VILNIUS UNIVERSITY

Eglė Nedzinskienė

PECULIARITIES OF ULTRASTRUCTURE OF TOOTH ROOT CANALS TREATED WITH RESORCINOL FORMALDEHYDE, EVALUATION OF INFLUENCE OF ENDODONTIC RETREATMENT PROCEDURES AND RESISTANCE TO FRACTURE. STUDY *IN VITRO*

Summary of Doctoral Dissertation

Biomedical sciences, Odontology (07 B)

Vilnius, 2018

The dissertation was prepared at Vilnius University during 2013–2018.

The dissertation is defended extramurally.

Scientific consultant:

Prof. Dr. Vytautė Pečiulienė (Vilnius University, Biomedical Sciences, Odontology – 07 B)

The dissertation is defended at the Dissertation Defence Board:

Chairman:

Assoc. Prof. Dr. Vilma Brukienė (Vilnius University, Biomedical Sciences, Odontology – 07 B)

Members:

Prof. Dr. Mare Saag (Tartu University, Estonia, Biomedical Sciences, Odontology – 07 B)

Prof. Dr. Alina Pūrienė (Vilnius University, Biomedical Sciences, Odontology – 07 B)

Prof. Dr. Nomeda Rima Valevičienė (Vilnius University, Biomedical Sciences, Medicine – 06 B)

Assoc. Prof. Dr. Arūnas Barkus (Vilnius University, Biomedical Sciences, Medicine – 06 B)

The dissertation will be defended at the public meeting of the Dissertation Defence Board on the 1st of June, 2018, at 10 a.m. in the Main lecture hall of the Faculty of Medicine, Vilnius University.

Address: M.K.Čiurlionis str. 21, LT – 03101 Vilnius, Lithuania.

The summary of the dissertation was sent out to relevant institutions on the 2nd of May, 2018.

The dissertation is available at the library of Vilnius University and Vilnius University website: *http://www.vu.lt/lt/naujienos/ivykiu-kalendorius*

VILNIAUS UNIVERSITETAS

Eglė Nedzinskienė

REZORCINO FORMALINU GYDYTŲ DANTŲ ŠAKNŲ KANALŲ ULTRASTRUKTŪROS YPATUMAI, ENDODONTINIO PERGYDYMO PROCEDŪRŲ POVEIKIO IR ATSPARUMO SKILIMUI VERTINIMAS. TYRIMAS *IN VITRO*

Daktaro disertacijos santrauka

Biomedicinos mokslai, odontologija (07 B)

Vilnius, 2018

Disertacija rengta 2013–2018 metais Vilniaus universitete.

Disertacija ginama eksternu.

Mokslinė konsultantė:

Prof. dr. Vytautė Pečiulienė (Vilniaus universitetas, biomedicinos mokslai, odontologija – 07 B)

Disertacija ginama viešame disertacijos Gynimo tarybos posėdyje:

Pirmininkė:

Doc. dr. Vilma Brukienė (Vilniaus universitetas, biomedicinos mokslai, odontologija – 07 B)

Nariai:

Prof. dr. Mare Saag (Tartu universitetas, Estija, biomedicinos mokslai, odontologija – 07 B)

Prof. dr. Alina Pūrienė (Vilniaus universitetas, biomedicinos mokslai, odontologija – 07 B)

Prof. dr. Nomeda Rima Valevičienė (Vilniaus universitetas, biomedicinos mokslai, medicina – 06 B)

Doc. dr. Arūnas Barkus (Vilniaus universitetas, biomedicinos mokslai, medicina – 06 B)

Disertacija ginama viešame disertacijos Gynimo tarybos posėdyje 2018 m. birželio 1 d. 10 val. Vilniaus universiteto Medicinos fakulteto Didžiojoje auditorijoje.

Adresas: M.K.Čiurlionio 21, LT – 03101 Vilnius.

Disertacijos santrauka išsiųsta 2018 m. gegužės 2 d.

Su disertacija galima susipažinti Vilniaus universiteto bibliotekoje ir VU interneto svetainėje adresu: *http://www.vu.lt/lt/naujienos/ivykiu-kalendorius*

Abbreviations

BL – buccolingual direction
EN – Eglė Nedzinskienė
MD – mesiodistal direction
mm – millimeter
N – newtons
NiTi – nickel-titanium
RF – resorcinol-formaldehyde
s – second
SEM – scanning electron microscope
VRF – vertical root fracture
w/o – without

1. INTRODUCTION

1.1. Research question and its relevance

In China, India, France and all the Eastern Europe countries, resorcinol-formaldehyde (RF) paste has been used for root canal treatment for many years (1, 2). In Lithuania this method was widely applied almost until 1994. Consequently, dentists are still struggling to resolve endodontic retreatment problems related to the use of this material in clinical practice. Based on clinical experience, the retreatment of teeth that previously were treated with RF paste has unpredictable outcomes. Until today there have been no studies carried out proving the impact of the treatment procedures on the dentin surface of such teeth and its mechanical properties. Still, the results of other studies show that teeth after endodontic treatment are more prone to fracture (3).

During the last couple of decades the attitude towards the principles of root canal mechanical preparation has changed dramatically. This change is thought to be related to the implementation of new alloys, such as nickel-titanium (NiTi), into the manufacturing of endodontic instruments. A huge impact was also made by machine-driven instruments that started to be used for root canal preparation and significantly reduced the time of the procedure as well as the rate of the most common complications (4, 5, 6). As all new technologies, apart from the discussed benefits, this one also has certain drawbacks. One of them is an increased probability of root dentin surface cracks. As regards the main principles of mechanical root fracture, it has already been established that dentin surface defects, such as cracks or unevens in the canal wall, have influence on the decrease of tooth resistance to fracture (4, 10). In addition, in the instance of functional load, it can proliferate and become the reason for vertical root fracture (VRF) (11, 12, 13, 14).

Apart from the impact on the mechanical resistance of a tooth, cracks have one more important influence on the outcomes of endodontic treatment.

They become a niche for accumulation of bacterial colonies which resist treatment and may become the main reason of unsuccessful treatment (11).

The probability of dentin cracks during endodontic retreatment procedures is another important factor determining the prognosis of the treatment. First of all, it is related to a possibly harmful impact on the structures of the tooth which may be caused by the following factors: the instruments and materials that were used during the initial endodontic treatment, vertical force pressure used by the dentist, the way and quality of tooth crown restoration, occlusion forces load, and finally, the usage of aggressive instruments during retreatment procedures.

No studies have been carried out on the physical properties of RF paste as well as its impact on tooth tissue and ultrastructure of dentin and, therefore, the impact of the treatment using RF paste on tooth tissue is still unknown. Based on individual experience of dentists, it has been attested that such teeth are more brittle. The present study *in vitro* was aimed to evaluate the impact of endodontic retreatment procedures on teeth previously treated with resorcinol-formaldehyde paste.

1.2. The aim and objectives

The aim of the present study is to evaluate the peculiarities of the dentin surface ultrastructure in teeth previously treated with resorcinol-formaldehyde paste, test the reliability of diagnostic methods that are used in endodontology and compare the influence of endodontic retreatment procedures on dentin and its resistance to fracture *in vitro*.

To achieve the aim of this study, the following objectives were set:

1. To evaluate the number, types, localization of dentin cracks after endodontic retreatment procedures have been performed.
2. To investigate the effectiveness of dye and different levels of microscopic magnification in order to detect dentin cracks of teeth previously treated with resorcinol-formaldehyde paste.

3. To investigate the resistance to fracture of root dentin in teeth previously treated with resorcinol-formaldehyde paste and pulp-vital teeth.
4. To evaluate the resistance to fracture of teeth previously treated with resorcinol-formaldehyde paste after endodontic retreatment procedures were performed, taking into account whether there are or no micro-cracks in the dentin.
5. To investigate the root canal surface and dentin ultrastructure of teeth previously treated with resorcinol-formaldehyde paste containing different tissue discoloration.

1.3. Scientific novelty and relevance

In the scientific literature there are only a few studies discussing the problems related to the treatment of teeth previously filled with RF paste. This may be related to the rare usage of this material type in dental practice. Even though this material has been used in Lithuania for a long time, there seem to be no studies carried out on its impact on the properties of the tooth tissue. In modern dentistry such teeth are considered to be in need of endodontic retreatment as well as coronal restoration procedures. In order to evaluate a possible impact of retreatment procedures on root dentin it was decided to carry out this study.

Rapid advance in technologies offers dentists more possibilities of using different combinations of instruments during treatment. The most common one is the combination of ultrasound devices and machine-driven instruments. Because of its wide application, this particular combination was chosen in the study to evaluate its impact on the root dentin surface of teeth previously treated with RF paste. The present study gives an in-depth analysis of the overall impact of the combination of both machine-driven and ultrasound instruments on root dentin. In addition, the reliability of diagnostic methods (i.e. dyeing with methylene blue and inspection under microscope), which are commonly used in dental clinical practice in order

to detect dentin cracks in teeth previously treated with RF paste when their colour differs from the neutral colour of the tooth tissue, have been tested in the study.

Moreover, a study of resistance to fracture was carried out not only to evaluate the impact of load on root dentin after the endodontic retreatment but also to assess the level of the impact that dentin surface defects resulting from the retreatment can have on the tooth strength.

The results of the present study *in vitro* are difficult to be interpreted and directly related to the real clinical situation. Still, the results allow to make certain assumptions when it comes to the prognosis of endodontic retreatment of such teeth.

