ORIGINAL ARTICLE

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The effect of storage time on dislocation resistance of core material to root canal sealer in standardized root canals: A laboratory study

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Purpose: To evaluate the effect of storage time on the dislocation resistance of core material to root canal sealer in standardized artificial root canals.

Methods: A single root canal with a round shape was selected using cone-beam computed tomography imaging, and root canal treatment was performed. The root was scanned with micro-computed tomography, and the data were exported as a stereolithography file. Forty artificial roots were manufactured using 3D printing technology. The artificial root canals were obturated using a single cone technique. The roots were divided into two groups according to the storage time of filled roots: 7 days and 30 days (n = 20), and stored at 37°C and 100% humidity. After each storage period, 2-mm sections were taken from the middle part of the roots. The sections were tested on a universal testing machine. The dislocation resistances (MPa) were calculated, and the data were analyzed using the Shapiro–Wilk test and independent samples t-test ($\alpha = .05$).

Results: The dislocation resistance of the filling material was significantly higher in the 30-day storage time group compared to the 7-day group ($p < .05$).

Conclusion: The storage time of the root fillings affected the dislocation resistance in the standardized experimental setup with artificial roots.

Keywords: Artificial root canal; dislocation resistance; micro-computed tomography; push-out test; storage time.

Introduction

Three-dimensional hermetic obturation that completely seals the entire root canal system against any bacterial entry is important for successful endodontic treatment (1). In endodontics, adhesion represents the ability of a sealer to bond to dentine and the core material (2). Filling the root canal with a thermoplastic core material and sealer is still the most widely used approach (3). An ideal sealer should adhere to both the dentin and core material, remain intact over time (4), and be resistant to dislodgement during operative procedures such as post-space preparation (5,6). AH Plus (Dentsply Sirona, Ballaigues, Switzerland) is an epoxy resin-based root canal sealer that is frequently

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used in endodontic research as a control material (3). It offers advantages such as long-term dimensional stability and better adhesion to root canal walls compared to other materials generally used (7). Although AH Plus showed superior adhesive properties to gutta-percha compared with calcium silicate sealers (8), gutta-percha itself lacks adhesion to either dentine or sealer (9). Therefore, the sealing ability of AH Plus remains partly controversial due to its lack of bonding to gutta-percha (10,11).

The push-out test is widely used in endodontic research to assess the bond strength between the root canal filling materials and/or dentin interface (12). Although it has been stated that push-out tests are reliable in terms of ranking the quality of the tested materials or techniques (13), a large number of methodological variables (plunger size, slice thickness, storage time, tooth portion, tooth type, load velocity, sealer, and core material) prevents the comparison of the results. The storage time of samples is one of the main factors that can influence the push-out bond strength (14). Studies have shown that pretreatment immersion time has a significant impact on the outcome of bond strength tests and the permeability of root dentin (15,16). In addition, the literature reveals that interface aging (storage time) substantially affects dislocation resistance values over time (14), but the results are not consistent (17-19).

Because natural teeth have potential confounding factors such as tooth age, the amount and distribution of sclerotic dentin, microhardness, and the modulus of elasticity of dentin, their usage in these types of studies may cause some differences in findings (13). It has been stated that the presence of calcospherites along the canal wall can increase the retention of the sealer in areas where root canal preparation cannot reach, and this may have a random effect on push tests (5). On the other hand, it has been reported that matrix metalloproteinase (MMP) enzyme activation in demineralized dentin is one of the reasons for bond failure between resin-based materials and dentin over time (20). To eliminate such confounding factors of dentin and overcome individual anatomical differences and conditions, it has been suggested to create balanced experimental groups using artificial canal spaces in push-out tests (13).

To the best of the authors' knowledge, there is no study evaluating both the storage time and the adhesion of the core material to the sealer in artificial root canals. Therefore, the aim of the present study was to evaluate the effect of storage time on the dislocation resistance of the core material to root canal sealer in standardized artificial root canals. The null hypothesis was that the different storage times of root fillings have no effect on the dislocation resistance of the filling material.

