks microbial resistance. It has been shown to be safer to use and less cytotoxic than NaOCl and doesn’t significantly affect the mechanical properties of the root like EDTA. [6] It has been recommended as an alternative to EDTA due of its potent antimicrobial capability, especially against E. faecalis & C. albicans, & its chelating ability to remove smear layer. [7] In previous studies, it has been shown that Triphala contains tannic acid and gallic acid, which might be beneficial in removal of smear layer. [8] Presence of citrusacid present in the fruits of Triphala acts as a weak chelator which explains the minimal effect on microhardness of dentin. [7] Commercial preparations are easily available, straight-forward to use and have longer shelf life. [8]

BioRootTM RCS is a hydraulic tricalcium-based sealer from Septodont that is comprised of tricalcium silicate, zirconium oxide, calcium chloride, polycarboxylate, and povidone. It demonstrates high antimicrobial activity & low cytotoxicity due to alkalinity of the sealer and prolonged release of Ca+ ions after setting, which may lead to endodontic & periodontal regeneration. [9] BioRootTM RCS has been reported to induce angiogenic and osteogenic growth factors in vitro and has been shown to be least cytotoxic compared to other sealers [10] and may also induce hard tissue deposition. BioRootTM RCS has been gaining tremendous interest owing to its ability to continue sealing in the presence of hydrophilic environment by apatite deposition and mineralization at the root canal wall interface. [9] Push-out bond strength (POBS) is often deemed to be an important foretelling factor for checking the connection between canal wall & the core material to the sealer evaluating shear bond strength interfacially between radicular dentin & filling material of the canal by computing the force necessary to displace the filling material. [11] Good POBS to dentin is considered to be a crucial attribute for the sealer as it leads to voidless interface between dentin & obturating material which is important for establishment of an impervious seal & providing dislocation resistance for the root filling during flexure of tooth. [12]

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Till date, no studies that have evaluated the effect of 10% Triphala used as an endodontic irrigant on the POBS of BioRootTM RCS sealer to radicular dentin. The null hypothesis was final irrigating solutions do not cause any significant difference in the POBS of BioRootTM RCS sealer to radicular dentin.

Materials and methods

Sample Size Estimation

The size of the sample for this study was estimated on the basis of a prior study which measured POBS of Bio-ceramic sealer to radicular dentin conditioned with different final irrigants. [4] On the basis of this, the size of the sample was calculated at a confidence interval of 95% & 90% power, leading to 12 samples in each group.

Sample Selection

Ethical clearance from the institutional review board was taken for the usage of human teeth which were extracted. (IEC2 642/20202) In totality, 36 extracted teeth with one root, one canal, were chosen. Tissue attachments & debris on the samples were eliminated with the use of ultrasonic scalers. The teeth storage was done in a 0.2% sodium azide solution at 4^{o}C till use. Radiographs were obtained from labial & mesial-proximal aspect of teeth to re-affirm a straight, one canal, mature apices with no calcifications. Samples were then categorized into 3 groups (n=12), group A (10 % Triphala), group B (17% EDTA), group C (0.9% saline).

Root Canal Preparation

With the help of a diamond disc, all the teeth were decoronated. Working length (WL) was measured using a size 10-K file till it became visible at the apex (observed using magnification) followed by subtraction of 1 mm from the length recorded. The canal width was standardized using a size 25-K file. Sticky wax was applied at the end to close the apices of every tooth & also to avoid any irrigant flow through the apical foramen of samples so as to enable reverse irrigant flow to replicate a closed end root canal morphological system. ProTaper Gold rotary files (Dentsply) were used in crown-down technique till F3 (30/0.09) for shaping and cleaning. Irrigation was done with 5 mL of 2.5% NaOCl for one minute for every change of instrument and then a rinse of 5 mL of 2.5% NaOCl at the end of shaping & cleaning. For the final rinse, the irrigation regimen was followed as given below:

- GROUP A – 5mL of 10% Triphala for 1 min as final rinse
- GROUP B –5mL of 17% EDTA for 1 min as final rinse
- GROUP C –5mL of 0.9% saline for 1 min as final rinse

After the above regimen, 5 mL of distilled water was used for one min. Entire irrigation procedure was done with the help of a 30-G side-vented needle. It was placed one mm short of WL. Following the final irrigation protocol, with the help of paper points #40, the canals were dried.