2. MATERIAL AND METHODS

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

2.1. The impact of endodontic retreatment procedures on root dentin surface of teeth previously treated with resorcinol-formaldehyde paste

2.1.1. Preparation of tooth roots

Eighty permanent first mandibular molars with mature apices previously filled with resorcinol-formaldehyde paste were chosen. After extraction, all teeth were gently cleaned with a gauze and immediately placed into distilled water to avoid dehydration and were kept in it during the study. Such categories as patient age, sex and the reasons of extraction were unknown.

Distal roots were sectioned from the tooth crown using a water-cooled diamond bur *(MASTERtorque LUX M9000L, KaVo Dental GmbH, Bismarcking 39, D-88400 Biberach, Germany*) and only roots with a length of 12 mm were used in this study. Only roots with one canal (I type) (15)

and without cracks were included. External surface and coronal section of the roots were observed with a microscope at the x 16 magnifications (*Zeiss Stemi SV6, Carl Zeiss, Jena*, *Germany*). The number of canals was evaluated radiographically. Radiographs were taken in the buccolingual and mesiodistal directions with a 3 cm distance between the X-ray tubuse and the root with a 0.08 ms exposition (*Planmeca Only, Helsinkii, Finland*). Roots were coded and immersed in the contents accordingly coded to roots.

2.1.2. Preparation of root specimens

2 mm of root coronal parts were fixed in resin for stability and then sectioned horizontally at the 3 mm and 9 mm distances from the anatomical apexes using a water-cooled low-speed saw (*Leica SP 1600*, *Wetzlar, Germany*). The surfaces of apical and coronal sections of the specimens were evaluated and photographs were taken under the x 10 and x 16 magnifications (*Zeiss Stemi SV6, Carl Zeiss, Jena*, *Germany*) using the same distance between the specimens and microscope object-glass. Two observers (endodontists) independently inspected all specimens in order to exclude the ones with cracks prior to retreatment procedures. Images of the specimens prior to the procedures (no cracks) served as the controls.

Mesiodistal (MD) and buccolingual (BL) diameters of the apical and coronal sections were measured three times and an average estimate was calculated in oder to avoid deviations of root diameters (range of variations: MD 3.62 – 3.98 mm BL 6.34 – 6.69 mm, MD 2.99 – 3.14 mm BL 4.61 – 4.83 mm). The specimens were divided into two groups according to the hardness of resorcinol-formaldehyde paste in the root canals: soft filling material (Group 1) and hard filling material (Group 2). If a root canal patency could be gained with a #15 *K file* (*Dentsplay Maillefer, Bellaigues, Switzerland*), the filling material was considered soft and, if no patency was gained, the filling material was considered hard.

2.1.3. Chemomechanical preparation of specimen root canals

In order to simulate periodontal ligament space and mimic the mechanisms of stress distribution, a silicon impression material (*Panasil Putty Soft, Kettenbach, Gmbh and Co, Germany*) was used as matrix around roots during their canal preparation procedures.

Group 1 contained 40 specimens where the filling material was soft, i.e. a canal patency was easy to gain with a #15 *K file* (*Densplay Maillefer, Ballaigues, Switzerland*). The mechanical preparation of the root canals was done with *ProTaper* rotary system using a full sequence of rotary files (SX, S1, S2, F1, F2, F3, F4, F5) (*Densplay Maillefer, Ballaigues, Switzerland*) following the manufacturer's recommendations. For this root canal preparation, an electric motor (*Densplay Maillefer, Ballaigues, Switzerland*) with a torque control at a constant speed of 300 rpm and gentle in-and-out motions were employed.

Group 2 contained 40 specimens where the filling material was hard and the *ProUltra Endo* tip # 4 (*Densplay Maillefer, Ballaigues, Switzerland*) and *Piezon Master* 400 scaler (*EMS SA, CH-1260 Nyon, Switzerland*) were used to remove the filling material. Subsequently, the *ProTaper* instruments were used to enlarge all the canals as it was done for the specimens in Group 1.

A 2 ml 2% sodium hypochlorite rinse prior to each subsequent instrument was used and after the completion of procedures, the canals were rinsed with 2 ml of distilled water. All the treatment procedures were completed by the same operator (EN).

2.1.4. Evaluation of flat surfaces of root specimens

After the preparation of the root canal specimens, the images of apical and coronal flat surfaces of both group 1 and group 2 were taken under the x10 and x16 levels of magnification: (*Zeiss Stemi SV6, Carl Zeiss, Jena, Germany*). In order to evaluate the use of dye as an aid for the detection of dentin cracks in teeth previously treated with resorcinol-formaldehyde paste, the coronal and apical flat surfaces of slices of root dentin were stained

for two minutes with *Vista-Blue* 2% methylene blue dye (*Inter-Med/Vista Dental Products, Racine, JAV*) and subsequently rinsed with water. Similarly to the undyed specimens, the stained images were also examined under x10 and x16 levels of magnification (*Zeiss Stemi SV6, Carl Zeiss, Jena, Germany*).

Three observers (endodontists) independently evaluated the microphotographs twice, with a two week interval between these evaluations. The number and type of dentin defects observed after the retreatment and dyeing were compared with the same specimen prior to the procedures (control condition). In cases of discrepancy among the three examiners, the images were re-inspected and a consensus was reached.

The following scheme for recording root defects was prepared, where the number of dentin defects in each specimen surface was recorded (if there were no defects – 0; if some defects were present, numbers 1, 2, 3, etc. were ascribed). Four types of defects were singled out for the evaluation. No defect – root dentin devoid of any cracks or lines (Figure 1). In complete fractures, the line extended from an inner root canal wall to an outer root surface (Figure 2). In incomplete (partial) fractures, the line started from an outer or inner root surface but it did not extend throughout the whole dentin surface (Figure 3). In intradental fractures, the line was localized inside the dentin without reaching the outer or inner surface of the root (Figure 4).

Figure 1. No defect

Figure 2. Complete fracture

Figure 3. Incomplete fracture

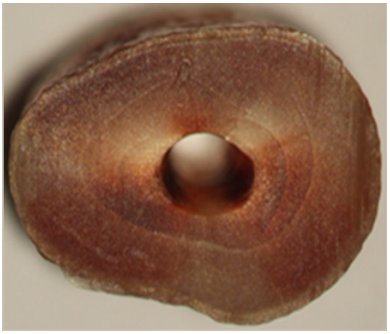


Figure 4. Intradental fracture

2.2. Investigation of root dentin resistance to fracture

2.2.1. Preparation and selection of teeth

One hundred twenty permanent first mandibular molars with mature apices previously filled with resorcinol-formaldehyde paste (where a filling material was soft, i.e. a canal patency was easy to gain with a #15 *K file* (*Densplay Maillefer, Ballaigues, Switzerland*)) were chosen. For the negative control group, 40 permanent pulp-vital first mandibular molars with mature apices were chosen. The roots were prepared as it was done in 2.1.1 part., coded and immersed in the contents accordingly coded to roots.

2.2.2. Preparation of specimens

A total of 160 standardized roots were used to form four study groups (C1, E1, E2, and E3), each comprising 40 roots. 80 distal roots previously treated with RF paste were chosen randomly and root canal preparation was performed the way it was done in 2.1.3 part. The preparation and evaluation of root specimens were performed as described above in 2.1.2 part. The negative control group (C1) included the roots with intact pulp. 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 RF paste and were retreated. The third experimental group (E3) included roots with micro-cracks which were previously treated with RF and received a similar retreatment as the roots from the E2 group.

2.2.3. The loading test

Prior to the loading, each root was embedded in a silicon impression material block (*Panasil Putty Soft, 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 directions for the apical and coronal sections of the specimens. 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 the 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 the specimen fractured. The "fracturing moment" was determined when a sudden drop in the force occurred that was observed in the display of the testing machine. The maximum force (F) required to fracture each specimen was recorded in Newtons (N).

2.2.4. Calculations of dentin strength index

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 the red line and subsequently an area of a synapse $\boldsymbol{A_0}$ was calculated (Figure 5). The green line corresponds to an apical outline of a specimen and the $\boldsymbol{A_{real}}$ 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 BL (a) and MD (b) diameter via:

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

The fracture to resistance was calculated using the following formula:

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

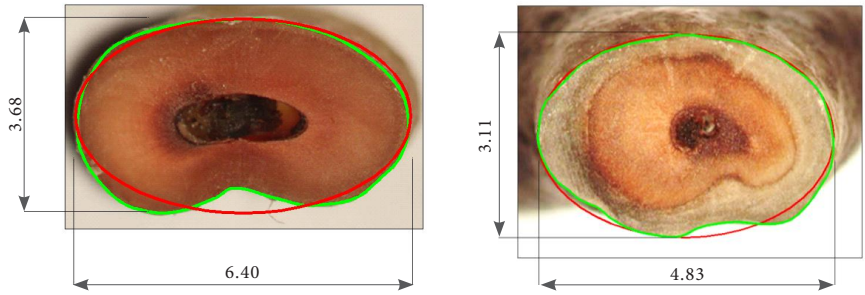


Image A
3.68 × 6.4 mm.
A_0=18.49 mm^2; A_{real}=18,65 mm^2
Error Δ=0.86%.

Image B
3.11 × 4.83 mm.
A_0=11.79 mm^2; A_{real}=11.51 mm^2
Error Δ=2.4%.

