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Resistance to Fracture of Roots Treated with Resorcinol Formaldehyde Resin: a Comparative *in Vitro* Study

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Summary

Hypotheses. There were three hypotheses in this study: H1) roots treated with resorcinol formaldehyde resin (RFR) are less resistant to fracture than roots with an intact pulp, H2) RFR roots are weakened by the re-treatment, and H3) re-treated RFR roots are further weakened by the presence of micro cracks.

Methods. A total of 160 specimens from single roots were selected and divided into four groups: the negative control group (C1) which comprised of roots with an intact pulp, the E1 group which had RFR teeth without cracks, the E2 group which had re-treated RFR teeth without micro cracks, and the E3 group which had re-treated RFR teeth with micro cracks. The resistance to fracture (RtoF) was evaluated after applying a vertical compressive force (250 N/s) until a specimen fractured.

Results. Bivariate analyses (one-way ANOVA with Dunnett adjustment) showed that the resistance to fracture was significantly ($P < 0.001$) higher in the control group than in the E1-E3 groups. The mean $\pm$ sd for C1 was $(227.5 \pm 29.6 \text{ N/mm}^2)$, for E1 $174.7 \pm 41.5 \text{ N/mm}^2$, for E2 $169.7 \pm 31.4 \text{ N/mm}^2$, and for E3 it was $150.5 \pm 11.4 \text{ N/mm}^2$. The linear multiple regression analyses with the three contrasts showed that the RtoF was affected by the RFR ($\beta = -0.56$; $P < 0.001$), the re-treatment of the RFR roots ($\beta = -0.17$; $P = 0.018$), and the presence of micro cracks in the RFR roots ($\beta = -0.17$; $P = 0.003$).

Conclusion. The RtoF was substantially affected by RFR material and to a lesser extent, by the re-treatment of the RFR roots and by the presence of micro cracks in the RFR roots.

Keywords: resorcinol formaldehyde resin, resistance to fracture, cracks, retreatment.

INTRODUCTION

Endodontic treatments may negatively affect the properties of root dentine such as microhardness and resistance to fracture [1]. A multifactorial etiology has been suggested where root preparation and its irrigation and the use of medications play important roles in the root fracture process [2]. For example, a tooth's mechanical strength may decrease due to removal of hard tissues, alterations in physical

properties of dentine during endodontic treatments, and/or occlusal loading [3]. It has been reported that dentine of endodontically treated teeth may have similar mechanical properties to that of vital teeth [3, 4]. Root canal enlargements and reductions of tooth structures have been considered as main risks for vertical root fractures [5, 6]. Excessive widening of root canals, particularly during the re-treatment procedures, requires an additional mechanical preparation

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that results in substantial dentine removal, which weakens roots [7]. The range of endodontic materials that have been used during root canal treatments include formaldehyde-containing materials, which have known cytotoxic and allergic properties [8, 9]. In Eastern Europe and in other post-Soviet countries, resorcinol-formaldehyde resin (RFR) has been widely used in endodontic therapy for many years [10]. Such teeth treated with RFR have been described as “red teeth”, “pink teeth” or “Russian Red teeth” [11]. RFR was mainly recommended and used for the treatment of narrow and curved root canals [12, 13]. In most cases, only pulp amputation was performed and in cases of large root canals, RFR was placed on a pulp chamber floor [10, 12]. Consequently, root canals were left either unfilled or were only partially obturated with the rationale of having a fixation of the residual pulp tissues and to ensure prolonged antibacterial activity [12]. It is important to consider that the re-treatment of RFR roots is challenging at best and may be unpredictable due to a large variation in the provision of previous treatments including the use of RFR.

From a clinical standpoint, it is important to examine the resistance to fracture (RtoF) of RFR-treated teeth and concomitantly discriminate the magnitude of negative effects due to the material and re-treatment of such teeth as well as the impact of micro cracks and how they may affect the resistance to fracture.