Materials and Methods

The manuscript of this laboratory study has been written according to the Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines (21). Figure 1 presents a visual representation of the study design and its outcomes.

Sample size calculation

According to a previous study (19), the minimum sample size required to detect a significant difference should be at least 17 in each group (34 in total), considering a type I error (alpha) of 0.05, power (1-beta) of 0.85, effect size of 1.09, and a two-sided alternative hypothesis (H1) (WSSPAS; Web-Based Sample Size & Power Analysis Software (22)). In the study, 20 samples were used in each group (40 in total).

Selection and preparation of samples

This study was approved by the Non-Interventional Research Ethics Committee of Fırat University (no. 2021/07-24). Cone-beam computed tomography (Planmeca; ProMax 3D Mid, Helsinki, Finland) was used to select single-rooted teeth with a round root canal anatomy. The selected root had no caries, microcracks, internal/external resorption, root canal treatment, or immature root apex. The coronal segment of the tooth was removed, and a size 15 K-file (Dentsply Sirona, Ballaigues, Switzerland) was introduced into the root canal to confirm the working length (18 mm). The root was instrumented up to the ProTaper Universal F5 file (Dentsply Maillefer; Ballagiues, Switzerland). The root canals were irrigated with 2 mL of 2.5% sodium hypochlorite at each file. For the final irrigation, 2 mL of 17% ethylenediaminetetraacetic acid (Microvem, İstanbul, Turkiye), 5 mL of 2.5% sodium hypochlorite, and 2 mL of distilled water were used, and the root canal was dried using absorbent paper points (#50) (Diadent; Chongju, Korea).

Obtaining 3D-printed artificial roots

The root was scanned by micro-computed tomography (SkyScan 1272; Bruker micro-CT, Kontich, Belgium) at a 10-µm slice thickness with the following parameters: 80 kV and 125 mA, rotation angles of 0.4 degrees with an aluminum filter. A 3D high-resolution image of the root was created using CTAn software (Skyscan; Aartselaar, Belgium) and converted to a stereolithography file after the images were reconstructed (NRecon v.1.6.9; Bruker micro-CT) (Figure 2a-d). The STL file of the prototype tooth was