Figure 5. Calculation of the adjusted areas in the cross-sectional sections of specimens

2.3. SEM investigation of root canal surface of teeth previously treated with resorcinol-formaldehyde paste

Twenty extracted mandibular molars with mature apices previously treated with RF paste were used in this study. The removal of distal roots and observation for cracks were done as it was described above (2.1.1 part). Two groups according to the colour of the root were formed: group A–10 roots with discoloration of brownish-dark or red and group B–10 roots with conventional yellowish color. To facilitate vertical root fracture into two separate parts, longitudinal grooves on buccal and lingual surfaces of the root with a small fissure diamond bur were made avoiding penetration into the root canal space. The roots were splinted with a small chisel into

two parts. These specimens were dehydrated in a graded series of ethanol solutions, attached to coded stubs, vacuum dried and observed with a scanning electron microscope (*ZEISS 1550VP, Zeiss, Germany*) in a back-scattered electron (BSE) mode. Photomicrographs at the apical, middle and coronal third of the root canals were taken at x200, x1000 and x2000 magnification.

Statistical analysis

The SPSS 21.0 software was used for all statistical analyses with a threshold for statistical significance set at $p<0.05$. For the comparison of defects identified after employing the two different methods (*ProTaper* versus *ProTaper*+Ultrasound) and for the evaluation of effectiveness of magnification to aid the crack identification, Chi-square or Fisher's exact tests were used. For the assessment of RR Risk Ratio having fractures after the two different preparation methods (*ProTaper* vs. *ProTaper*+Ultrasound), logistic regression was employed.

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. The multivariate analysis (linear multiple regression with three contrasts) was used to test the three study hypotheses. The absence of collinearity was considered if the tolerance values were below 0.500 and the Variance Inflation Factor (VIF) values were below 2.0.

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 ($p=0.565$).

3. RESULTS

3.1 The impact of endodontic retreatment procedures on root dentin surface of teeth previously treated with resorcinol-formaldehyde paste

The percentage distribution of cracks in coronal third cross-section of the root is shown in figure 6. There were statistically significantly more complete fractures (p=0.05) and incomplete fractures from the outer root surface (p=0.01) in cases where methylene blue was used.

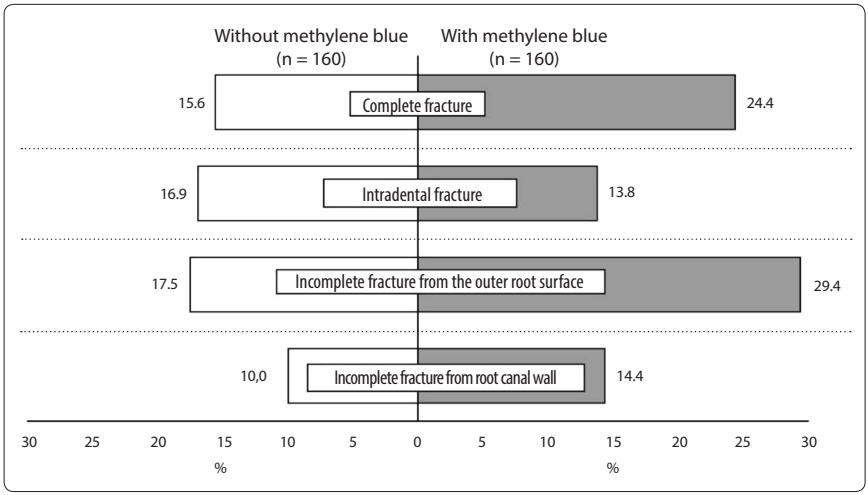


Figure 6. The percentage of cracks of root coronal surface

Table 1 compares the total number of root fractures of coronal cross-sectional surfaces after the root canal preparation with NiTi rotary *ProTaper* instruments (Table 1). There were statistically significantly more cracks (p=0.008) using x10 magnification after dyeing with methylene blue than in the cases when dye was not used.

Also more statistically significant (p<0.001) cracks were found after dyeing using x16 magnification.

An overall statistically significant trend was that more cracks were observed in the coronal surface as compared to the apical surface of the root. These findings were consistent with and without dyeing with methylene blue (Table 1).

Table 1. A total of root fractures of the first group (x10 and x16 magnification, with and without dye)

RESULTS: Any Root Fractures			**Magnification x10#**		
			#No (%)	**Yes (%)**	**p value**
ProTaper	Coronal	Without Methylene	32 (80.0)	8 (20.0)	0.008
		With Methylene	25 (62.5)	15 (37.5)	
	Apical	Without Methylene	40 (100.0)	0	1.000
		With Methylene	40 (100.0)	0	
COMPARISONS		**ProTaper: Coronal vs. Apical:** w/o Methylene (p=0.003) with Methylene (p<0.001)			
			Magnification x16#		
ProTaper	Coronal	Without Methylene	35 (87.5)	5 (12.5)	<0.001
		With Methylene	24 (60.0)	16 (40.0)	
	Apical	Without Methylene	40 (100.0)	0	0.314
		With Methylene	39 (97.5)	1 (2.5)	
COMPARISONS		**ProTaper: Coronal vs. Apical:** w/o Methylene (p=0.021) with Methylene (p<0.001)			

Chi Square Test or Fisher Exact Test

The use of dye under x10 magnification did not significantly aid crack identification at any of root location in the second group, where the combination of *ProTaper* and Ultrasound instruments was used for root canal retreatment (Table 2). The use of dye statistically significanly (p=0.025) help to identify more cracks at the apical sross-sectional surface under x16 magnification.

Concomitantly, there were detected more cracks at coronal surface between two magnification levels in the *ProTaper*+Ultrasound group as it was in the *ProTaper* group (Table 2).

Table 2. A total of root fractures of the second group (x10 and x16 magnification, with and without dye)

RESULTS: Any Root Fractures			**Magnification x10#**		
			No (%)	**Yes (%)**	**p value#**
ProTaper+ Ultrasound	Coronal	Without Methylene	21 (52.5)	19 (47.5)	0.655
		With Methylene	19 (47.5)	21 (52.5)	
	Apical	Without Methylene	37 (92.5)	3 (7.5)	0.456
		With Methylene	35 (87.5)	5 (12.5)	
COMPARISONS		**ProTaper+Ultrasound: Coronal vs. Apical** w/o Methylene (p<0.001) with Methylene (p<0.001)			
			Magnification x16#		
ProTaper+ Ultrasound	Coronal	Without Methylene	20 (50.0)	20 (50.0)	0.654
		With Methylene	18 (45.0)	22 (55.0)	
	Apical	Without Methylene	39 (97.5)	1 (2.5)	0.025
		With Methylene	33 (82.5)	7 (17.5)	
COMPARISONS		**ProTaper+Ultrasound: Coronal vs. Apical** w/o Methylene (p<0.001) with Methylene (p=0.001)			

Chi Square Test or Fisher Exact Test

Comparing the total number of root fractures after the two different root canal retreatment methods, in both specimens groups there were found more cracks at the coronal cross-sectional surfaces of roots.

The use of dye was effective in crack detection in the first (*ProTaper*) group, however in the second specimen group (*ProTaper*+Ultrasound) statistically significantly more cracks were identified without the use of methylene blue (Table 3).

When comparing the occurrence of cracks at the apical surfaces of the root between the groups, with or without the use of dye, more cracks were detected after *ProTaper*+Ultrasound preparation (Table 3), but there were no significant differences. However, the use of dye for crack identification at the apical surface of the root was statistically significant (x10 - p=0.021, x16 – p=0.025).

Table 3. A total of root fractures – a comparison between the first and the second groups (x10 and x16 magnification, with and without dye)

RESULTS: Any Root Fractures		**MAGNIFICATION x10#**		
		1 group ProTaper N (%)	**2 group ProTaper+Ultrasound N (%)**	**p value#**
Coronal surface	Without Methylene	5 (12.5)	19 (47.5)	0.009
	With Methylene	16 (40.0)	21 (52.5)	0.178
Apical surface	Without Methylene	0 (0.0)	3 (7.5)	0.078
	With Methylene	1 (2.5)	5 (12.5)	0.021
		MAGNIFICATION x16#		
Coronal surface	Without Methylene	5 (12.5)	20 (50.0)	<0.001
	With Methylene	16 (40.0)	22 (55.0)	0.179
Apical surface	Without Methylene	0 (0.0)	1 (2.5)	0.315
	With Methylene	1 (2.5)	7 (17.5)	0.025

Chi Square Test or Fisher Exact Test

Comparing the different types of root fracture after the two different preparation techniques between the groups, with or without methylene blue under both magnifications, there were statistically significantly more complete fractures in the second specimen group (Table 4).

When comparing the occurrence of intradental cracks, in both specimen groups with or without dye and under both magnifications, there were statistically significantly more intradental fractures in the *ProTaper*+Ultrasound group (Table 5).

