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EFFECT OF TWO CALCIUM-SILICATE AND ONE RESIN SEALERS ON THE FRACTURE RESISTANCE OF ROOT DENTIN USING DIFFERENT TREATMENTS IN DOG'S TEETH (AN IN VIVO STUDY)

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Calcium silicate sealers, Dentin, Fracture resistance, Root canal obturation.

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ABSTRACT

Introduction: Calcium silicate sealers have been widely used to do their bioactivity, but their effect on the fracture resistance of endodontically treated teeth is still unclear. **Aim:** This search compared the effect of calcium silicate sealers and resin sealer on the fracture resistance of dog's root, using different irrigants. **Materials and methods:** Sixty-five incisors of six dogs were randomly allocated into three experimental (n=15), positive (n=15), and (n=5) negative control groups according to the type of sealer that was used for obturation; group A: BioRoot RCS, group B: TotalFill BC, group C: AH plus, group D: positive control prepared-unfilled and group E: negative control intact teeth. Each group was then classified into 3 subgroups (n=5) according to the final irrigation used; subgroup 1: 2.5% NaOCl, subgroup 2: 17% EDTA, subgroup 3: Saline. After three months of preparation, irrigation, and filling, dogs were euthanized, and teeth were extracted for assessment of root fracture resistance using Instronrsal testing machine. Two-way analysis of variance (ANOVA) was used to study the effect of sealer type, irrigant, and their interaction on mean fracture resistance. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. The significance level was set at $P \leq 0.05$. **Results:** There was no statistically significant difference between roots obturated with the three tested sealers after different irrigants and the negative control (intact roots) group. All showed higher statistically significant mean fracture resistance than positive control (prepared / unfilled) group. **Conclusion:** Root canal preparation decreases the fracture resistance of root canal treated teeth. However, obturation with all tested sealers increased the force values needed to fracture the filled samples when compared to unfilled ones.

INTRODUCTION

Endodontically treated teeth seem to be more prone to vertical root fracture than vital teeth [1,2] that results in tooth extraction [3]. This is mainly due to the removal of tooth structure during mechanical preparation, dentin dehydration after the endodontic treatment [4], excessive pressure during canal filling procedures [4,5]. Additionally, chemical substances used for chemo-mechanical preparation and intracanal medications may alter the physical and mechanical properties of dentin, increasing the possibility of root fracture [6].

Following the concept; in endodontically treated teeth, the root canal system is reinforced by canal filling materials with the aid of root canal sealers. The rationale of using endodontic sealers is to provide good adhesion, create monoblock and attain impervious seal between the core material and radicular dentin. This had led to the development of adhesive root canal sealers (7, 8).

Calcium silicate sealers based on dentin adhesion technology were recently introduced. The potential advantages of these sealers are related to the biocompatible and bioactive di- and tricalcium silicate constituents. Also, the chemical interaction at the interfacial dentin along with the micromechanical tag-like structures result in effective adhesion of sealer to root dentin. This adhesion strengthens the root canal treated teeth, and thus reduces fracture risk (9).

The release of calcium hydroxide from di- and tricalcium silicate cements due to hydration and the contact with phosphate from dentinal fluids leads to a precipitation of calcium phosphate or calcium carbonate on the material's surface (10,11) and formation of hydroxyapatite (12). Furthermore, calcium silicates form an interfacial layer at the dentin wall denoted as "mineral infiltration zone" (9). Despite of calcium silicate material's bioactivity, other research showed that, the high alkaline effect of released calcium hydroxide may have an adverse effect on dentin collagen matrix. They degenerate the collagenous component of the interfacial dentin and raising their permeability by the breakdown of collagen fibrils intermolecular bonds and hence they may exert negative effect on root mechanical properties (13).

Lately, different calcium silicate-based sealers were presented. TotalFill® BC Sealer™ (FKG Swiss endo), in the form of a premixed single syringe which contains calcium silicates, calcium phosphate monobasic, zirconium oxide, tantalum oxide and thickening agents. It forms hydroxyapatite upon setting and chemically bonds to both dentin and bioceramicmic points (TotalFill® BC Points™) ensuring 3D filling and increasing the root fracture resistance (14,15). BioRoot RCS (Septodont, Saint Maur-des-Fosses, France) is composed of powder of tricalcium silicate and zirconium oxide and liquid composed of water, calcium chloride and polycarboxylate. t is free of monomer, aluminate and calcium sulfate. It does not shrink during setting allowing a tight seal to the root canal (16- 19). AH plus sealer is considered the gold standard root canal sealer due to its low solubility, disintegration, and excellent sealing ability, forming good adhesion to dentin (20).