We formulated three hypotheses for the present *in vitro* study: H1) roots of teeth treated with RFR are less resistant to fracture than roots with intact pulps, H2) the RFR roots are further weakened if re-treatment of such roots is performed, and H3) the re-treated RFR roots with micro cracks are less resistant to fracture as compared to the re-treated RFR roots without micro cracks.

MATERIAL AND METHODS

The institutional Ethics committee, Vilnius University hospital “Zalgirio clinic”, approved the study.

Selection of teeth

A total of 247 extracted teeth were inspected, of which 120 fulfilled our selection criteria of i) permanent first mandibular molars, ii) mature apices, and iii) previous treatments with RFR. These 120 teeth with RFR were

chosen to represent three experimental groups (E1-E3), each containing a total of 40 teeth. For the negative control group (C1), we chose another 40 permanent first mandibular molars (with mature apices and untreated pulp) which were extracted for periodontal reasons. After extractions, teeth were gently cleaned with a gauze in order to avoid damage to root surfaces and immediately placed into containers with a distilled water to protect root dentine from dehydration.

Subsequently, digital radiographs (Planmeca Only, Helsinki, Finland) of each root were taken in buccolingual and mesiodistal positions. For both positions, the distance between film and root was held at 3 cm. External root surfaces were inspected with a microscope (Zeiss Stemi SV6, Carl Zeiss, Jena, Germany) under 16× magnification in order to identify the presence of resorcinol formaldehyde, confirm the presence of only one root canal, and examine roots for micro cracks and/or infractions.

Preparation of roots

Using a water-cooling diamond bur, single distal roots of lower permanent molars were sectioned from a crown to obtain a standardized 12 mm root length. A total of 160 standardized roots were used to form four study groups (C1, E1, E2, and E3), each comprising 40 roots. The negative control group (C1) included the roots with intact pulps. The first experimental group (E1) included roots without micro cracks which were previously treated with resorcinol formaldehyde. No further treatments for the E1 group were initiated. The second experimental group (E2) included roots without micro cracks which had previously been treated with RFR and were re-treated. The third experimental group (E3) included roots with micro cracks which were previously treated with RFR and received a similar re-treatment as the roots from the E2 group.

Root re-treatment procedures (Groups E2-E3)

The root canal patency was established with a size 15 K-File (Dentsply Maillefer, Bellai-

gues, Switzerland) using a torque-control motor (Dentsply Maillefer, Bellaiques, Switzerland). Following the manufacturer's recommendations, the full sequence of ProTaper rotary instruments was used to enlarge canals employing a speed of 300 rpm and the following files: Sx, S1, S2, F1, F2, F3, F4, and F5. During the mechanical preparation, each canal was irrigated with a 2 mL of 2% sodium hypochlorite between the uses of each instrument. After the completion of the instrumentation, the root canals were rinsed with 2 mL of distilled water.

Preparation of specimens

A total of 160 roots (40 for each group) were fixed in resin and then sectioned horizontally at 3 mm and 9 mm distance from an anatomical apex using a low-speed saw under water-cooling (Leica SP, Wetzlar, Germany). This way, 6 mm length dentine specimens with two smooth surfaces were acquired which helped to prevent interference from the jaws of a test machine (Toni Technik GmbH, Gustav-Meyer-Allee 25-D-13355, Berlin, Germany). Subsequently, external root surfaces and flat surfaces of these specimens were inspected with a microscope under 16× magnifications (Zeiss Stemi SV6, Carl Zeiss, Jena, Germany). The images of flat surfaces were taken and examined for the presence/absence of a crack. All procedures related to the preparation of roots and specimens were performed by the same operator (EN). Another observer performed an independent examination for the presence/absence of cracks.