Table 4. Complete fractures – a comparison between the first and the second groups (x10 and x16 magnification, with and without dye)

RESULTS: Complete fractures			MAGNIFICATION x10#		
			1 group ProTaper N (%)	**2 group ProTaper+Ultrasound N (%)**	**p value#**
	Without Methylene	No	39 (58.2)	28 (41.8)	p=0.001
		Yes	1 (7.7)	12 (92.3)	
	With Methylene	No	35 (57.4)	26 (42.6)	p=0.020
		Yes	5 (26.3)	14 (73.7)	
p value#			p=0.090	p=0.633	
			MAGNIFICATION x16#		
	Without Methylene	No	40 (58.8)	28 (41.2)	p <0.001
		Yes	0 (0.0)	12 (100.0)	
	With Methylene	No	35 (58.3)	25 (41.7)	p=0.010
		Yes	5 (25.0)	15 (75.0)	
p value#			p=0.021	p=0.478	

Chi Square Test or Fisher Exact Test

Table 5. Intradental fractures – a comparison between the first and the second groups (x10 and x16 magnification, with and without dye)

RESULTS: Intradental fractures			MAGNIFICATION x10#		
			1 group ProTaper N (%)	**2 group ProTaper+Ultrasound N (%)**	**p value#**
	Without Methylene	No	40 (58.8)	28 (41.2)	p<0.001
		Yes	0 (0.0)	12 (100.0)	
	With Methylene	No	40 (55.6)	32 (44.4)	p=0.003
		Yes	0 (0.0)	8 (100.0)	
p value#			p=1.000	p=0.302	
			MAGNIFICATION x16#		
	Without Methylene	No	40 (61.5)	25 (38.5)	p<0.001
		Yes	0 (0.0)	15 (100.0)	
	With Methylene	No	40 (60.6)	26 (39.4)	p<0.001
		Yes	0 (0.0)	14 (100.0)	
p value#			p=1.000	p=0.816	

Chi Square Test or Fisher Exact Test

Similarly, although statistically non-significant findings were indicated when incomplete fractures from the outer root surface were compared between the *ProTaper* and the *ProTaper*+Ultrasound groups, using dye or not under both magnifications, there were more cracks detected in the *ProTaper*+Ultrasound group (Table 6). Identifications of incomplete fractures from the outer root surface were better when the dye was not used in both specimen groups.

Table 6. Incomplete fractures from the outer root surface – a comparison between the first and the second groups (x10 and x16 magnification, with and without dye)

RESULTS: Incomplete fractures from the outer root surface			**MAGNIFICATION x10#**		
			1 group ProTaper N (%)	**2 group ProTaper+Ultrasound N (%)**	**p value#**
	Without Methylene	No	35 (54.7)	29 (45.3)	p=0.093
		Yes	5 (31.3)	11 (68.8)	
	With Methylene	No	30 (54.5)	25 (45.5)	p=0.228
		Yes	10 (40.0)	15 (60.0)	
	p value#		p=0.152	p=0.340	
			MAGNIFICATION x16#		
	Without Methylene	No	38 (55.9)	30 (44.1)	p=0.012
		Yes	2 (16.7)	10 (83.3)	
	With Methylene	No	32 (55.2)	26 (44.8)	p=0.133
		Yes	8 (36.4)	14 (63.6)	
	p value#		p=0.043	p=0.329	

Chi Square Test or Fisher Exact Test

Concomitantly, there were identified more incomplete fractures from the root canal in the *ProTaper*+Ultrasound group when comparisons were made between the two specimen groups with or without dye under both magnifications, but findings were not statistically significant (Table 7).

Table 8 presents RR Risk Ratio for different types of fractures in the coronal third of roots comparisons between the two groups (*ProTaper* vs. *ProTaper*+Ultrasound) using methylene blue and without it. The risk ratio of complete fractures was in the *ProTaper*+Ultrasound group.

Table 7. Incomplete fractures from the root canal – a comparison between the first and the second groups (x10 and x16 magnification, with and without dye)

RESULTS: Incomplete fractures from the root canal			MAGNIFICATION x10#		
			1 group ProTaper N (%)	**2 group ProTaper+Ultrasound N (%)**	**p value#**
	Without Methylene	No	38 (53.5)	33 (46.5)	p=0.077
		Yes	2 (22.2)	7 (77.8)	
	With Methylene	No	37 (52.9)	33 (47.1)	p=0.176
		Yes	3 (30.0)	7 (70.0)	
p value#			p=0.064	p=1.000	
			MAGNIFICATION x16#		
	Without Methylene	No	37 (50.7)	36 (49.3)	p=0.692
		Yes	3 (42.9)	4 (57.1)	
	With Methylene	No	33 (49.3)	34 (50.7)	p=0.762
		Yes	7 (53.8)	6 (46.2)	
p value#			p=1.000	p=0.500	

Chi Square Test or Fisher Exact Test

Table 8. The risk of crack type of root coronal surface – a comparison between two groups using x10 and x16 magnification

ProTaper vs. ProTaper+Ultrasound Location: Root's Coronal Third		**MAGNIFICATION** x10			**MAGNIFICATION** x16		
		RR*	p-value	(95% CI)	RR*	p-value	(95% CI)
Complete fractures	Without Methylene	1.3	0.033	(1.0; 1.7)	0.1	<0.001	(0.0; 0.6)
	With Methylene	0.2	0.011	(0.1; 0.5)	0.3	0.009	(0.1; 0.8)
Incomplete fractures from the outer root surface	Without Methylene	0.5	0.081	(0.2; 1.2)	0.2	0.018	(0.1; 0.9)
	With Methylene	0.7	0.167	(0.3; 1.3)	0.6	0.105	(0.3; 1.2)
Incomplete fractures from the root canal	Without Methylene	0.3	0.077	(0.1; 1.3)	0.8	0.499	(0.2; 3.1)
	With Methylene	0.4	0.155	(0.1; 1.5)	1.2	0.500	(0.4; 3.2)

Chi Square Test or Fisher Exact Test *RR Risk Ratio

3.2. Investigation of root dentin resistance to fracture

The results of this testing are presented in Table 9. 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 retreated RF roots with micro cracks. For the C1 group, the mean ± sd was 227.5 ± 29.6 N/mm^2, for the E1 group it was 174.7±41.5 N/mm^2, for the E2 group it was 169.7± 31.4 N/mm^2, and for the E3 it was 150.5±11.4 N/mm^2.

Table 9. Resistance to fracture (N/mm^2) – comparisons among the study groups

Groups	Mean±sd	Difference #	95% CI#	p value*
C1-Negative control	227.5±29.6			
E1-Resorcinol formaldehyde w/o cracks	174.7±41.5	-53.2	-69.4; -37.1	<0.001
E2-Re-treated resorcinol formaldehyde w/o cracks	169.7±31.4	-57.2	-73.3; -41.1	<0.001
E2-Re-treated resorcinol formaldehyde with cracks	150.5±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

The linear multiple regression (LMR) employing the three independent contrasts tested the three study hypotheses. The results of this testing are presented in Table 10. 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 summary of the LMR model indicates that an overall linear regression model was highly statistically significant (p<0.001) and that the three contrasts, namely 1) the treatment with RF, 2) the retreatment of RF teeth without micro cracks, and 3) the retreatment of RF teeth with micro cracks, jointly explained 47% of variance (adjusted R-squared=0.47) in the study outcome (root 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 (β=-0.56; p<0.001). The second contrast which represents the negative effect due to the retreatment of non-cracked RFR roots was also statistically significant (β=-0.17; P=0.0018). The third contrast or the detrimental effect due to the retreatment of cracked RFR roots was also statistically significant (β=-0.17; P=0.003).

Table 10. Resistance to fracture (N/mm2) – comparisons among experimental groups (Linear Multiple Regression Analysis)

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

Condition	B (se)#	β (95% CI) *	p value	Tole-rance	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)
β (95% CI) * Standardized regression coefficient (95% Confidence Interval)
^ VIF Factor Variance Inflation Factor

More details about the resistance to fracture observed in different groups and their visual comparison are presented in Figure 7, where the box and 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 the cracked roots of the RF teeth that were also retreated.

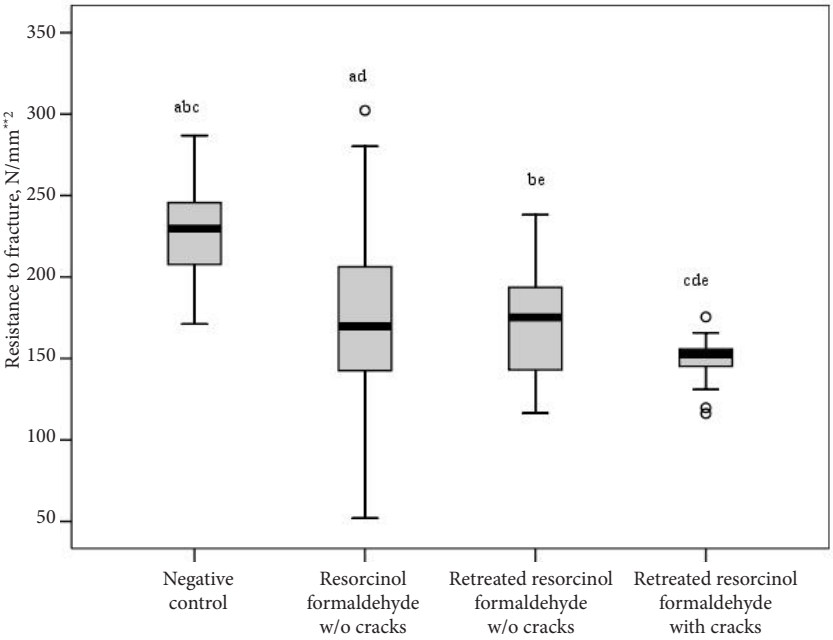
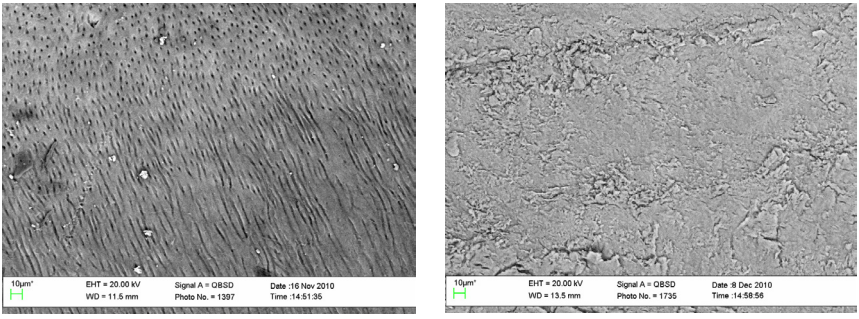