Preparation of Dentin Disks

Sectioning of the tooth was performed using hard tissue microtome under continuous water cooling to achieve a slice of thickness 2.0 ± 0.1mm from the middle third. Sealer was mixed as per the manufacturer’s instructions & compacted into the dentin disks using a hand plugger. Then, for 7 days it was allowed to set at 37º C & 100% humidity before push-out assessment.

Evaluation of Push out Bond Strength

Height of each disc & diameter of the canal were noted with the help of digital caliper. The adhesion surface area was calculated: Adhesion surface area (mm sq.) = 2 x $\boldsymbol{\pi}$ x h x r, wherein, $\boldsymbol{\pi}$ is the constant 3.14, r is radius of the root canal preparation & h is thickness of the root disc. The push- out testing was done with the help of Universal Testing Machine (UTM). Force was directed in the apico-coronal direction at a crosshead speed of one mm per minute with the help of 0.6 mm stainless-steel plungers. It was placed such that it came in contact only with the obturating cement. The highest force (F) that was applied at the time of bond failure was noted in Newton. The POBS was measured in mega Pascals (MPa): Push-out BS (MPa) = Force (N)/ Adhesion surface area (mm sq.). POBS values with respect to BioRootTM RCS sealer to radicular dentin was given as means ± standard deviation.

Fractographic Analysis

Every sample of all the groups was analyzed under stereomicroscopic at 40x magnification to ascertain the type of bond failure. The types of bond failures were classified as:

1. Mixed failure: Both adhesive and cohesive failure.

2. Cohesive failure: Within BioRootTM RCS.

3. Adhesive failure: At canal walls and & BioRootTM RCS interface.

Statistical Analysis

Data was statistically analysed with the help of SPSS Statistics Version 24.0 software (IBM Corp, Armonk, NY). Normality was evaluated using Kolmogorov-

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Smirnov test. Push- out BS data was analysed using one-way ANOVA with post hoc Tukey Honest Significant Difference test. In order to analyse bond failures, Chi-square test was done. P value < .05 was considered to be statistically important. (95% confidence)

Results

Push-out bond strength analysis

On intergroup comparison, Group B (17 % EDTA) had highest POBS (3.569 MPa), followed by Group A (10% Triphala) with POBS (3.458 MPa) and Group C (0.9% saline) had lowest BS (2.243 MPa) in comparison to remaining groups (P< .05). No significant difference was observed amongst groups A (10% Triphala) [3.458 MPa] and B (17 % EDTA) [3.569 MPa] (P > .05). However, significant difference was noticed between group A (10% Triphala) and Group C (0.9% Saline) & a very highly significant difference between group B (17 % EDTA) and Group C (0.9% saline).

Fractographic Analysis

Chi-square test demonstrated that there wasn't a significant difference amongst the groups with respect to the disposition of failure types. The results of the fractographic analysis showed that out of a total 36 samples, 22.3% showed adhesive failure, 17.1% showed cohesive failure and 60.6% showed mixed failure.

Discussion

The present in-vitro study evaluated the effect of different final irrigation protocols on the POBS of BioRootTM RCS to radicular dentin. The different irrigation protocols showed variable influence on the POBS of BioRootTM RCS.

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Hence the null hypothesis was rejected which stated that the irrigation regimen does not influence the POBS of the sealers to radicular dentin. Similar results have been obtained by Srivastava et al., Alfawaz et al and Türker et al, with BioRootTM RCS on testing with 17% EDTA. [4,13,14]

A successful root canal treatment is dependent on lot of factors, which include, through and proper biomechanical preparation, 3-dimensional impervious obturation seal, and ultimately a good post endodontic restoration. The main aim of this therapy precludes the re-entry of any pathogens and prevention of any microleakage which might predispose the treatment for a failure. Investigators have stated that most of the unsuccessful RCTs are probably due to microleakage of irritants and microbes into the periapical region. Thus, adhesive properties of endodontic sealers play a vital role in minimising the detachment of material from the dentin, & to prevent microleakage. [15] BS tests are utilised in Endodontics to check for the adhesiveness of the sealer to the tooth surface. Increased adhesion of the material may provide better strength to the restored teeth [16].