The loading test

Prior to the loading, each root was embedded in a silicon impression material (Panasil, Kettenbach, GmbH and Co, Germany) to simulate a periodontal ligament space by mimicking its mechanism of stress distribution. The root width was measured at the buccolingual and mesiodistal positions for the apical and coronal sections of the specimens. In the loading test, a simulated periodontal ligament – represented by a silicon impression material – allowed a specimen to move within a limited space. The specimens were placed between the jaws of a universal testing machine (Toni Technik GmbH, Gustav-Meyer-Allee 25-D-13355, Berlin, Germany) and the loading forces were applied from a coronal end of a specimen to a slowly increasing vertical direction with the point of application being centered in

the root canal. The loading speed of 250 N/s was applied until a specimen fractured. The “fracturing moment” was determined when a sudden drop in a force occurred that was observed in the display of the testing machine. The maximum force required to fracture each specimen was recorded in Newtons (N).

Calculations of apical flats of specimens

An image of an apical surface of a specimen was evaluated in the graphic AutoCAD program. Using the buccolingual and mesiodistal diameters as landmarks, the synapse was drawn as indicated by a red line and subsequently an area of a synapse A_0 was calculated (Fig. 1). The green line corresponds to an apical outline of a specimen and the A_{real} is calculated automatically by the AutoCAD program. Considering the discrepancy (error) between the A_0 and A_{real} measurements, formulas were used to calculate the apical areas using the synapse (oval), the area of which was calculated from the buccolingual (a) and mesiodistal (b) diameter via:

$$A_0 = \frac{a \cdot b}{4}$$

The fracture to resistance was calculated using the following formula:

$$fr = \frac{F_u}{A_0}$$

Statistical analyses

All statistical analyses were performed employing the SPSS software (IBM, version 22.0) and the significance for all tests was set as $P < 0.05$. The univariate analysis (Shapiro-Wilk test) tested the data for normality in preparation for the inferential analyses (bivariate and multivariate).

The bivariate analysis (one-way ANOVA with a post hoc Dunnett adjustment) compared the resistance to fracture among the four study groups. The assumption regarding the homogeneity of variance was tested employing the Levene test.

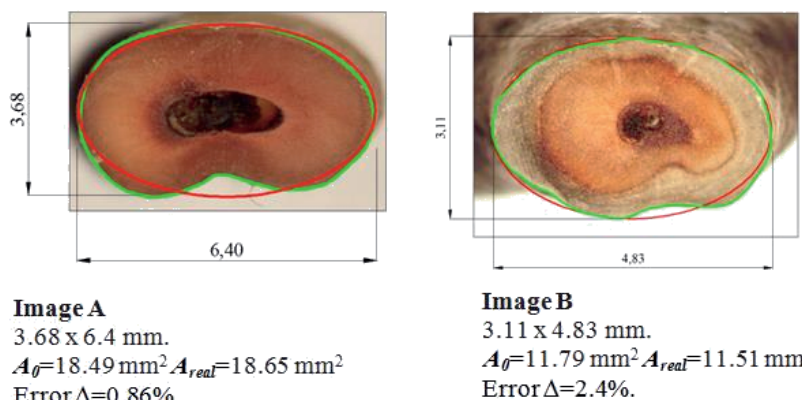


Fig. 1. Calculation of the adjusted areas in the cross-sectional sections of specimens

The multivariate analysis (linear multiple regression with three contrasts) was used to test the three study hypotheses which postulated three potentially detrimental effects (here, three contrasts), each independently reducing the root's resistance to fracture. To support the testing with contrasts, three dummy variables were prepared and their coding is presented in Table 1. The assumption of the independence among the independent variables (here among the three contrasts) was tested employing the collinearity diagnostics. The absence of collinearity was considered if tolerance values were below 0.500 and the Variance Inflation Factor (VIF) values were below 2.0.