However, the mutual effect of different irrigants with calcium silicate sealers on the root fracture resistance was not clearly studied, especially in vivo. Thus, this research was carried out in vivo to analyze the biomechanical effect of BioRoot (RCS) and TotalFill BC sealers in comparison to AH Plus resin sealer on fracture resistance of dog's root after treatment with different irrigating solutions. The null hypothesis of this study was that, there is no difference in fracture resistance between roots filled with either BioRoot RCS, TotalFill BC or AH Plus sealers.

MATERIALS AND METHODS

Ethical approval was granted for the use of dog's incisors teeth by the Research Ethics Committee of the Faculty of Dentistry, Suez Canal University (61/ 2017).

Sample size calculation:

To evaluate and compare the effect of two calcium silicate sealers (TotalFill® BC Sealer and BioRoot sealer) and resin sealer (AH plus) on dog's teeth using different irrigants (NaOCl and EDTA); a repeated measures analysis of variance (repeated measures ANOVA) is proposed. A minimum calculated total sample size of 65 samples were sufficient to detect the effect size of 0.25, a power (1-β) of 85 % with a partial eta-squared of 0.06 and at a significant level of $p<0.05$. A total sample size of 65 was applied, each irrigant type (A, B, and C) was represented by 20 samples. Each sealer type subgroup (1, 2, 3 and 4) was represented by 5 samples. The intact teeth negative control subgroup 5 was represented by five samples for fracture resistance. The sample size was calculated according to G*Power software version 3.1.9.5 [21-24].

Selection of the Samples:

Sixty-five intact healthy upper and lower incisors of six mongrel dogs aged between three and seven years and with a mean weight of 15 kg were used in this study. Dogs have six incisors at each arch with only one canal each. The animals were quarantined in separate cages at the Faculty of Veterinary Medicine, Suez Canal University.

Randomization:

The study was double - blinded by the operator and the assessor. Teeth were randomly assigned to be filled with either BioRoot RCS, Total Fill BC, AH plus sealer or left unfilled. Masking tape concealed the contents of the obturating materials from the operator was kept with the allocator. After mechanical preparation and irrigation, the allocator mixed the sealer and then gave it to the operator as a ready mixed paste at the time of obturation. The operator did not know the type of sealer used. A random sequence was generated by computer software, (http://www.random.org/).

Grouping and randomization of teeth:

Teeth of each dog were randomly and blindly classified into three experimental groups (n =20 each) according to the irrigation protocol that was used during and after preparation using 30-gauge needles as follow:

- **Group A**: Samples were irrigated with 6 mL 2.5% sodium hypochlorite (NaOCl) (Clorox, Egyptian company for house hold products, Egypt) during preparation and 3 mL 17% ethylenediaminetetraacetic acid (EDTA) (Maillefer, Dentsply, Ballaigues, Switzerland) as final irrigant.
- **Group B:** Samples were irrigated with 6 mL 2.5% NaOCl during preparation and 3 mL 2.5% NaOCl as final irrigant.
- **Group C (control):** Samples were irrigated with 6 mL 0.9% saline solution during preparation and 3 mL saline as final irrigant. Samples in each group were then subdivided into four subgroups (n = 5) according to the type of sealer that was used for obturation as follow:

* **Subgroup 1:** teeth were filled with BioRoot RCS. (Septodont, Saint Maur-des-Fosses, France).
* **Subgroup 2:** teeth were filled with Total Fill BC sealer FKG Dentaire, La-Chaux-de-Fonds, Switzerland).
* **Subgroup 3:** teeth were filled with AH Plus sealer (Dentsply De Trey, Konstanz, Germany).
* **Subgroup 4 (+ve control):** teeth were left without filling.
* **Subgroup 5 (-ve control):** intact teeth.

Preoperative considerations and anesthesia of the dogs:

Food was withheld 6-8 hours prior to operation. Fifteen minutes before induction of general anesthesia, each dog was premedicated with I/M injection of chloropromazine hydrochloride (Hikma Maple; Usl Pharma; Sandoz) in a dose of 1 mg/kg. General anesthesia was accomplished by I/V injection of thiopental sodium (EPICO, Egypt) 2.5% solution until the main reflexes were abolished [(25)].

Mechanical preparation of Dog's teeth:

Isolation with sterile cotton rolls was preformed, then access preparation was carried out using round bur ≠2 (KG Sorensen Ind. e Com. Ltda., Barueri, Brazil.). Root canal preparation was performed with I Race rotary files (FKG Dentaire, La-Chaux-de-Fonds, Switzerland) and finishing up using ≠ 35 file 4% [(26)]. Each root canal was irrigated with its corresponding irrigation protocol using 30-gauge needles, then flushed with sterile distilled water to remove any residual of the irrigant. Absorbent paper points # 35 (Dentsply Maillefer; Ballaigues, Switzerland) were used for gentle dryness of each root canal.