To evaluate resistance of root canal filling material against dislodgement forces push-out tests have been commonly utilised. [17] Resistance to displacement forces provided by the root canal filling material is successful in keeping the integrity of the sealer-dentin interface intact during flexure of the tooth and intra-radicular procedures. [18] The POBS values offer valuable insight regarding the sealer retention on the root canal walls. To prevent sealer detachment or premature debonding while slicing, thick slices of 2 mm were preferred. [19] The pin chosen for every disk, that is, 0.6 mm for middle third, was approximately

90% of the diameter of the canal, so that it contacts maximum of the sealer material without putting undue pressure on root canal walls. [20]

NaOCl was used in the present study as it is often deemed as most ideal irrigant & can serve as a benchmark for any endodontic therapy. As an irrigant, it has good antimicrobial action and is relatively cheap, it has low viscosity, can dissolve proteins, and has a good shelf life. It is also very effective in destroying bacterial biofilms. [21] NaOCl is commercially available in the market at various concentrations including 0.5%, 1%, 2.5%, 5.25%. NaOCl can lead to destruction of the intratubular surface near the root canal and, eventually cause reduction in the radicular dentin's mechanical strength. [22] The higher the concentration of NaOCl, the more profound is its effect.

Based on the literature review by Jefferson et al., they concluded that the 2.5% NaOCl is the most suitable for endodontic treatment of root canals as it shows good bactericidal as well as potent tissue dissolving ability and low cytotoxicity. [23] Thus, in this study 2.5% NaOCl was used as irrigant in between instrument change. Along with the tissue dissolving capability of NaOCl, a chelating agent is often necessary for removal of smear layer produced for better penetration of sealers to root canal dentin. [24] Hence, chelating agents such as 17 % EDTA is commonly preferred as the final rinse during the irrigation protocol. It can remove the dentinal debris as well as smear layer produced during instrumentation. The length of application determines the effectiveness of the solution. Longer the EDTA application, more is the depth & percentage of sealer penetrability, more smear layer will be removed & sealer penetration will be deeper, thus leading to better bond strength of sealer to radicular dentin. [25]

In the current study, irrigation with 17% EDTA was done for only 1 min. A longer irrigation time often leads to demineralization and eventually to erosion of dentin, leading to reduction in flexural strength of dentin & modulus of elasticity, eventually affecting the dentinal microhardness & increasing the odds of root fracture. [26,27]

Due to the presence of high amount of citric acid present in the constituents of Triphala, it acts as a chelator and that may be reason for good the smear layer removal. [28] Along with this the acidic constituents like tannic acid and gallic acid can cause smear layer removal. [7] This might be the reason for the high POBS for 10% Triphala group. In the present study,17% EDTA showed the best POBS followed by 10% Triphala & 0.9% Saline. This can be attributed to better smear layer removal, which eventually resulted in better penetration of sealer, leading to formation of a stronger bond. Results showed with 10% Triphala didn’t show a significant difference, owing to its weak chelating ability.[28]

Removal of smear layer allows better penetrability of the sealer into the dentinal tubules, leading to better adhesion to the radicular dentin according to Bayram et al. [29] In the present study, samples irrigated with 0.9% saline exhibited lower bond strength probably due to lack of smear layer removal from radicular dentin, leading to sealer not able to penetrate into the dentinal tubules. [4] BioRootTM RCS was preferred in this study over other sealers such as AH Plus as it demonstrated better POBS. [4, 30] It also shows bioactivity with sustained Ca+2 ion release, low cytotoxicity & minimal irritation to the periapex and its tissues. [31] According to the results of fractographic analysis, mixed failure was seen in majority of the samples which accounted to 60.6%, followed by adhesive failures which summed up to a 22.3% and cohesive failures being the least only making it to 17.1% of all the samples. A similar observation was seen in a study done by Donnermeyer et al. [32]

Conclusions

Within the limitations of the study, 10% Triphala is comparable to 17% EDTA as the final irrigant with respect to POBS of BioRootTM RCS to root canal dentin.