Fig. 2 demonstrates how the three contrasts were designed and their sequence of testing. The dummy 1 variable represents contrast 1 (effect due to the treatment with RFR material),

which compares the resistance to fracture of teeth treated with RFR to the resistance to fracture of teeth not treated with the RFR. Contrast 1 compared the combined resistance to fracture values of three experimental groups (E1, E2, and E3) to the resistance to fracture values of the negative control group (C1). Contrast 2 (effect due to the re-treatment of the RFR) evaluated the RFR roots without micro cracks which also received a re-treatment. Contrast 2 compared the combined E2 and E3 values (re-treated RFR roots) with the E1 group that also comprised the RFR roots but which were not re-treated. Contrast 3 (effect due to the presence of cracks) evaluated the effect of the re-treatment of the RFR teeth with cracks. This contrast compared the RFR re-treated roots without micro cracks (E2) with the similar group of roots with micro cracks (E3).

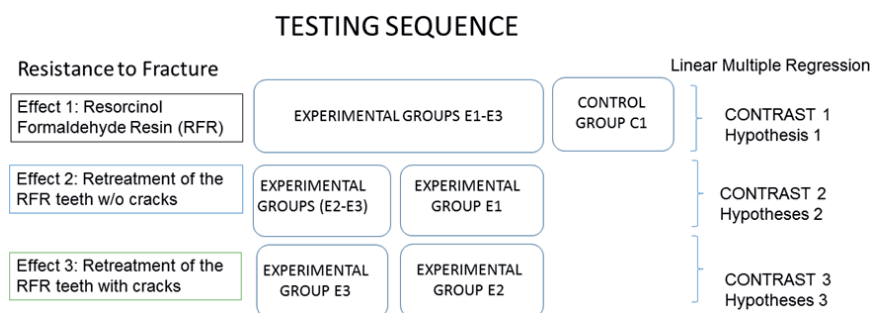


Fig. 2. The testing sequencing of the study hypotheses (Linear Multiple Regression)

Table 1. Coding of dummy variables for the Linear Multiple Regression with 3 contrasts

Groups	Dummy variable 3	Dummy variable 3	Dummy Variable 3
	Contrast 1	Contrast 2	Contrast 3
C1: Negative control (roots with an intact pulp)	-3	0	0
E1: Resorcinol formaldehyde roots without cracks	1	-2	0
E2: Re-treated resorcinol formaldehyde roots without cracks	1	1	-1
E3: Re-treated resorcinol formaldehyde roots with cracks	1	1	1
Total sum	0	0	0

RESULTS

Univariate and bivariate analyses

The assumption of normality was fulfilled as indicated by a non-significant value according to the Shapiro-Wilk test ($P=0.549$). According to Levene's test, the assumption of the homogeneity of variance was also fulfilled ($P=0.565$). The bivariate analysis (one-way ANOVA with a post hoc Dunnett adjustment) compared the resistance to fracture among the four study groups. It is important to note that each of the study groups were exposed to only one experimental condition.

The results of this testing are presented in Table 2. As expected, the highest resistance to fracture was found for the teeth with an intact pulp (C1 group) and the lowest resistance to fracture was observed in the E3 group which contained the re-treated RFR roots with micro cracks. For the C1 group, the mean $\pm$ sd was 227.5 ± 29.6 N/mm², for the E1 group it was 174.7 ± 41.5 N/mm², for the E2 group it was 169.7 ± 31.4 N/mm², and for the E3 it was 150.5 ± 11.4 N/mm².

More details about the resistance to fracture observed in different groups and their visual comparison are presented in Fig. 3, where box and