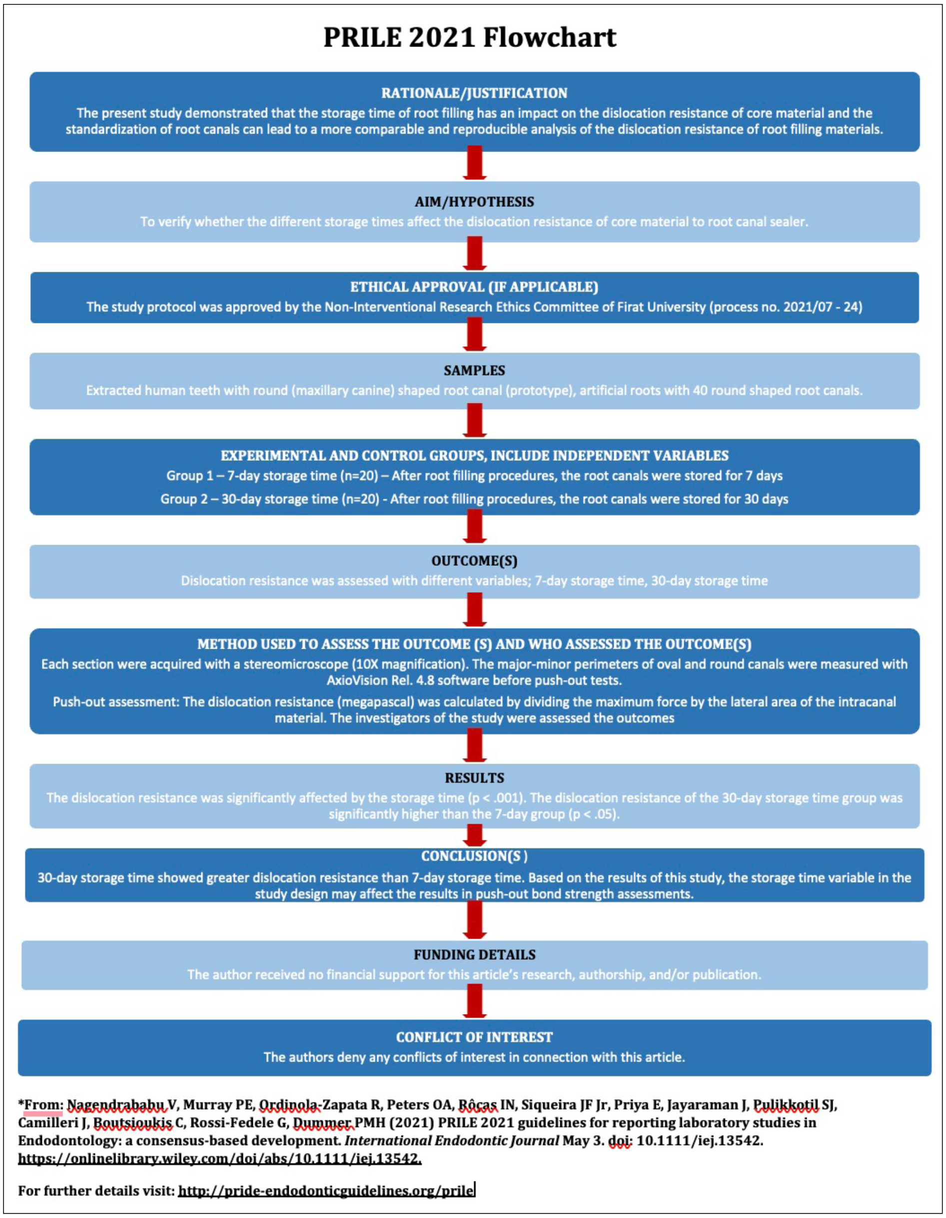

Fig. 1. PRILE 2021 flowchart: A visual representation of the study design and its outcomes.

transferred to a DentaFab Sega DLP 3D printer (3bFab; Istanbul, Turkiye). Forty root replicas were manufactured at 50-µm resolution (PowerResins Model resin; 3bFab).

Obturation and slicing of artificial roots

Root canals were filled using the single-cone technique. A sonic activation system (EndoActivator; Dentsply Tulsa Dental Specialties, Tulsa, OK) was used to homogeneously distribute the sealer into the root canal. The medium tip of the EndoActivator was smeared with AH Plus sealer (Dentsply De Trey, GmbH, Konstanz, Germany) and inserted 2 mm below the root canal length. The Endo-Activator was operated with an in-and-out motion at an amplitude of 3 mm for 5 s. The F5 ProTaper Universal (Dentsply Maillefer) gutta-percha cone was also coated with sealer and inserted into the root canal at the working length. The gutta-percha was removed to the orifice level and vertically condensed using a plugger. The orifice was sealed with temporary filling material (Coltosol; Coltene, Altstätten, Switzerland).

The roots were then divided into two groups according to storage time (group 1: 7 days, group 2: 30 days) (n = 20) and stored at 37°C with 100% humidity. After each storage period, the roots were fixed on acrylic resin rods. Two-mm horizontal slices were obtained from the middle part of the roots using a low-speed diamond saw (Accutom-3;