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TABLES

Table I. POBS of different groups in this study (All values in MPa)

Sample no	10% Triphala	17% EDTA	0.9% Saline
1	2.32	4.12	1.36
2	5.95	3.83	1.78
3	3.44	3.78	1.91
4	3.27	2.46	3.27
5	3.39	2.97	1.89
6	3.14	3.63	2.47
7	3.70	3.21	2.23
8	2.84	4.27	2.97
9	4.13	5.03	1.94
10	2.98	3.72	2.51
11	3.11	3.45	3.27
12	3.23	2.36	1.32

Table 2 represents the mean POBS of different groups in this study along with standard deviation. (All values in MPa)

	N^a	Mean	Std. Deviation
Pushout Bond Strength for 10% Triphala	12	3.458	.902
Pushout Bond Strength for 17 % EDTA	12	3.569	.755
Pushout Bond Strength for Saline	12	2.243	.670

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FIGURES

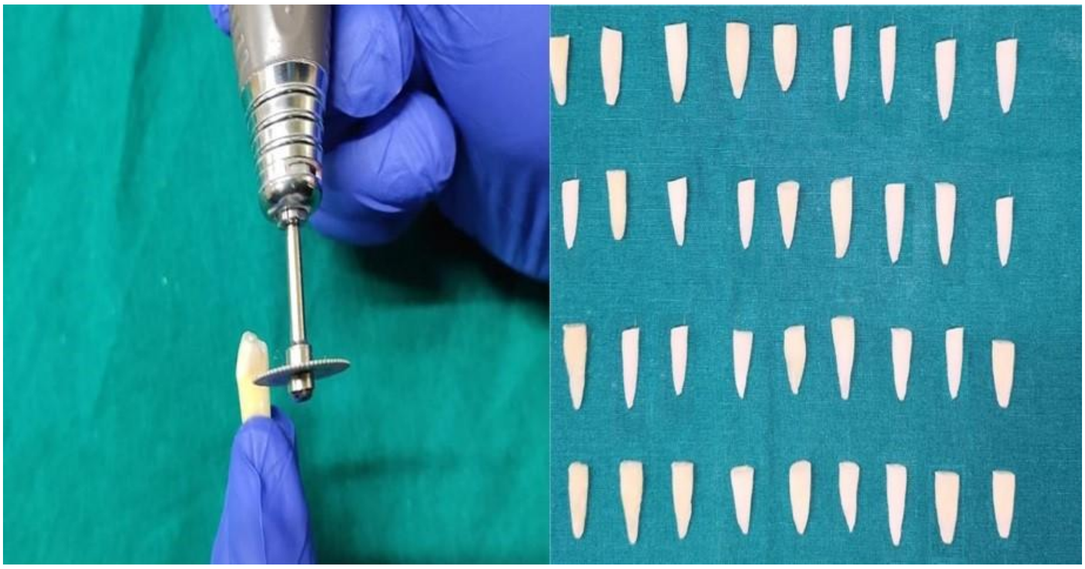

Figure 1. Decoronated tooth samples

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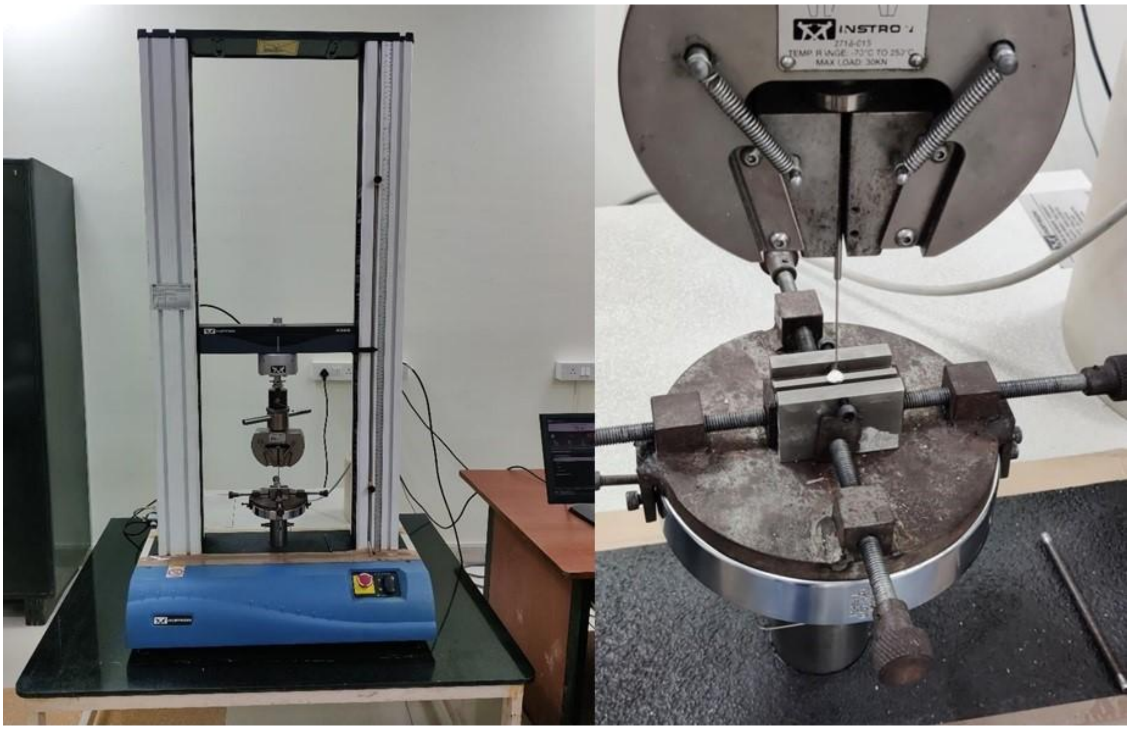