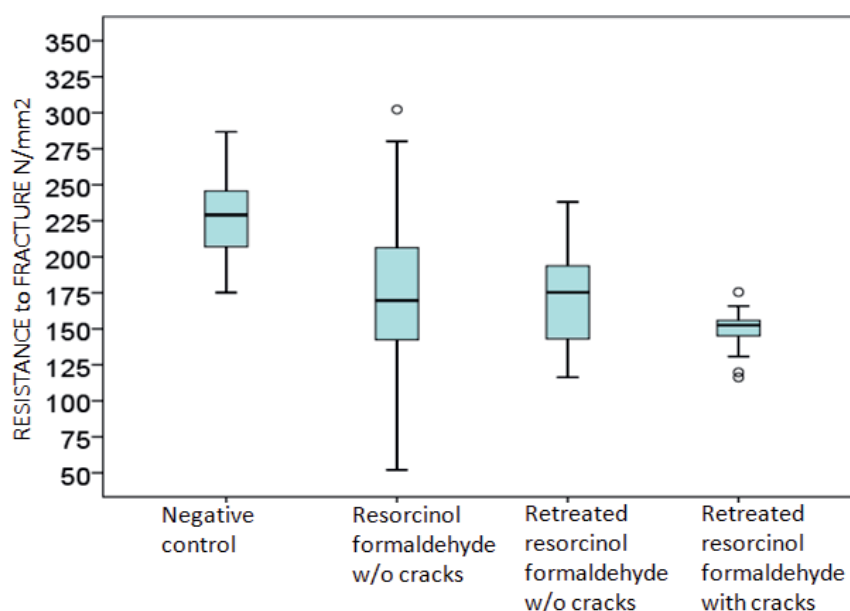


Fig. 3. Study groups and their sequencing in the linear regression analysis

whisker plots, one for each group, represent the distribution of the resistance to fracture dependent upon the experimental condition. There was a clear, increasing pattern of decreased resistance to fracture as more risks were involved. Roots in the negative control group (C1) were most resistant to fracture and this group had a substantially less intra-group variation as compared to the other groups. The least resistant group to fracture was cracked roots of the RFR teeth that were also re-treated.

Multivariate analyses

The linear multiple regression (LMR) employing the three independent contrasts tested the three study hypotheses. The results of this testing are presented in Table 3. The collinearity diagnostics showed that the assumption for the independence among the three contrasts was fulfilled, as indicated by the relatively high tolerance values (>0.65) and by the low VIF values (<1.0). The LMR model's summary indicates that an overall linear regres-

Table 2. Resistance to fracture (N/mm²) – comparisons among the study groups

Groups	Mean $\pm$ sd	Difference #	95% CI#	P values*
C1-Negative control	227.5 $\pm$ 29.6			
E1-Resorcinol formaldehyde w/o cracks	174.7 $\pm$ 41.5	-53.2	-69.4; -37.1	<0.001
E2-Re-treated resorcinol formaldehyde w/o cracks	169.7 $\pm$ 31.4	-57.2	-73.3; -41.1	<0.001
E2-Re-treated resorcinol formaldehyde with cracks	150.5 $\pm$ 11.4	-77.5	-93.9; -61.3	<0.001

Mean difference from the mean of the control;

*One Way ANOVA with a Post Hoc Dunnett adjustment: comparisons with the control group

Table 3. Resistance to fracture (N/mm²) – comparisons among experimental teeth fractured under different study conditions (Linear Multiple Regression Analysis)

Summary: Adjusted R-Squared 0.47; $F=47.221$ (df=3); $P<0.001$

Condition	B (se)#	(95% CI)*	P value	Tolerance	VIF Factor^
Constant	227.9 (4.8)		<0.001		
Contrast 1: Effect of the treatment with resorcinol formaldehyde (RFR) (controlled for the resistance to fracture of teeth with an intact pulp)	-53.2 (6.8)	-0.56 (-66.7; -39.8)	<0.001	0.67	1.50
Contrast 2: Effect of the retreating the RFR teeth (controlled for the resistance to fracture of teeth with an intact pulp and for the treatment with RFR)	-14.1 (5.9)	-0.17 (-25.7; -2.5)	0.018	0.68	1.5
Contrast 3: Effect of the cracks (controlled for the resistance to fracture of teeth with an intact pulp, for the treatment with RFR and for the re-treatment of RFR teeth).	-10.1 (3.4)	-0.17 (-16.9; -3.4)	0.003	1.00	1.0

B (se)# Unstandardized regression coefficient (standard error)