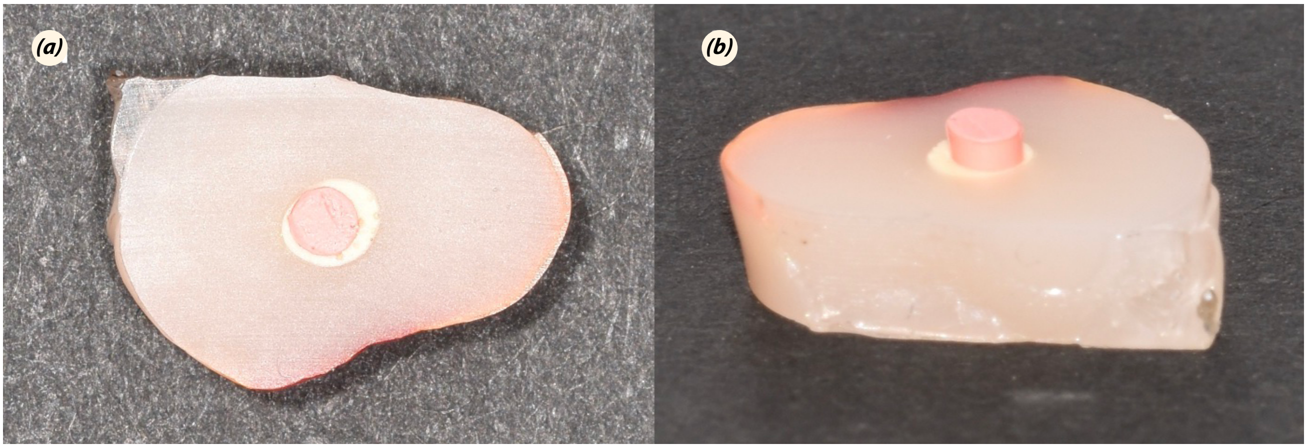

Fig. 2. Representative images of **(a)** section sample obtained from the artificial root canal and **(b)** gutta-percha dislodged cleanly from the sealer after the push-out test.

Struers, Copenhagen, Denmark) at 350 rpm under water cooling, considering the disc thickness (0.3 mm) (Fig. 2a). The coronal surface of each slice was marked to determine the push-out test direction. Each slice thickness was measured using a digital caliper (± 0.1 mm) (Max-Extra Professional Tools, Guangzhou, China). Images of the slices were obtained using a stereomicroscope (10X) (Olympus BX43; Olympus Co., Tokyo, Japan) to measure the root canal areas of both surfaces (major and minor perimeters). The root canal areas were measured using AxioVision Rel. 4.8 software (Zeiss, Göttingen, Germany) before the push-out tests.

Push-out test and assessment

Each slice was mounted on a support, and the load was applied by a cylindrical stainless steel plunger tip (0.75-mm diameter) (Fig. 3b) over the gutta-percha in an apical-coronal direction. The plunger tip was positioned to avoid any contact with the root canal walls. The push-out test was performed at a crosshead speed of 0.5 mm min-1 on an Autograph AG-X universal testing machine (Shimadzu, Tokyo, Japan) (Fig. 3a) until the gutta-percha dislocated (Fig. 2b). The maximum force (F) at the point of dislocation of the material from the slice was recorded in Newtons (N). The lateral area (A, in mm^2) was calculated using the following formula (23):

$$A = \frac{h(B+b)}{2}$$

In this formula, 'h' is the height measurement of the filling material, 'B' is the major perimeter (coronal side), and 'b' is the minor perimeter (apical side). The dislocation resistance was calculated in megapascal (MPa) by dividing the maximum force (F) by its lateral area (DR = F / A).

Data Presentation and Statistical Analysis

The dislocation resistance data were normally distributed (Shapiro-Wilk, $p > 0.05$) and had homogeneity of variance (Levene's test, $p > 0.05$). Therefore, parametric tests were used. An independent sample t-test was used to evaluate the effect of storage time on the dislocation resistance values. Statistical tests were performed using SPSS for Windows, Version 26.0 (IBM Corp., Armonk, NY), and the cut-off level for significance was set at $\alpha = 5\%$.