Figure 2. UTM (Instron) used to evaluate POBS

Figure 3. Stereomicroscope used for bond failure analysis

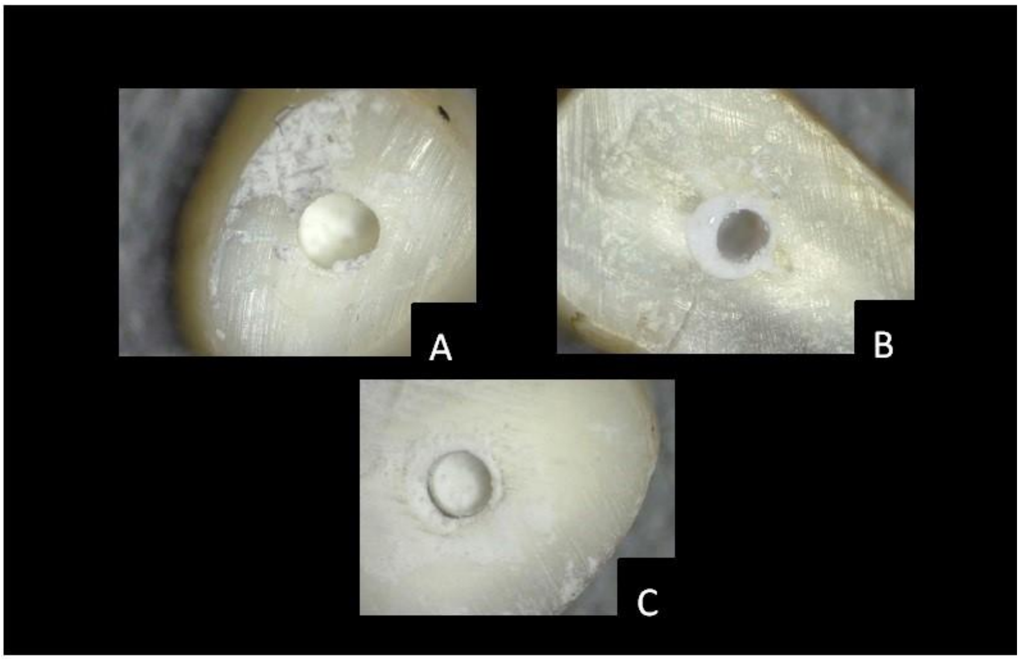

Figure 4. Representative stereomicroscopic images of the bond failures types. (A) Adhesive failure, (B) cohesive failure, and (C) mixed failure