Thereafter, each root canal was obturated using size 35 gutta-percha points 4% (Dentsply Maillefer; Ballaigues, Switzerland) and the corresponding sealer according to its group using single cone technique. A periapical radiograph was taken to check the adequacy of the root canal filling. Each tooth received a glass ionomer coronal seal using Fuji® IX GP FAST (Fuji® IX, GC Corporation, USA). After three months the dogs were euthanized using an intravenous barbiturate overdose of 6% pentobarbital (Butler Company, Columbus, Ohio, United States) dose (120 mg/kg) [(26)]. The incisors were extracted, decoronated and placed in capped test tubes with saline to avoid dehydration of samples and properly labelled.

Assessment of root fracture resistance:

Five roots from each subgroup and five roots of the –ve control group were used for assessment of fracture resistance. Each root was adjusted to a standardized length of 12 mm. The apical root ends were embedded individually in copper rings (25 mm high and 10 mm in diameter) filled with acrylic resin, leaving 7 mm of each root exposed. The copper rings with the vertically aligned roots were mounted in Instron universal testing machine. The application of vertical loading force to fracture was according to Sedgley & Messer [(27)] to test the brittleness of endodontically treated teeth.

A loading fixture with a spherical tip (r = 2 mm) were mounted and aligned in the center of the canal opening of each root. Then each specimen was subjected to a slowly increasing vertical load at a crosshead speed of (1m/min) until the root fractured or an audible crack was heard. The force required to fracture each specimen was recorded and measured in Newton [(28)]. The data thus obtained was recorded, tabulated and subjected to statistical evaluation. Analysis of variance was used to analyze the difference between various test groups.

Statistical Analysis

Data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Fracture resistance data were parametric data, presented as mean and standard deviation (SD) values. Two-way Analysis of Variance (ANOVA) was used to study the effect of sealer type, irrigant and their interaction on mean fracture resistance. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

a. Effect of sealer type:

There was no statistically significant difference between the three sealers (BioRoot RCS, TotalFill BC and AH plus) with different irrigants and the negative control (intact teeth) subgroup. All showed higher statistically significant mean fracture resistance than +ve control (prepared / unfilled) subgroup (Table 1).

b. Effect of irrigants type:

In experimental groups (instrumented, irrigated and obturated) there was no statistically significant difference between EDTA, NaOCl and the negative control group, all showed the highest statistically significant mean fracture resistance than saline (Table 2).

While in positive control subgroup (instrumented and unfilled group) saline demonstrated highest mean fracture resistance followed by EDTA then NaOCl with no statistically significant difference between the three irrigants (Table 2).

Table (1) *Mean and standard deviation (SD) values for the effect of the three selected sealers with different irrigating solutions on root fracture resistance.*

Irrigant	SG1 BioRoot		SG2 TotalFil		SG3 AH Plus		SG4 +ve Control +ve Control		SG5 -ve Control		P-value	Effect size (Partial eta squared)
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
EDTA	664.5[a]	113.1	675.8[a]	86.9	659.4[a]	123.6	411.7[b]	157.3	735.8[a]	195.9	0.0371*	0.049
NaOCl	654.9[a]	116.4	661.8[a]	120	647.5[a]	132.9	409.3[b]	63.7	735.8[a]	195.9	0.0368*	0.049
Saline	549.3[a]	66.7	573.2[a]	190.7	531.4[a]	84.5	453.7[b]	132.3	735.8[a]	195.9	0.0494*	0.038

**: Significant at $P \leq 0.05$. Means with different superscripts in the same row are statistically significant different.*

Table (2) *The mean and standard deviation (SD) values for the effect of the three selected irrigants on root fracture resistance.*

Sealer	GA EDTA		GB NaOCl		GC Saline		P-value	Effect size (Partial eta squared)
	Mean	SD	Mean	SD	Mean	SD		
BioRoot	664.5[a]	113.1	654.9 [a]	116.4	549.3[b]	66.7	0.0109*	0.069
TotalFill	675.8[a]	86.9	661.8[a]	120	573.2[b]	190.7	0.0421*	0.028
AH-Plus	659.4[a]	123.6	647.5[a]	132.9	531.4[b]	84.5	0.0109*	0.058
+ve Control	411.7[a]	157.3	409.3[a]	63.7	453.7[a]	132.3	0.317	0.036
ve Control	735.8[a]	195.9	735.8[a]	195.9	735.8[a]	195.9	0.612	0.012

**: Significant at $P \leq 0.05$. Means with different superscripts in the same row are statistically significant different.*

DISCUSSION

The results of this study support the null hypothesis that, there is no significant difference in fracture resistance between roots filled with either BioRoot RCS, TotalFill BC or AH Plus sealers.