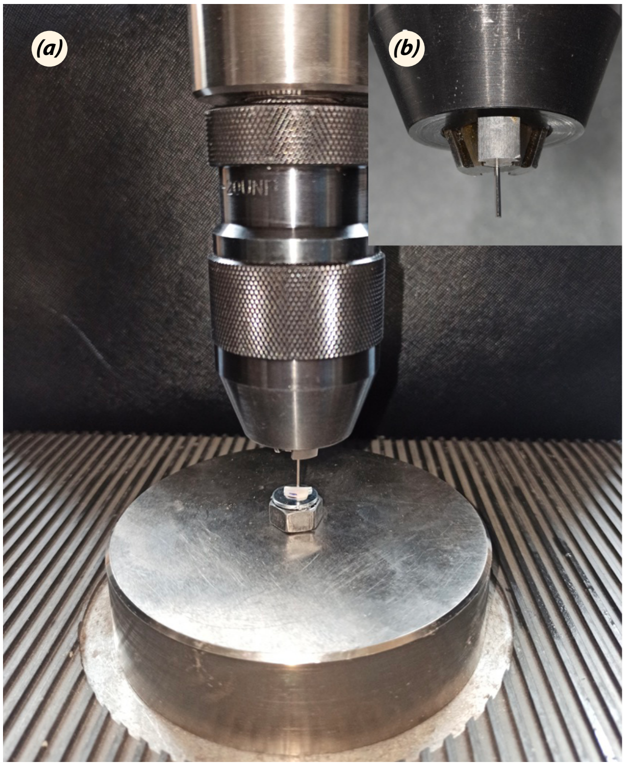

Fig. 3. Representative images of **(a)** the push-out test design of universal testing machine and **(b)** cylindrical stainless steel plunger tip (0.75-mm diameter).

Table 1. Mean and standard deviation (SD) of the dislocation resistance (MPa) of the tested root canals at different storage times (n=20).

	7-days	30-days	
Storage Time	Mean (SD)		p-value
	0.19 (0.06)[a]	0.35 (0.11)[b]	<0.001

Different letters indicate significant differences between storage times ($p < 0.05$).

Results

The mean, standard deviation, and p values of the dislocation resistance of the tested root canals at different storage times are shown in Table 1. Dislocation resistance was significantly affected by storage time ($p < .001$). The dislocation resistance of the 30-day storage time group was significantly higher than that of the 7-day group ($p < .05$).

Discussion

The effect of storage time on the dislocation resistance of the core material to root canal sealer was investigated in the current study. The results revealed that dislocation resistance increased significantly as storage time increased. Therefore, the three null hypotheses were rejected.

The push-out test can be used to measure the interfacial shear strength between different surfaces, such as dentin-sealer and sealer-core material (2). In post-space preparation procedures, sealer-core material adhesion is as important as dentin-sealer adhesion to prevent the gutta-percha from dislodging the entire canal. In this context, if the bond strength of the filling material is inadequate during post-space preparation, gaps may be created between the sealer-core material or dentine-sealer, jeopardizing the preservation of sealing integrity (24,25).

Although many studies have investigated bond strength, it is unclear whether the failure modes in push tests are adhesive (sealer-dentin) or cohesive (sealer-core material) (26) and are at the same level of importance (8). While sealer adhesion occurs in the form of mechanical bonding to the dentinal tubules or chemical bonding, the core gutta-percha lacks the ability to adhere to dentin or sealer (9). To overcome this situation and to prevent bias in push-out bond strength studies, it is recommended to use only sealer to fill the entire root canal space (26). However, others have reported that the use of sealer alone does not represent actual clinical conditions and that not using gutta-percha may affect the results of push tests (27). Even if all these confounding factors are excluded, situations arising from the nature of dentin, such as physical properties resulting from dentin structure, tooth age, tubule structure (13), MMP activity and collagen degradation (19), and different brands of gutta-percha (8), may affect push-out bond strength. Therefore, we used standardized artificial root canals to both simulate the root canal and eliminate confounding factors of dentin structure.