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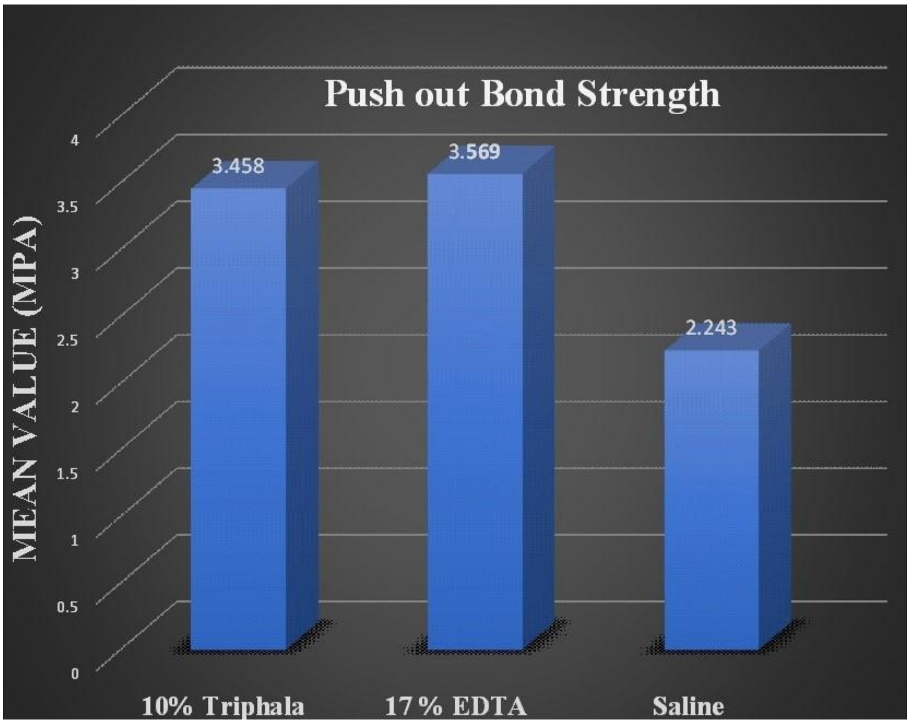

Figure 5. Graph representing Mean Push out bond strength of BioRootTM RCS

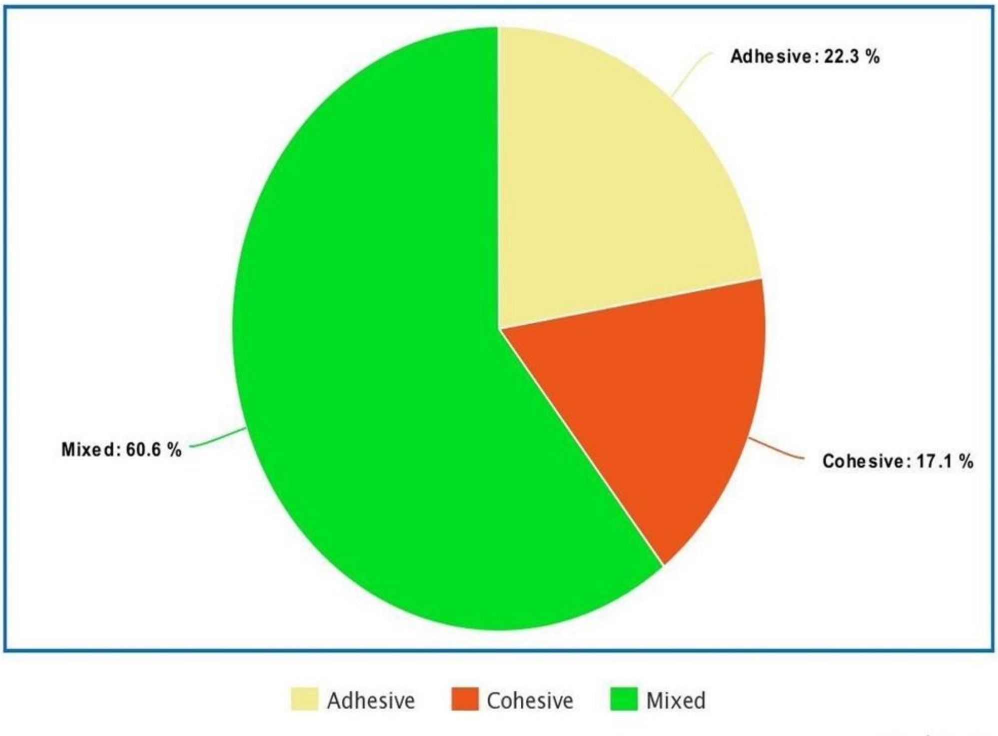

Figure 6. Pie chart of fractographic analysis of BioRoot RCS sealer