Figure 7. Study groups and their sequencing in the linear regression analysis

3.3 SEM investigation of root canal surface of teeth previously treated with resorcinol-formaldehyde paste

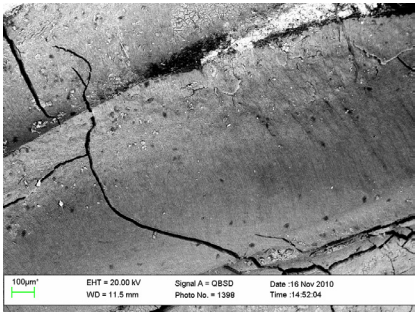
In the brownish-red root scanned specimens smear layer was not detected, but exhibited varying amounts of remaining debris and RF paste on the surface of the root canals. The fields without coverage with filling material were found in all the samples. In those areas the morphology of dentin was ordinary and open dentinal tubules were clearly seen (Figure 8a). While in the samples of non-stained roots the dentin profile was sufficiently unusual. In the coronal thirds just few open dentinal tubules were detected while in the middle and apical thirds no open dentinal tubules were observed and dentin profile was similar to sclerotic dentin (Figure 8b).

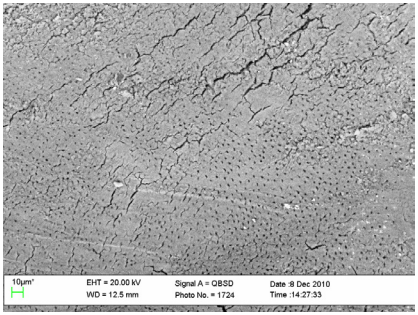


a b

Figure 8. The appearance of root wall dentin of RF treated tooth. Ordinary dentin morphology of colored root sample at the middle third of the root canal (a). The apical third of root canal of non-colored sample: dentin profile similar to sclerotic dentin (b). Original magnification x1000

The multiple dentinal defects (infractions and cracks) were detected in all thirds of scanned specimens at all magnifications, however visually significantly more dentinal defects were detected in the brownish-red roots in comparison with the non-colored root samples (Figure 9).

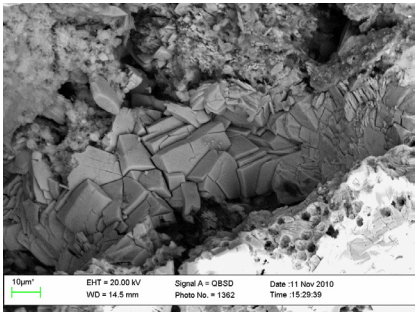


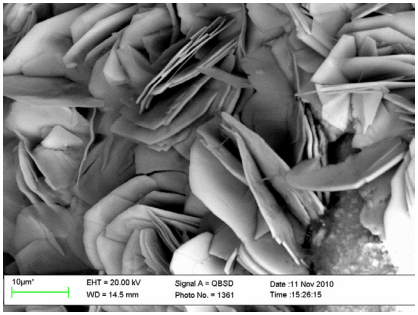


a b

Figure 9. Multiple dentinal defects of discolored root dentin. Original magnification x200 (a) ir x1000 (b)

The mass of RF paste lying on the root canal surface was detected in all the specimens of colored roots, however usually just in the coronal and partially in the middle third of root canals (Figure 10a). At a higher magnification the crystals of RF paste compounds were detected (Figure 10b).

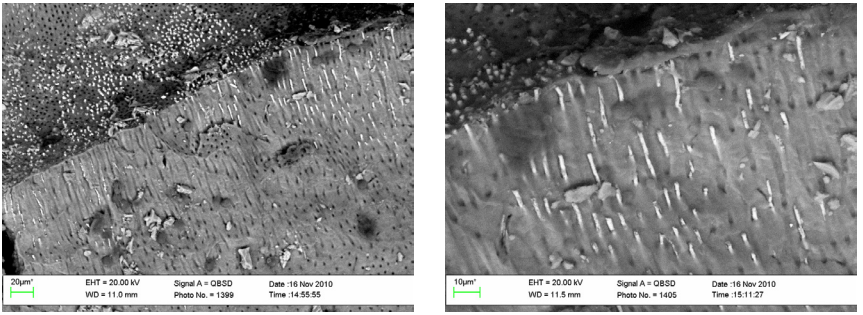




a b

Figure 10. The mass of RF paste on the root canal wall (a). Original magnification x1000. Crystals in RF based material (b). Original magnification x2000

Examination with SEM revealed good adherence of RF resin to the root canal dentin wall. The multiple solid and quite polymerized tags of RF paste deeply penetrated into the dentinal tubules in all root canal third of the brown or red root sample (Figure 11).



a b

Figure 11. Multiple tags of RF paste penetrated in dentinal tubules. Original magnification x1000 (a) and x2000 (b).

3. DISCUSSION

Endodontic retreatment involves a number of procedures where different instruments and materials are used and all of them have different effect on the surface of the tooth root canal wall. During the mechanical root canal preparation for the initial endodontic treatment, the dentin surface is first exposed to direct mechanical forces (vertical instrument pressure, friction that is created between the instrument and the root walls, etc.). In cases of retreatment the tooth is already experiencing repeated mechanical force as well as ultrasound vibration. The use of different combinations of instruments for treatment determines the accumulative effect of performed treatment on the root dentin surface (7, 16). Root canal filling with RF paste techniques differed not only in their specifics but also in the ability to change the tooth colour. When using this technique, modern chemomechanical standards of the root canal preparation were not applied (1, 2). Therefore it can be stated that, differently from many conventional endodontic treatments, the dentin of the tooth root canal has been affected by long-term chemical effect of RF paste.

Retreatment procedures typically make up a large portion of a dentist's daily work load. Despite the prohibition by both the European Union Directive (17) and the European Society of Endodontists (18), on the use

of resorcinol-formaldehyde paste as root canal filling material, roots filled with this material still constitute a substantial part of retreatment cases in clinical dental practice.

In the present study, the machine-driven system *ProTaper* (*Densplay Maillefer, Ballaigues, Switzerland*) for mechanical preparation of the root canal was chosen because these instruments are used in many countries. The previous *in vitro* studies have reported that the use of rotary instruments may cause cracks in root dentin (19, 20). The machine-driven system has progressively varying taper and removes relatively more of the dentin coronally as compared to other systems (19). Thus, the taper preparation could be a contributing factor to the generation of dentin cracks (19). During canal preparation, the canal is shaped via contact between the instrument and the dentinal walls, creating a momentary stress concentration in the dentin which may lead to dentinal defects (19, 20).

Similar to other *in vitro* studies (13, 14, 16, 19), we employed the sectioning method that allows a direct inspection of root dentin, which is impossible in clinical practice. This method allowed us to evaluate the impact of the retreatment on root dentin of teeth previously treated with RF paste.

The recent study attempted to mimic the technical procedure of the root canal mechanical preparation and load testing as it is done in a clinical setting. In order to simulate stress absorption, during the present experiment a silicon layer surrounding the specimen was used as it was done in the other studies (13, 20, 22-24).

It is also important to emphasize that irrigation with NaOCl can significantly decrease the elastic modulus and flexural strength of dentin (25-27). In order to mimic the clinical situation where 2% NaOCl is commonly used, the solution of the same concentration was used in this study.

The important findings of the present *in vitro* study were that both the *ProTaper* and *ProTaper* combined with ultrasound preparation techniques had harmful effects on root dentin, as both techniques led to crack development in roots previously treated with resorcinol-formaldehyde paste. Most of the cracks were observed in the coronal rather than in the apical location of roots.

Seemingly the *ProTaper* combined with the ultrasound preparation technique is more damaging to roots as compared to the *ProTaper* alone. Namely, the use of combination of instruments induced complete fractures and intradental cracks in root dentin. To identify cracks in such roots, two types of aids were considered; the use of different (x10 and x16) magnifications and the use of dye. Seemingly, there was no benefit in using higher level of magnification to identify the cracks. But, the use of methylene blue dye to assist crack identification was more effective in the group where the *ProTaper* alone was used for root canal preparation of teeth previously treated with RF paste. It is likely that methylene blue can be effectively used for a small craze line detection.

Shemesh et al. also found that the retreatment groups developed more defects than the primary treated groups and stated that retreatment procedures require more mechanical manipulations in the root canal; consequently, more dentin tissue is removed from root canal walls in retreatment cases (7).

It is still considered that teeth after endodontic treatments become weaker and are more prone to fracture than vital teeth (28). The strength of endodontically treated teeth has been analysed in several studies and has been associated with proprioreceptors (29), dehydration (30), lost of tooth tissue (29), mechanical preparation (31), irrigation (26) and intracanal medicaments (32), and extent of remaining tissues (33). Consequently, it is important to note that the strength of a tooth may be compromised due to mechanical root preparations and it can be concluded that the remaining volume of the dentin after root canal preparation is most relevant to tooth strength (34).

The present experiment evaluated only vertical loading, even though occlusal forces in oral environment are complex and diverse (35). We studied resistance to fracture of teeth previously treated with RF paste under different study conditions. Comparisons of resistance to fracture were made not only among the three experimental groups but also with pulp-vital teeth. The control group of teeth with pulp-vital demonstrated the highest resistance to fracture, and the least resistance to fracture was found in the group which contained retreated roots with micro-cracks.