In this study preparation of root canals was done with I Race rotary files, this resulted in a uniform, standardized preparation that leads to equal distribution of stresses in the root during filling [29]. To avoid excessive dentin removal, maintaining the coronal dentin and to minimize the wedging forces of the spreaders during lateral and vertical compaction, single cone technique was used for canal obturation [30]. The effect of tested sealers on fracture resistance of the roots was judged using the universal testing machine [28].

Based on the results of the present study, the lower fracture resistance of prepared unfilled roots was attributed to canal preparation resulting in excessive loss of dentin and weakening of the roots along with absence of filling material needed to reinforce tooth structure [31].

Whereas the high fracture resistance of the –ve control group (intact teeth) was due to that, in intact roots, no instrumentation was done, no force was imparted in the teeth, and there was no loss of dentin so that the tooth integrity is intact [32].

The prepared filled roots reported high fracture resistance that was insignificantly different than intact teeth (-ve control). The high fracture resistance of both bioceramic sealers could be due to the nature of calcium silicate sealer's composition, which does not shrink during setting and hardens in presence of moisture. The sealer absorbs water from dentinal tubules to initiate the setting reaction producing a composite of calcium silicate hydrogel and hydroxyapatite. Both of these compounds form a strong chemical and micromechanical bonding with the dentin hydroxyapatite. This chemical bonding along with the deep penetration of the sealer into canal irregularities and dentinal tubules enhances the fracture resistance of teeth [33,34]. So, calcium silicate root canal sealers might have the potential to reinforce the instrumented teeth against vertical root fracture [35,36].

While the high fracture resistance of AH Plus group might be related to formation of a covalent bond by an open epoxide ring to any exposed amino groups in the collagen. AH Plus has a better penetration into the micro-irregularities because of its creeping property and long polymerization period, which increases the mechanical interlocking between the sealer and root dentin. In addition, AH Plus has low shrinkage while setting and long-term dimensional stability. It is resilient, and in combination to gutta-percha, it forms a perfect seal with dentinal walls giving it a good strength and resistance to fracture [37].

The results of present study came in accordance with **Osiri et al.,** [38] and **Guneser et al.,** [39] who reported that, the fracture resistance of roots obturated with Totalfill BC, BioRoot RCS or AH plus sealer was not significantly different from that of intact roots.

Considering the different irrigants used in this study, in the positive control group (instrumented and unfilled group) the highest mean root fracture resistance (RFR) was seen in teeth finally irrigated with 0.9% saline followed by teeth finally irrigated with 17% EDTA then teeth irrigated with 2.5%NaOCl with no statistically significant difference between the three irrigants.

This might be attributed to that, 2.5% NaOCl resulted in deproteination of dentin by dissolution of collagen matrix, leaving behind a brittle layer of hydroxyapatite crystals that are not supported by the collagen matrix. Destruction of the matrix in mineralized tissues decreases the elastic modulus and flexural strength of dentin (40,41), results in a less tough, more brittle substrate that might precipitate fatigue crack propagation during cyclic loading and might be the main cause of decreasing toughness and compressive strength of the dentin (42,43).

On the other hand, EDTA demineralizes the inorganic components of dentin by chelating calcium ions in the hydroxyapatite crystals and resulted in changes in the microstructure of dentin and its calcium/phosphorus ratio (44). This mineral loss leads to reduction in compressive strength of dentin (43).

In this study, experimental groups (irrigated and obturated), roots irrigated with 17 % EDTA presented non-significant higher RFR than roots irrigated with 2.5% NaOCl. These findings confirmed the results of **Beltz et al., (45)** who reported that, samples in which the smear layer were removed exhibited higher fracture resistance, which might be attributed to the demineralizing ability of 17% EDTA and removal of inorganic components of the smear layer with exposure of collagen matrix. EDTA improved sealer penetration when compared to 2.5% NaOCl (46). Thereby, increases sealing efficiency and strength of the roots (47).

However other studies showed that, long-term exposure of 17% EDTA (10-min) can result in dissolution of peritubular and intertubular dentin (48) resulting in decreased modulus of elasticity and flexure strength (49). This in turn can reduce the microhardness and resistance to fracture (50).

Knowing that the bond strength of the filling material influences the fracture resistance of the tooth (51-53). Hence, the limitation of our study was that the effect of dentin bonding with the tested sealers, degree of collagen degradation and tooth fracture resistance was not correlated. Accordingly, further studies are required to cover this limitation and to correlate also between the long-term clinical use of calcium silicate sealers and the risk of root fracture in endodontically treated teeth.

CONCLUSION

Within the limitation of this in vivo study, it can be concluded that TotalFill BC and Bioroot RCS, are able to reinforce the tooth against fracture as good as AH Plus.

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