Original Article

The influence of humidity on bond strength of AH Plus, BioRoot RCS, and Nanoseal-S sealers: An *in vitro* study

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ABSTRACT

Aim: This study aimed to evaluate the influence of humidity on the bond strength of AH Plus, BioRoot RCS, and Nanoseal-S sealers.

Materials and Methods: Sixty root slices, each 1 ± 0.1 mm thick, were prepared from the middle-third of 20 mandibular premolars. Three holes, 0.8 mm wide each, were drilled on each slice. These holes were subjected to standardized irrigations and dried. Each hole in each slice was filled with the three individual sealers, AH Plus, BioRoot RCS, and Nanoseal-S. The samples were then stored in saline for 7 days at 37 C. The push-out bond strength of each sealer in each hole of all samples was tested. The differences in push-out bond strengths between the three sealer samples were assessed using the unpaired *t*-test and *post hoc* Turkey's HSD test. The significance level was set at 5%.

Results: The results of the *post hoc* Tukey's HSD test ($P < 0.05$) indicated that the mean and median push-out bond strength were highest for BioRoot RCS, irrespective of the moisture conditions.

Conclusion: Humidity conditions did not influence the bond strength of AH Plus, BioRoot RCS, and Nanoseal-S sealers.

Keywords: AH Plus sealer, bioceramic sealer, Nanoseal-S sealer, push-out bond strength

INTRODUCTION

A successful root canal treatment can be achieved when sealers are used to prevent the residual bacteria and endotoxins from crossing the root apex.[1] Nevertheless, after a thorough cleaning and shaping of the root canal, some sealers may not be successful in preventing the foregoing infection.[2,3] In this regard, it is necessary to identify an optimum sealing material that is capable of maintaining bond to the dentinal wall, preventing the preceding infection, and resisting dislodgement of the filling.

Typically, push-out bond strength determines the extent of resistance to dislodgement of a filling material when applied to the root canal dentin. Although push-out bond strength might not be reliable in terms of representing clinical conditions of the sealers, according to Uregan *et al.*, it showed a better assessment of the bond stronger than the conventional shear tests.[4]

A new sealer, Nanoseal-S (Prevest DenPro, Digiana, India) was introduced, composed of polydimethylsiloxane matrix and micro-silver in chemical form. Micro-silver particles are distributed equally in the sealer. The presence of micro-silver in chemical form means that it does not cause corrosion or color changes, and hence, it is also highly biocompatible preventing further bacterial progression.[5]

Polydimethylsiloxane-based sealers have a distinct feature of minor expansion of approximately 0.2% on the setting, providing firm adaptation to dentinal walls and hence decreasing the apical leakage. Punjabi *et al*. found this

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adaptation of silicone sealers to provide higher fracture resistance of treated teeth, potentially strengthening it to a level comparable to that of intact teeth.[6]

Epoxy-based sealers are widely used in endodontics because of their excellent physicochemical and biological properties. Because of its low solubility, adequate dimensional stability, and micro-retention to dentin, AH Plus is still considered the gold standard.[7]

The tricalcium silicate sealers are distinguished by their bioactivity, that is, their ability to form hydroxyapatite on their surface and an osteogenic effect, and hence are very accurately mentioned as bioactive materials.[8] BioRoot RCS (Septodont, St. Maur-des-Fossés, France) is one such hydraulic tricalcium silicate-based sealer containing tricalcium silicate, zirconium oxide, and povidone. Due to the prolonged release of Ca^{+} ions after setting and alkalinity of the sealer, it possesses high antimicrobial and low cytotoxic properties, promoting endodontic and periodontal regeneration. However, most importantly, it has gained popularity because of its ability to create a seal in the presence of hydrophilic atmosphere by mineralization and apatite deposition at canal wall interface.[9]

In the literature, there have been discussions regarding the different levels of residual humidity and how they could interfere with the sealing ability of endodontic sealers. This is particularly true about the resin-based sealers that are known for their hydrophobic properties, whereby moist conditions could lead to their bond failures and hence leakage. Indeed, the bond strength between the sealer and the dentinal walls could be affected by the presence of humidity.[7]

Therefore, this study aimed to evaluate the influence of humidity on the push-out bond strength associated with AH Plus, BioRoot RCS, and Nanoseal-S sealers.