It is important to note that the present study *in vitro* clearly demonstated a cumulative detrimental effect to the risks of tooth strength, namely the use of the RF material, the retreatment of teeth treated with RF paste, and the impact of the micro-cracks present in such retreated teeth. It is known that there is a possibility of undiagnosed cracks during endodontic retreatment. Our experiment demonstrated that the presence of cracks after retreatment poses an additional risk factor to the resistance to fracture of root dentin. 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 to test the resistance to fracture.

In order to better investigate the root canal surface of teeth previously treated with RF paste, observation with a scanning electron microscope (SEM) was done. SEM is a popular tool for the visualization of surfaces of the root canal wall in order to observe dentinal tubules, the smear layer, various root canal filling materials and their contact with root dentin (36). In multi-rooted teeth treated with RF paste different discoloration of roots is often observed. Subsequently, it could be assumed that a possible effect of RF paste on dentin might be different. Therefore extensively discolored roots and roots without discoloration were used in this study in order to identify possible differences of the effect of RF paste on root dentin.

The root canal surfaces of scanned discolored root specimens had a usual profile, which could be observed by SEM. The smear layer was not detected as root canals were not cleaned and shaped. It should be mentioned that often clinicians did not have a goal to obturate root canals with RF paste to the entire length due to a strong believe that underlying pulp tissue could be fixated and this would ensure good treatment results (1). The most surprising finding of this study was the profile of root canal dentin of non-discolored root specimens. The morphology of dentin was quite unusual and similar to sclerotic dentin as only a few open dentin tubules were detected in the coronal part. It is quite difficult to explain our findings. It could be that sclerotic dentin is related to tubular sclerosis – a well known physiological

phenomenon that starts in the apical root part and adverse coronally (37). Another factor influencing obliteration of tubules could be the toxicity of components of RF paste. It could be supposed that underlying pulp-vital tissues could provoke obliteration of dentinal tubules, because in the non-discolored roots endodontic treatment procedures were not performed as after amputation of the pulp the mixed RF paste was placed on the floor of pulp chamber.

In the present study multiple dentin defects were detected in all the scanned specimens despite the colour of roots. It should be mentioned that the defects were localized on the inner region of root canal walls. It might be assumed that the reason for the observed dentin defects could be the breakdown of the protein structure caused by the components of RF paste. Obviously more dentin defects were observed in the discolored roots where the canals were partially obturated with RF paste.

The results of this study showed that RF paste possesses good adhesion and deep penetration into dentinal tubules. The penetration depth could be directly related to flow ability. However, RF paste is not a premixed material and due to the lack of strict mixing recommendations the consistency could vary from thin to thick and it directly relies on liquid/power ratio.

Even though teeth that were treated with RF paste started getting a historical aspect, dentists still face a number of challenges in this field. Our study evaluates the impact of retreatment procedures on teeth previously treated with RF paste. The study investigates the following issues: the impact of retreatment procedures on frequency of tooth root cracks, the resistance of the root to fracture as well as the effect of RF paste on root dentin. All these factors could be important from the clinical perspective as they may be the reason of non-healing periapical destruction. The outcome of endodontic treatment depends on a multitude of mutually related factors that cannot be accounted for as a whole in one study. Thus the results obtained from both *in vitro* and *in vivo* studies could help a dentist to get a more accurate view towards evaluating the influence these factors may have on the outcome of the treatment.

4. CONCLUSIONS

1. The use of NiTi instruments alone as well as the combination of NiTi instruments and ultrasound devices during endodontic retreatment results in a damaging effect on root dentin. The usage of both methods cause more dentin cracks in coronal third of the root. The usage of the instruments combination method causes a higher number of cracks in the root – complete fractures as well as intradental fractures of dentin being more frequent among them.
2. The identification of dentin cracks in teeth previously treated with resorcinol-formaldehyde paste was more effective when a combination of dye and microscope inspection was used.
3. The roots of teeth previously treated with resorcinol-formaldehyde paste are less resistant to fracture than teeth with vital-pulp.
4. The endodontic retreatment of teeth previously treated with resorcinol-formaldehyde paste reduces the resistance to fracture of root dentin and increases the risk of vertical fracture. Resistance to fracture significantly decreases by the presence of micro-cracks during retreatment.
5. Resorcinol-formaldehyde paste not only causes different colour changes of the tooth tissue but also has a different impact on dentinal tubules. The structure of root dentin of discoloured teeth is typical, while the dentinal tubules of teeth that have no changes in colour are totally sclerotic. It is thought that the chemical changes in dentin that were caused by this material have an impact on defects in the dentin of both discoloured teeth and teeth with normal colour. Resorcinol-formaldehyde paste has features of homogeniety as well as penetration into dentinal tubules.

6. PRACTICAL RECOMMENDATIONS

1. During endodontic retreatment procedures of teeth that were previously treated with resorcinol-formaldehyde paste, dentists are recommended to avoid any long term usage of ultrasound instruments as well as rotary machine-driven instruments that have a large taper. These systems can be used after assesing the clinical need and reducing the time of usage to minimum. During the treatment with ultrasound instruments a continuous water cooling is necessary. It is also recommended to use instrument systems of smaller taper for root canal preparation.
2. In instances when any type of instrument is used it is recommended to carry out an early diagnostics on possible cracks after every additional endodontic retreatment procedure. The best way to perform diagnostics is by using dye – methylene blue – and magnification. This should be done keeping in mind the fact that the possibility of undiagnosed cracks still remains.
3. To evaluate the prognosis of a tooth previously treated with resorcinol-formaldehyde paste after the performed endodontic retreatment as well as risks and its suitability for abutment. It is recommended to avoid a large fixed dental prosthesis on such teeth.

REFERENCES

1. Schwandt N, Gound T. Resorcinol-formaldehyde resin ("Russian Red") endodontic Therapy. J Endod 2003;29:435-7.
2. Matthews JD. Pink teeth resulting from Russian endodontic therapy. J Am Dent Assoc 2000;131:1598-9.
3. Kishen A. Biomechanics of fractures in endodontically treated teeth. Endod Topics 2015;33:3-13.
4. Devale MR, Mahesh MC, Bhandary S. Effect of instrumentation length and instrumentation systems: hand versus rotary files on apical crack formation-An in vitro study. J of Clinical and Diagnostic Research 2017;11(1):ZC15-8.
5. Zhou X, Jiang S, Wang S, Zhu X, Zhang C. Comparison of dentinal and apical crack formation caused by four different nickel-titanium rotary and reciprocating systems in large and small canals. Dental Materials Journal 2015;34(6):903-9.
6. Karatas E, Arslan H, Alsancak M, Kirici DO, Ersoy I. Incidence of dentinal cracks after root canal preparation with Twisted file adaptive instruments using different kinematics. J Endod 2015;41:1130-3.
7. Shemesh H, Roeleveld AC, Wesselink PR, Wu MK. Demage to root dentin during retreatment procedures. J Endod 2011;37:63-6.
8. Capar ID, Arslan H, Akcay M, Uysal B. Effects of *ProTaper* Universal, *ProTaper* Next, and HyFlex instruments on crack formation in dentin. J Endod 2014;40:1482-4.
9. Kim HC, Lee MH, Yum J, et al. Potential relationship between design of nickel-titanium rotary instruments and vertical root fracrure. J Endod 2010;36:1195-9.
10. Soni D, Reisingani D, Mathur R, Madan N, Visnoi S. Incidence of apical crack initiation during canal preparation using hand stainless steel (K-file) and Hand NiTi (*ProTaper*) files. Int J Pediatr Dent 2016;9(4):303-7.

11. Tsesis I, Rosen E, Tamse A, et al. Diagnosis of vertical root fractures in endodontically treated teeth based on clinical and radiographic indices: a systematic review. J Endod 2010;36:1455-8.
12. Baretto MS, Moraes RA, Rosa RA, et al. Vertical root fractures and dentin defects: effects of root canal preparation, filling and mechanical cycling. J Endod 2012;38:1135-9.
13. Yoldas O, Yilmaz S, Atakan G, et al. Dentinal microcrack formation during root canal preparations by different NiTi rotary instruments and the self-adjusting file. J Endod 2012;38:232-5.
14. Burklein S, Tsotsis P, Schafer E. Incidence of dentinal defects after root canal preparation: reciprocating versus rotary instrumentation. J Endod 2013;39:501-4.
15. Versiani MA, De-Deus G, Dummer PMH. A new system for classifying root canal morphology. Int Endod J 2017;50(8):761-70.
16. Shemesh H, Bier CA, Wu MK, et al. The effects of canal preparation and filling on the incidence of dentinal defects. Int Endod J 2009;42:208-13.
17. Medical Device Directive 93/42 EEC.
18. Quality guidelines for endodontic treatment: consensus report of European Society of Endodontology. Int Endod 2006; 39: 921-30.
19. Bier CAS, Shemesh H, Tanomaru-Filho M, Wesselink PR, Wu MK. The ability of different nickel-titanium rotary instrument to induce dentinal damage during canal preparation. J Endod 2009; 35: 236–8.
20. Liu R, Kaiwar A, Shemesh H, et al. Incidence of apical root cracks and apical dentinal detachments after canal preparation with hand and rotary files at different instrumentation length. J Endod 2013; 39: 129–32.
21. Hin ES, Wu MK, Wesselink PR, Shemesh H. Effect of Self-Adjusting file, Mtwo, and *ProTaper* on the root canal wall. J Endod 2013,39:262-64.
22. Adorno CG, Yoshioka T, Jindan P, et al. The effect of endodontic procedures on apical crack initiation and propagation ex vivo. Int Endod J 2013;46:763-8.