(95% CI)* Standardized regression coefficient (95% Confidence Interval)

^ VIF Factor Variance Inflation Factor

sion model was highly statistically significant ($P < 0.001$) and that the three contrasts, namely 1) the treatment with RFR, 2) the re-treatment of the RFR teeth without micro cracks, and 3) the re-treatment of RFR teeth with micro cracks, jointly explained 47% of variance (adjusted R-squared=0.47) in the study outcome (roots' resistance to fracture). It is important to note that each of the three contrasts, namely the risks decreasing the resistance to fracture as postulated by the three study hypotheses, had an independent effect even when controlled for the other two contrasts. The constant in the LMR model represents the value of the first contrast indicating the magnitude of RFR's detrimental effect ($\beta = -0.56$; $p < 0.001$). The second contrast which represents the negative effect due to the re-treatment of non-cracked RFR roots was also statistically significant ($\beta = -0.17$; $P = 0.0018$). The third contrast or the detrimental effect due to the re-treatment of cracked RFR roots was also statistically significant ($\beta = -0.17$; $P = 0.003$).

DISCUSSION

Endodontic treatments with the RFR technique were common root canal treatment modalities in Eastern Europe [10]. Teeth after endodontic treatments become weaker and are more prone to fracture than vital teeth [4]. The strength of endodontically treated teeth has been analysed in several studies and has been associated with the extent of remaining tissues [6]. Instrumented roots were found to be significantly weaker than roots with an intact pulp [14]. Consequently, it is important to consider that a tooth's strength may be compromised due to mechanical root preparations [5, 15]. The present *in vitro* study was designed to examine the strength of teeth treated with RFR and thus resistance to fracture (RtoF), which was used as a proxy for a root's mechanical strength [16].

We studied RtoF of the RFR teeth under different study conditions. In order to support this testing, we postulated three study hypotheses, each of

them related to an independent detrimental effect contributing to the reduction of a root's resistance to fracture. The first hypothesis stated that a root is weakened because of the treatment with the RFR material; this was confirmed because the RtoF was substantially lower in the roots that had previous RFR treatments compared to the roots with intact pulps. The second study hypothesis tested an additional detrimental effect that occurred due to the re-treatment of RFR teeth. This hypothesis was also confirmed since the re-treatment of the RFT teeth showed the further weakening of the RFR roots. The third hypothesis considered an additional risk factor of the presence of micro cracks while re-treating the RFR teeth; this hypothesis was also confirmed. It is important to consider that the present experiment clearly demonstrated the cumulative detrimental effect of the three risks, namely the use of the RFR material, the re-treatment of the teeth treated with the RFR material, and the impact of the micro cracks present in the RFR teeth. These are considerable risks that may be encountered in clinical endodontic practices. However, any broad generalizations from the present findings should be drawn with caution, mainly due to the fact that we used an *in vitro* design for the testing of our hypotheses. To compensate for this limitation, we tried to mimic the clinical environment as much as possible. Knowing that a periodontal ligament plays an important role in a tooth's resistance to fracture, we embedded each root into a silicon impression material prior to the loading. We also acknowledge that it is impossible to recreate fully the complexity of the periodontal ligament [17, 18]. Another important consideration of the present study was to avoid the dehydration of roots. Thus after an extraction, each tooth was immediately placed into a container with distilled water. To minimize the potential for information bias, we standardised the procedures for the selection of teeth, preparation of roots and specimens, and outcome assessments. However, the lack of standardisation among different studies [19] makes it

difficult to compare our study with other studies.

The present experiment only evaluated vertical loading, even though occlusal forces in oral environment are complex and diverse [20]. We chose this approach based on the evidence that vertical root fractures have been mainly associated with procedures during root canal treatment [21].