SUBJECTS AND METHODS

Sample selection and preparation

The local ethics committee of MIDSR Dental College and Hospital, Latur, Maharashtra, India, approved this study (MIDSR/IEC/836/86/2019).

Sample size was calculated using following formula:[10]

$$n = 2\frac{S^2 (Z1 + Z2)^2}{(M1 - M2)^2}$$

By using above formula and putting the values of variables given in Table 1, the minimum sample size of 10 in each group was obtained.

For this purpose, 20 mandibular first and second premolar teeth extracted for orthodontic purpose were included in this study.

All the teeth were cleaned for removal of calculus and debris if any. Crowns of all teeth were sectioned using the slow-speed circular diamond disc to standardize the root length to 16 mm, confirmed using a digital Vernier caliper. All roots were then subjected for intraoral periapical (IOPA) radiograph examination from the buccolingual direction. Based on physical examination and the IOPA, the roots with aberrant anatomy, calcified canals, fused roots, cracks and fractures, root resorption, and root caries were excluded from the study.

The roots were immediately soaked in sodium hypochlorite 5.25% for 10 min. This was done to clean the soft and hard tissue debris. Later, the water-cooled diamond disc was used to cutoff the coronal and apex sections of each root to obtain an intact middle-third root portion. Then, three slices (1 ± 0.1 mm thickness) were obtained from each of 20 such middle-third root portions using a low-speed diamond saw with a diamond disc (125 mm × 0.35 mm × 12.7 mm) in constant water irrigation. For confirming the final thickness of each slice, a digital Vernier caliper with an accuracy of 0.001 mm was used. A total of 60 dental slices were thus obtained from the initial 20 mandibular premolar teeth.

Each of 60 root slices was photographed. The photographs were analyzed using Adobe Photoshop (ADOBE System Inc., San Jose, California) to measure the diameter of each section with respect to the diameter of the canal at the center. This was done to standardize the width of dentin around the canal in each slice.

The 60 root specimens were randomly divided into six groups (N = 10) according to the endodontic sealers: AH Plus (Dentsply-Detrey, Konstanz, Germany), BioRoot RCS (Septodont, Saint-Maur-des Fosses, France), and Nanoseal-S (Prevest, DenPro), as well as according to the moisture conditions to be analyzed: G1 – AH/moist, G2 – AH/dry, G3 – Nanoseal-S/moist, G4 – Nanoseal/dry, G5 – BioRoot RCS/moist, and G6 – BioRoot RCS/dry.

Preparation of canal-like holes for push-out assay

Using a 0.8 mm cylindrical carbide bur, three canal-like holes, each to be filled with exactly one of the three materials, were drilled in coronal facing surface of each root slice to establish a fair comparison between the materials [Figure 1]. During this process, a minimum of 1 mm distance was maintained

Figure 1: Three canal-like holes, each to be filled with endodontic sealers, were drilled on each root slice

between the holes, external cementum, and the root canal wall[11] measured using a graduated probe.

After preparation, all the specimens were immersed in a beaker containing 100 ml of 2.5% sodium hypochlorite solution for 15 min and then rinsed in distilled water. The smear layer was removed by soaking the dental slices in 100 ml of 17% ethylenediaminetetraacetic acid for 3 min and subsequently in 100 ml of distilled water for a minute. Afterward, the root slices were transferred to 2.5% NaOCl for 1 min. Finally, rinse with distilled water for 1 min was performed and dried with paper points.