23. Adorno CG, Yoshioka T, Suda H. The effect of working length and root canal preparation technique on crack development in the apical root canal wall. Int Endod J 2010;43:321-7.
24. Ashwinkumar V, Krithikadatta J, Surendran S, Velmurugan N. Effect of reciprocating file motion on microcrack formation in root canals: an SEM study. Int Endod J 2013;47:622-7.
25. Souza EM, Calixto AM, Lima CN, Pappen FG, De-Deus G. Similar influence of stabilized alkaline and neutral sodium hypochlorite soliutions on the fracture resistance of root canal-treated bovine teeth. J Endod 2014;40:1600-3.
26. Pascon FM, Kantovitz KR, Sacramento PA, et al. Effect of sodium hypochlorite on dentine mechanical properties: A review. J Dent 2009;37:903-8.
27. Adorno CG, Yoshioka T, Suda H. Crack initiation on the apical root surface caused by three different nickel-titanium rotary files at different working lengths. J Endod 2011;37:522-5.
28. Topcuoglu HS, Tuncay O, Karatas E, Arslan H, Yeter K. In vitro fracture resistance of roots obturated with epoxy resin-based, mineral trioxide aggregate-based, and bioceramic root canal sealers. J Endod 2013;39:1630-1633.
29. Nauman M. Restorative procedures: effect on the mechanical integrity of root filled teeth. Endod Topics 2015;33:73-86.
30. Kishen A, Vedantam S. Hydromechanics in dentine: role of dentinal tubules and hydrostatic pressure on mechanical stress-strain distribution. Dent Mater 2007;23:1296-306.
31. Cicek E, Ali Aslan M, Akkocan O. Comparison of the resistance of teeth instrumented with different nickel-titanium systems to vertical root fracture: an in vitro study. J Endod 2015;41:1682-5.
32. Zarei M, Afkhami F, Malek Poor Z. Fracture resistance of human root dentin exposed to calcium hydroxide intervisit medication at various time periods: An in vitro study. Dent Traumatol. 2013;29:156-60.

33. Zicari F, Van Meerbeek B, Scotti R, Naert I. Effect of ferrule and post placement on fracture resistance of endodontically treated teeth after fatigue loading. J Dent 2013;41:207-15.
34. Genesh A, Venkateshbabu N, John A, Deenadhayalan G, Kandaswamy D. A comparative assessment of fracture resistance of endodontically treated and re-treated teeth: An in vitro study J Conserv Dent 2014;17(1):61-4.
35. Landys Boren D, Jonasson P, Kvist T. Long-term survival of endodontically treated teeth at a public dental specialist clinic. J Endod 2015;41:176-81.
36. Paradella TC, Bottino MA. Scanning Electron Microscopy in modern dentistry research. Braz Dent Sci 2012;15:43-48.
37. Yan W, Montoya C, Oilo M, Ossa A, et al. Reduction in fracture resistance of root with aging. J Endod 2017;43(9):1494-8.

PUBLICATIONS ON THE TOPIC OF DISERTATION

1. Eglė Nedzinskienė, Vytautė Pečiulienė, Rasmutė Manelienė, Saulius Drukteinis, Jolanta Aleksejūnienė, Audronė Jakaitienė. Potential to induce dentinal cracks during retreatment procedures of teeth with "Russian red": an ex vivo study. Medicina Journal. 2017;53:166-172.
2. Eglė Nedzinskienė, Vytautė Pečiulienė, Saulius Drukteinis. The appearance of root canal surfaces in teeth previously treated using resorcinol-formaldehyde resin: a preliminary SEM investigation. Stomatologija, Baltic Dental and Maxillofacial Journal. 2016;18:92-7.
3. Eglė Nedzinskienė, Vytautė Pečiulienė, Jolanta Aleksejūnienė. Resistance to fracture of roots treated with resorcinol formaldehyde resin: a comparative *in vitro* study. Laboratorinė medicina. 2017, t. 19, Nr. 4 (76), p. 234-239.

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to:

Prof. Dr. Vytautė Pečiulienė, DDs, PhD, MD, Head of Institute of Odontology Faculty of Medicine of Vilnius University, my scientific consultant and supervisor of my PhD thesis. I would like to thank her for sharing invaluable scientific knowledge and priceless experience. Her energy and wisdom inspired me. I will always treasure her time.

Assoc. Prof. Dr. Jolanta Aleksejūnienė, DDS, PhD, MD (The University of British Columbia) for passionate scientific discussions and help analyzing research results and preparing publications.

Assoc. Prof. Dr. Saulius Drukteinis, DDS, PhD, MD and Assoc. Prof. Dr. Rasmutė Manelienė, DDS, PhD, MD for their professional help and valuable constructive criticism.

Aušra Šaikuvienė, the chief of the consulting department of Vilnius University Hospital Zalgiris Clinic and Rūta Rastenienė, PhD, for help with collecting data and encouragement.

Also I would like to thank my colleagues for support, valuable advice and positivity.

To my parents and son for their warm support, patience and unconditional understanding.

CURRICULUM VITAE

Eglė Nedzinskienė was born in 1978, in Varėna.

Educational background:

- Varėna 2 nd secondary school, Lithuania (1985 – 1996).
- Faculty of Stomatology, Kaunas University of Medicine, Lithuania (1996 – 2001); Diploma – Medical Doctor in Stomatology.
- Faculty of Stomatology, Kaunas Universityy of Medicine, Lithuania (2001 – 2002); Internship Certificate – Dentist General Practitioner.
- Institute of Odontology, Faculty of Medicine, Vilnius University, Lithuania, postgraduate studies (2002 – 2005); Certificate – Specialist in Endodontology (Endodontist).

Occupations:

- 2005 to current date, endodontist at the Hospital of Vilnius University, Zalgiris Clinic.
- 2011 to current date, an assistant at the Institute of Odontology, Faculty of Medicine, Vilnius University (Field: Endodontology).

SANTRAUKA

Įvadas

Ilgą laiką Kinijoje, Indijoje, Prancūzijoje ir visose Rytų Europos šalyse šaknų kanalų gydymui buvo naudojama rezorcino formalino (RF) pasta (1, 2). Lietuvoje šis metodas buvo plačiai taikomas beveik iki 1994 metų. Todėl gydytojai odontologai iki šiol klinikinėje praktikoje sprendžia su šios medžiagos naudojimu susijusias endodontinio pergydymo problemas. Klinikinė patirtis leidžia teigti, kad rezorcino formalino pasta gydytų dantų pergydymas siejamas su sunkiai nuspėjama gydymo baigtimi. Iki šiol nebuvo atlikta tyrimų, nagrinėjančių gydymo procedūrų įtaką tokių dantų dentino paviršiui, jo mechaninėms savybėms. Tyrimų rezultatai rodo, kad endodontiškai gydytų dantų polinkis skilti yra didesnis (3).

Endodontinis (per)gydymas apima nemažai procedūrų, kurioms naudojamos skirtingos priemonės, pasižyminčios skirtingu poveikiu danties šaknies kanalo sienos paviršiui. Atliekant mechaninį šaknies kanalo ruošimą pirminio endodontinio gydymo metu, dentino paviršius pirmą kartą yra veikiamas tiesioginių mechaninių jėgų (vertikalaus instrumento spaudimo, trinties, sukuriamos tarp instrumento ir šaknies sienelių, ir kt.), o pergydymo atveju dantis patiria jau pakartotinį mechaninių jėgų, dažniausiai ir ultragarsinės vibracijos poveikį.

Per pastaruosius du dešimtmečius požiūris į šaknies kanalo mechaninio ruošimo principus pasikeitė iš esmės. Tai siejama su naujų lydinių, pavyzdžiui, nikelio ir titano (NiTi), įdiegimu į endodontinių instrumentų gamybą bei vis dažniau šaknų kanalų ruošimui naudojamais mašininiais instrumentais, kurie labai sutrumpino gydymo procedūros atlikimo trukmę, sumažino dažniausiai pasitaikančių komplikacijų skaičių (4, 5, 6). Technologijų pažanga gydytojams odontologams suteikia galimybę gydymui naudoti skirtingo tipo instrumentų derinius, o endodontiniam pergydymui daž-

niausiai naudojamas ultragarsinių ir mašininių instrumentų derinys. Kaip ir kiekviena nauja technologija, be minėtų pranašumų, ji yra siejama ir su tam tikrais trūkumais. Vienas iš jų yra šaknies dentino paviršiaus defektų didesnė tikimybė (4, 7, 8, 9). Dentino skilimų tikimybė atliekant endodontinio pergydymo procedūras yra dar vienas labai svarbus veiksnys, galintis turėti įtakos tokio tipo gydymo prognozei. Mikroskilimai, kanalo sienos paviršiaus netolygumai, turi įtakos danties atsparumo lūžiui mažėjimui (4, 10), o dėl funkcinio krūvio gali plisti ir sukelti vertikalų šaknies lūžį (11, 12, 13, 14).

Skilimai, be poveikio danties mechaniniam atsparumui, turi dar vieną svarbią reikšmę endodontinio gydymo baigčiai. Jie tampa bakterijų kolonijų sankaupų niša, apsaugota nuo gydomųjų veiksmų poveikio, o tai gali tapti tiesiogine gydymo nesėkmės priežastimi (11).