In previous studies, while evaluating the bond strength of the filling materials to the root canal, the relationship between the storage time of the samples and bond strength was also evaluated. However, they reported contradictory findings because of the heterogeneity of the methodologies. In some of these studies, it has been reported that the adhesion of AH Plus to dentin decreases with decreasing storage time (17,28), or there is no significant difference (18,19). In addition, although the sealer used in these studies was AH Plus, the filling methods were different from each other (single cone, lateral compaction, only sealer, or thermomechanical compaction). Although cold lateral compaction is a frequently studied method, its ability to adapt to the root canal system is questionable because of the formation of gaps and spreading tracts (29). Therefore, the single cone method was preferred to ensure homogeneity in the root canal filling in this study.

In previous studies using single cone and lateral compaction techniques with AH Plus and gutta-percha, bond strength increased with storage time, but there was no significant difference (17,18). It has been stated that the AH Plus sealer's satisfactory resistance values even after being stored for a long time are due to the stability of the sealer. It is also known that the AH Plus sealer does not shrink and even expands over time in a humid environment (30). In parallel with these findings, in our study, dislocation resistance increased in a 30-day storage time compared with 7 days. Contrary to the findings of our study, the bonding resistance decreased over time when the thermomechanical compaction method was used with the AH Plus sealer (17). In the thermomechanical method, since gutta-percha is compressed hot, it spreads more, and thus the amount of sealer decreases (31,32). In this method, the fact that less AH Plus sealer volume remains on the canal walls can be considered as less expansion of the sealer. Therefore, the low interfacial bond strength between the gutta-percha and sealer, and less sealer on the dentine surface in this technique, may be the reason for the decrease in bond strength.

In a study in which root canal filling was performed with gutta-percha and AH Plus, the gutta-percha was separated from the sealer, and the sealer remained on the dentin surface (33). Since gutta-percha does not have any bond with the sealer and dentin, low interfacial strength is expected between gutta-percha and sealer because the resistance to dislocation results directly from Coulomb's friction (34,35). In studies conducted on real teeth, it has also been reported that the gutta-percha generally dislocates from the sealer, but there are also rare adhesive failures (18,19,36). In our study, the failure mode was not evaluated; however, in the visual examination, after the dislocation occurred, it was observed that the gutta-percha was separated cleanly from the sealer (Fig. 2b). The fact that dislocation resistance increased less over time in previous studies compared with our study may be due to the dentin-sealer adhesive failure in real teeth, despite the advantages of AH Plus sealers being stable and expanding with moisture.

It has been stated that the elastic modulus of the core materials can affect the dislocation resistance (37). This may bring to mind the question of whether the elastic modulus of gutta-percha changes over time and may affect the dislocation resistance values. In a study, after 3 months of storage, gutta-percha at room temperature had no effect on its modulus of elasticity (38). Therefore, in our study, the significant increase in dislocation resistance after 30 days is thought to be due to the properties of the sealer (stability and expansion in a humid environment) over time rather than the core material's elasticity.

The use of artificial root canals is one of the limitations of this study; however, it provides a standard experimental design for assessing the dislocation resistance of the core material from the sealer by eliminating other confounding factors. Further research is needed to determine whether the increased dislocation resistance of the core material to the sealer over time may negatively affect dentin-sealer adhesion during gutta-percha removal in post-space preparation processes.

Conclusion

In summary, the 30-day storage time showed greater dislocation resistance than the 7-day storage time. Within the limits of this study, it was concluded that the storage time variable in the study design may affect the results of push-out bond strength assessments.

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Conflict of Interest: None declared.

Ethical Approval: The study protocol was approved by the Fırat University Ethics Commitee (date: 27.05.2021, protocol no: 2021/07-24).

Informed consent: Written informed consent was obtained from patients who participated in this study.

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