The three dental sealers: Nanoseal-S, BioRoot RCS, and AH Plus Sealers, were randomly placed in the three holes of each of the root slice such that one sealer was precisely placed per hole. According to the manufacturer's guidelines, the sealers were placed in the holes by vibrating gently to prevent bubble formation when placing the materials. The sections under moist group were stored in an aqueous solution of 0.9% sodium chloride and 10 ml of deionized water.

Following this, the sections were then placed in an incubator at 37°C, 100% humidity for 72 h to allow complete setting of the sealers.[12]

Push-out assessment

A plunger tip of 0.6 mm was positioned above a test sealer while avoiding the surrounding dental structures. Using a universal testing machine, the pressure was applied in a coronal-to-apical direction at the rate of 0.5 mm min^{-1} till the sealer got dislodged.

Real-time software plotted the load × time curve while testing. The bond strength was measured and recorded in MPa.

At failure, the load (expressed in Newton's) was divided by the area of the bonded interface. Calculation of the adhesion area of the root canal sealer was done using the following formula: area = $2\pi r \times h$, where π =3.14, r = radius of the hole with the root canal sealer (0.4 mm), and h = material's height (1.0 mm).[11,13]

Statistical analysis

Data were collected by using a structure pro forma. Data thus were entered in MS Excel sheet and analyzed using SPSS 23.0 version, (IBM SPSS Statistics Software, USA).

Quantitative data were expressed in terms of mean and standard deviation (SD).

Comparison of mean and SD between two groups was done using an unpaired *t*-test to assess whether the mean difference between groups is significant or not.

Descriptive statistics of each variable were presented in terms of mean, SD, and standard error of the mean.

Comparison of mean and SD between all groups was done using a one-way ANOVA test.

$P < 0.05$ was considered statistically significant whereas $P < 0.001$ was considered highly significant.

Post hoc Tukey's HSD test was used to see whether the mean difference between the individual group is significant or not.

RESULTS

A summary of the push-out bond strengths of Nanoseal-S, AH Plus, and BioRoot RCS sealers for dry and moist conditions is given in Tables 2 and 3.

The results of the *post hoc* Tukey's HSD test ($P < 0.05$) indicated that the mean and the median push-out bond strength were highest for BioRoot RCS in both dry and moist conditions.

DISCUSSION

A root canal sealer must adapt well to the canal wall to create a strong sealer–dentine interface that can withstand mechanical stress.[14] The push-out bond strength test conducted in this study is relatively easy to perform, can replicate similar clinical conditions, has accurate specimen standardization, has minimal stress, and is less technique sensitive.[15-17] Above all, it measures the material–dentine interfacial bond strength in all surfaces of the root canal.[18]

In contrast, other established methods for assessing push-out bond strength involve the canal preparation, obturation, and analysis of the different root sections of extracted teeth.[19,20] These techniques have the disadvantage of causing inconsistent baseline measurements due to a lack of proper standardization of the root canal anatomy. Hence, this study used single dental slices each with three standardized holes to test the push-out bond strength of three different sealers.[11,13] This was done to eliminate the complicating elements such as the age of tooth, canal shape, sclerosis, and differences in micro-hardness and other variables observed in the clinical cases concerning root canal treatment. In addition, a distance of 1 mm was maintained between any two holes, the external cementum, and the root canal surface to avoid fracture of the dental slice.[11]

Table 1: Variables used for sample size calculation

Variables	Description	Std. Deviation & Mean Values
M1	Mean test intervention	1.17
M2	Mean control intervention	12.20
S1	Standard deviation of M1	0.47
S2	Standard deviation of M2	4.9
S	Pooled SD	3.48073
1-α	Set level of confidence. Usual values 0.95; 0.99	0.95
1-β	Set level of power of test. Usual values 0.8, 0.9	0.8
Z1	*Z* value associated with alpha**	1.64485
Z2	*Z* value associated with beta	0.84162
n1	Minimum sample size	2
d	Absolute precision	2.44
n2	Minimum sample size	10

**,Significance-0.10

Table 2: Intergroup comparison of mean push-out bond strength under dry conditions (MPa)