Rezorcino formalino pastos fizikinės savybės, jos poveikis danties audiniams ir dentino ultrastruktūrai nebuvo tirti ir todėl visiškai nežinomas gydymo naudojant šią medžiagą poveikis danties audiniams. Tik remiantis individualia klinikine gydytojų patirtimi yra teigiama, kad tokie dantys pasižymi didesniu trapumu. Šiuo tyrimu *in vitro* buvo siekiama ištirti atliekamų endodontinio pergydymo procedūrų įtaką dantims, gydytiems rezorcino formalino pasta.

Darbo tikslas – ištirti dantų, gydytų rezorcino formalino pasta, dentino paviršiaus ultrastruktūros ypatumus, naudojamų endodontijoje diagnostikos metodų patikimumą, endodontinio pergydymo procedūrų poveikį dentinui ir jo atsparumą skilimui *in vitro*.

Darbo uždaviniai

1. Įvertinti dentino skilimų skaičių, tipus, jų lokalizaciją po atliktų endodontinio pergydymo procedūrų.
2. Ištirti dažo medžiagos ir skirtingų mikroskopo didinimų efektyvumą vertinant rezorcino formalino pasta gydytų dantų skilimus.

3. Ištirti dantų, gydytų rezorcino formalino pasta ir turinčių gyvą pulpą, šaknies dentino atsparumą skilimui.
4. Įvertinti dantų gydytų rezorcino formalino pasta atsparumą skilimui po atlikto endodontinio pergydymo procedūrų, esant dentine mikropažaidų ir jų nesant.
5. Ištirti rezorcino formalino pasta gydytų dantų, turinčių skirtingą audinių spalvą, šaknų kanalų paviršių ir dentino ultrastruktūrą.

Medžiaga ir metodai

Tyrimui atlikti gautas VšĮ VUL Žalgirio klinikos etikos komiteto leidimas Nr. EK-2.

Endodontinio pergydymo procedūrų poveikio šaknies dentino paviršiui tyrimui buvo įtraukta rezorcino formalino pasta gydytų 80 pašalintų pirmųjų apatinio žandikaulio krūminių dantų susiformavusiomis šaknų viršūnėmis, kurių paviršiuje nebuvo matoma jokių skilimo požymių. Suformuotos dvi grupės, remiantis kanalo užpildo medžiagos kietumu ir tyrėjo gebėjimu praeiti šaknies mėginio kanalo spindį. Vertinta ir lyginta šaknies dentino skilimų skaičius ir tipai šaknies vainikinio ir viršūninio skerspjūvio paviršiuose, dažymo metileno mėlynuoju ir naudojimo skirtingo didinimo mikroskopą apžiūrai nauda skilimų diagnostikai.

Šaknies dentino atsparumo skilimui tyrimui buvo atrinkta 120 išrautų apatinio žandikaulio pirmųjų krūminių dantų susiformavusiomis šaknų viršūnėmis, kurių šaknų kanalai anksčiau pildyti minkštos konsistencijos rezorcino formalino pasta ir 40 apatinio žandikaulio pirmųjų krūminių dantų, turinčių susiformavusias šaknų viršūnes ir gyvybingą pulpą. Buvo suformuotos keturios mėginių grupės. Vertinta RF pastos ir endododontinio pergydymo (nesant ir atsiradus mikroskilimams jo metu) įtaka šaknies dentino atsparumo skilimui ir lyginta tarp eksperimentinių grupių ir su kontroline grupe – gyvybingą pulpą turinčių dantų.

Šaknų kanalų paviršiaus tyrimui SEM naudota 20 išrautų apatinio žandikaulio pirmų krūminių susiformavusiomis šaknų viršūnėmis dantų, kurių šaknų kanalai buvo pildyti rezorcino formalino pasta. Buvo sudarytos dvi mėginių grupės, priklausomai nuo danties audinių spalvos (1 grupė – būdingi šiai šaknų kanalų pildymo metodikai audinių spalvos pokyčiai – nuo rausvos iki tamsiai rudai raudonos spalvos, 2 grupė – turinčių natūralaus dentino audinių spalvą). Mikronuotraukos buvo atliktos x200, x1000 ir x2000 didinimais, vainikiniame, viduriniame ir viršūniniame šaknų kanalų paviršių trečdaliuose ir palyginta tarp grupių.

Rezultatai

Palyginus bendrą dentino skilimų skaičių po endodontinio pergydymo atlikto *ProTaper* NiTi mašininiais bei *ProTaper* NiTi mašininių ir ultragarsinių instrumentų deriniu, naudojant 10 ir 16 kartų didinimą ir dažo medžiagą ar jos nenaudojant, abiejose grupėse statistiškai reikšmingai daugiau skilimų buvo nustatyta vainikinio šaknų mėginių trečdalio skerspjūvių paviršiuose.

Identifikuotų skilimų skaičius buvo didesnis 1 mėginių grupėje naudojant dažo medžiagą, o 2 grupėje statistiškai reikšmingai daugiau skilimų buvo rasta nenaudojant dažo medžiagos.

Šaknų viršūninių trečdalių paviršiuose tiek naudojant dažo medžiagą tiek jos nenaudojant išliko tokia pati tendencija kaip ir vainikinių trečdalių paviršiuose – skilimų diagnozuota daugiau naudojant instrumentų derinį, bet skirtumas nebuvo statistiškai reikšmingas.

Atlikus skirtingų dentino skilimų tipų radimo dažnio palyginimą tarp 1 ir 2 mėginių grupių, naudojant 10 ir 16 kartų didinimą be dažo medžiagos ir su dažo medžiaga, statistiškai reikšmingai daugiau visiškų ir vidinių dentino skilimų rasta *ProTaper* ir ultragarsinių instrumentų derinio grupės mėginiuose. Statistiškai reikšmingai daugiau dalinių skilimų nuo išorinio šaknies paviršiaus rasta taip pat 2 mėginių grupėje, abiejose grupėse jų daugiau nustatyta naudojant dažo medžiagą, bet skirtumai nebuvo statistiškai reikšmingi.

Palyginus keturias tiriamąsias grupes, didžiausias šaknų mėginių dentino atsparumas skilimui buvo gyvybingą pulpą turinčių dantų (C1 grupėje, neigiama kontrolė), o mažiausias nustatytas grupėje, kurioje po pergydymo buvo rasta dentino skilimų (E3 grupėje). C1 grupėje atsparumo skilimui vidurkis buvo 227,5±29,6 N/mm^2, E1 grupėje – 174,7±41,5 N/mm^2, E2 grupėje – 169,7±31,5 4/mm^2 ir E3 grupėje – 150,5±11,4 N/mm^2.

Rudai raudonos spalvos šaknų skenuotų SEM mėginių kanalo paviršiuje dentino morfologija buvo įprasta ir buvo aiškiai matomi atviri dentino kanalėliai, o vertinant natūralaus dentino spalvos šaknų kanalų paviršius, dentino vaizdas buvo neįprastas. Visų mėginių vainikiniame šaknies kanalo trečdalyje buvo matomi pavieniai atviri dentino kanalėliai, o viduriniame ir viršūniniame kanalo trečdaliuose vaizdas buvo labai panašus į sklerozinio dentino

Visuose abiejų eksperimentinių grupių šaknų kanalų trečdaliuose buvo matomi daugybiniai šaknies dentino defektai, tačiau vizualiai gerokai daugiau defektų buvo matoma rudai raudonų šaknų mėginiuose negu natūralios dentino spalvos šaknų mėginiuose.

SEM vertinimas parodė rezorcino formalino pastos gerą sukibimą su šaknies kanalo dentinu. Visuose rudai raudonos spalvos šaknų mėginių kanalų trečdaliuose matomi daugybiniai vientisi ir gana polimerizuoti rezorcino formalino pastos „siūlai“ (angl. *tag*s), giliai įsiskverbiantys į dentino kanalėlius.

Išvados

1. Endodontiniam pergydymui naudojami mašininiai nikelio ir titano lydinio instrumentai bei jų ir ultragarsinių instrumentų derinys turi žalingą poveikį šaknies dentinui. Abu metodai sukelia daugiau skilimų šaknies vainikiniame trečdalyje. Naudojant instrumentų derinius, šaknyje susidaro daugiau skilimų, tarp kurių gerokai dažnesni yra visiški ir vidiniai dentino skilimai.

2. Rezorcino formalinu gydytų dantų dentino skilimai identifikuojami efektyviau, jei naudojama ir dažo medžiaga, ir apžiūra mikroskopu.
3. Rezorcino formalino pasta gydytų dantų šaknys mažiau atsparios skilimui nei gyvybingą pulpą turinčių dantų.
4. Rezorcino formalino pasta gydytų dantų endodontinis pergydymas sumažina atsparumą šaknies dentino skilimui, padidindamas vertikalaus skilimo riziką. Pergydant šaknis atsiradę dentino skilimai labai sumažina jo atsparumą skilimui.
5. Rezorcino formalino pasta ne tik sukelia skirtingus danties audinių spalvos pokyčius, bet ir turi skirtingą poveikį dentino kanalėliams. Pasikeitusios spalvos dantų šaknų dentino struktūra tipiška, o spalvos pokyčių neturinčių dantų šaknų dentino kanalėliai yra visiškai sklerozavę. Manoma, šios medžiagos sukelti cheminiai dentino pokyčiai turi įtakos ir pasikeitusios, ir nepakeitusios spalvos dantų šaknies dentino skilimams. Rezorcino formalino pasta pasižymi homogeniškumu ir skvarbumu į dentino kanalėlius.