Previous *in vitro* studies have reported that the use of rotary instruments may cause cracks in root dentine [22–24]. Similar to other *in vitro* studies, we employed the sectioning method that allows a direct inspection of a root dentine, which is impossible in clinical practice. This method allowed us to examine the detrimental effects of cracks in teeth already weakened by an endodontic treatment and the use of RFR and the re-treatment of such teeth. Our experiment demonstrated that the presence of cracks poses an additional risk factor to the RtoF. This important finding about inherent risks of treatment or re-treatment of teeth previously treated with RFR should inform a clinician's approach when treating patients who present with cases of RFR.

CONCLUSIONS

The three study hypotheses were confirmed by the present *in vitro* study that suggested there are three risk factors that reduce the root's RtoF: 1) using resorcinol-formaldehyde resin (RFR), 2) re-treating RFR teeth, and 3) re-treating RFR teeth with cracks. The strongest detrimental effect to RtoF was the use of the RFR material.

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Conflict of Interest Statement

The authors declare no conflicts of interest related to this study. ♦

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Santrauka

REZORCINO FORMALINO DERVA GYDYTŲ DANTŲ ŠAKNŲ ATSPARUMAS SKILIMUI: LYGINAMASIS *IN VITRO* TYRIMAS

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Hipotezės. Prieš atliekant tyrimą buvo sudarytos trys hipotezės: 1 hipotezė – dantys, gydyti rezorcino formalino derva, yra mažiau atsparūs skilimui nei dantys, turintys gyvybingą pulpą; 2 hipotezė – dantų, gydytų rezorcino formalino derva, endodontinio pergydymo procedūros silpnina atsparumą skilimui; 3 hipotezė – dantų, gydytų rezorcino formalino derva, atsparumas skilimui dar labiau sumažėja endodontinio pergydymo metu įvykus skilimams.

Tyrimo metodai. Buvo naudojama 160 apatinio žandikaulio pirmojo krūminio danties distalinių šaknų, turinčių vieną kanalą. Paruošti mėginiai suskirstyti

į keturias grupes: neigiama kontrolinė grupė (C1) – dantys, turintys gyvybingą pulpą, E1 grupė – dantys, gydyti rezorcino formalino derva, be skilimų, E2 grupė – dantys, gydyti rezorcino formalino derva, po endodontinio pergydymo neturintys skilimų, E3 grupė – dantys, gydyti rezorcino formalino derva, po endodontinio pergydymo turintys skilimų. Atsparumas skilimui buvo vertinamas atlikus gniuždymo tyrimą naudojant vertikalią didėjančią krūvį (250 N/s) iki mėginio skilimo momento.

Rezultatai. Dvimatė duomenų analizė: vieno faktoriaus ANOVA su post hoc Dunnett pataisymu naudota siekiant palyginti atsparumą skilimui tarp grupių. Sta-

tistiškai reikšmingai ($p < 0,001$) didesnis atsparumas buvo kontrolinėje grupėje (C1) negu E1–E3 grupėse. C1 grupėje atsparumo skilimui vidurkis reikšmė buvo $227,5 \pm 29,6$ N/mm², E1 grupėje – $174,7 \pm 41,5$ N/mm², E2 grupėje – $169,7 \pm 31,4$ N/mm² ir E3 grupėje – $150,5 \pm 11,4$ N/mm². Daugialypė tiesinė regresija naudojant tris skirtingus kontrastus parodė, kad skilimo atsparumui daro įtaką gydymas rezorcino formalino derva ($\beta = -0,56$; $p < 0,001$), rezorcino formalino derva gydytų dantų pergydymas ($\beta = -0,17$; $p = 0,018$) ir pergydymo metu įvykę skilimai ($\beta = -0,17$; $p = 0,003$).

Išvados. Gydymas rezorcino formalino derva veikia atsparumą skilimui, o šių dantų pergydymas esant skilimams ar jų nesant dar labiau mažina atsparumą skilimui.

Reikšminiai žodžiai: rezorcino formalino derva, atsparumas skilimui, skilimai, endodontinis pergydymas.

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