Dry push-out bond strength (Mpa)	*n*	Mean±SD	*P*
Nanoseal-S	11	1.75±0.35	0.0001 (<0.001)
AH Plus	11	3.07±0.34	
BioRoot RCS	11	3.63±0.49	
Total	33	2.81±0.89	

SD: Standard deviation

Table 3: Intergroup comparison of mean push-out bond strength under moist conditions (MPa)

Moist push-out bond strength (Mpa)	*n*	Mean±SD	*P*
Nanoseal-S	11	0.98±0.30	0.0001 (<0.001)
AH Plus	11	1.86±0.35	
BioRoot RCS	11	2.26±0.30	
Total	33	1.70±0.63	

SD: Standard deviation

The artificial canals, so prepared, were only filled with root canal sealers, which may lead to stress concentrating on the sealers, but not on other materials, such as gutta-percha, as in other techniques. Second, although the use of a sealer with gutta-percha would replicate more of a clinical situation, this procedure of using only a sealer would show the proper bond strength between root canal sealers and dentine.[11,13,21]

In this study, the three sealers under discussion exhibited different bond strengths. The order of bond strength is given as follows: BioRoot RCS > AH Plus > Nanoseal-S.

The exact mechanism of bioceramic-based sealer bonding to root dentin is unknown; however, the following mechanisms have been suggested for calcium silicate-based sealers:

1. Diffusion of the sealer particles into the dentinal tubules (tubular diffusion) to produce mechanical interlocking bonds[22]
2. Infiltration of the sealer's mineral content into the intertubular dentin results in the establishment of a mineral infiltration zone produced after denaturing the collagen fibers with a strong alkaline sealer[23,24]
3. Partial reaction of phosphate with calcium silicate hydrogel and calcium hydroxide, produced through the reaction of calcium silicates in the presence of the dentin's moisture, results in the formation of hydroxyapatite along the mineral infiltration zone.[25]

One factor that may account for the higher bond strength of BioRoot RCS is its zirconium oxide which induces a higher release of calcium ions maintaining a long-term bioactivity.[26] This continuous setting of calcium silicate-based sealers in the process of hydration and ion exchange with the medium could be related to the improvement of the bond strength and the stability of the sealing provided by the root canal filling, in the long term.[27] This may be the reason why BioRoot RCS showed the highest push-out bond strength, in this study, compared to even AH Plus and even in the presence of moisture in the canals. This result is in agreement with various studies done on this topic.[28-31] Further investigations are needed to clarify other physical properties of BioRoot RCS that may account for its increased bond strength.

The strong bond strength of AH Plus with root canal dentin, as seen in this study, is related to its capacity to interact chemically with the collagen network and form covalent bonds between the epoxy rings and the amine groups of the exposed collagen.[20] Similarly, AH Plus has been associated with long-term stability and efficient cohesion between molecules, increasing its micromechanical retention to the root dentin.

Accordingly, it has been suggested that it may be advantageous to leave root canals slightly moist before filling procedures to enhance the sealing properties of endodontic sealers.[14] This is in agreement with various other studies on AH Plus.[13,32-34]

In this study, Nanoseal-S showed the least push-out bond strength. This can be attributed to poor wetting of root dentin due to the presence of silicone, which possibly produces high surface tension forces, making the spreading of these materials difficult.[34] One such study on GuttaFlow-2, having similar contents such as Nanoseal-S, with polydimethylsiloxane and micro-silver, showed similar results with GuttaFlow-2 showing least push-out bond strength than AH plus and GuttaFlow Bioseal.[4]

CONCLUSION

Within the limitation of the present study, it may be concluded that humidity has no influence of the bond strength of AH Plus, BioRoot RCS, and Nanoseal-S sealers. BioRoot RCS showed significantly higher push out bond strength compared to AH Plus and Nanoseal-S sealers in both dry and moist conditions.

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Conflicts of interest
There are no conflicts of interest.

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