STUDIA UBB CHEMIA, LX, 2, Tom I, 2015 (p. 59-70)
(RECOMMENDED CITATION)

COMPARATIVE EVALUATION OF THE APICAL SEALING ABILITY OF FOUR DENTAL MATERIALS USED IN ENDODONTIC SURGERY – AN *IN VITRO* STUDY

ANDREEA IULIANA GULIE (căs. KUI)[a,*], GHEORGHE ZSOLT NICULA[b], CODRUTA POPESCU[c], EUGEN MIRONESCU[d], MANDRA BADEA[e]

ABSTRACT. The aim of this in vitro study was to assess dye microleakage and sealing ability of four dental materials: a polycarboxilate cement (Adhesor Carbofine® - Spofa Dental), a glass ionomer cement (Kavitan Plus®- Spofa Dental), a composite resin (Core-It®- SpiDent) and a MTA based cement (MTA Fillapex®- Angelus). Forty, extracted, human teeth with single root canals were selected for this study. The teeth were randomly divided into four study groups and one control group. The root canals were instrumented and filled with gutta-percha and sealer. Root-ends were resected and 3 mm deep cavities were prepared. Root-end cavities were filled, each with a type of material. Methylene blue dye was used for determination of dye leakage. Afterwards, Scanning Electron Microscopy was used to evaluate the sealing ability of each material. Kolgorow-smirnow z test was used to determine the type of data distribution. One-way analysis of variance (ANOVA) followed by a Tukey test were used to determine the statistical difference between groups, with $P < 0.05$ set as significant. All the four sealers produced apical leakage to a certain extent and there was no statistically significant difference between the five experimental groups. For SEM evaluation, the results showed that there is a statistically significant difference between the control group and the Adhesor Carbofine group. MTA based cement provides leakage results comparable to other commonly used root-end filling materials.

Keywords: *Electronic microscopy, Composite cements, Cement paste, MTA*

[a] *University of Medicine and Pharmacy "Iuliu Hatieganu", Faculty of Dentistry, Department of Prosthodontics, Cluj-Napoca, Romania*
[b] *University of Medicine and Pharmacy "Iuliu Hatieganu", Faculty of Medicine, Dept. of Cell and Molecular Biology, Pasteur St. No. 6, Cluj-Napoca, Romania*
[c] *University of Medicine and Pharmacy "Iuliu Hatieganu", Faculty of Medicine, Department of Socio-Humanistic Sciences – History of Medicine, Cluj-Napoca, Romania*
[d] *University of Medicine and Pharmacy "Iuliu Hatieganu", Faculty of Medicine, Dept. of Cell and Molecular Biology, Pasteur St. No. 6, Cluj-Napoca, Romania*
[e] *University of Medicine and Pharmacy "Iuliu Hatieganu", Faculty of Dentistry, Department of Dental Prevention, Cluj-Napoca, Romania*
* *Corresponding author: guile.andreea@umfcluj.ro*

INTRODUCTION

In endodontic therapy, non-surgical treatments and re-treatments are indicated and are considered a first option in the presence of persistent apical periodontitis. When optimal results cannot be achieved by a non-surgical orthograde re-treatment, then endodontic surgery is indicated. [1]

Apicoectomy (apicectomy/root-end resection) with retrograde obturation is a widely applied procedure in endodontics, when all efforts for the successful completion of orthograde endodontic therapy have failed. The main purpose of endodontic treatment is to eliminate micro-organisms from the root canal systems and prevent its re-infection. [2] In vitro studies suggest that a root-end filling is essential to prevent leakage from root canal space and dentinal tubes.

Placing a root-end filing material during periapical surgery should guarantee the complete sealing of the root canal. A correct apical sealing avoids recontamination and leads to a reduction of microorganisms, therefore to a successful treatment. [3]

Several materials have been indicated as root-end filling materials, though each of these materials has its own limitations. Different materials have been used in this direction, such as amalgams, ZOE cements, glass ionomer cements, composites. [5]

Zinc polycarboxylate cement consists of a powder which contains zinc-oxide, magnesium oxide, bismuth, aluminium oxides and stannous fluoride. The bond strength to enamel is greater than to dentin. The sealing ability of policarboxylate cement, using dye penetration methods is inferior to amalgam. [6]

Glass ionomer cement was introduced in the early 1970's as a new restorative material. The sealing ability of light-curing glass ionomer cements was significantly better than amalgam and also slightly better than conventional glass ionomer cements. [6]

The use of composite resin in addition to bonding agents is likely to produce a leak-resistant seal. There are studies showing an excellent long term success of composites along with dentin bonding agent, but presence of a dry field during placement is important. In addition, some components found in conventional composite resins, like inorganic fillers and silane coupling agent may be the reason why the materials presents anti-bacterial effects against bacteries like *P. gingivalis, P. intermedia, E. foecalis and P. endodontalis*. [6]

Mineral Trioxide Aggregate (MTA) has shown excellent seal and hard tissue repair compared with other root-end filling materials. Its main advantages are osteogenic and regenerative potential, biocompatibility and also anti-bacterial properties against *E. foecalis, S.aureus, P. aeruginosa,* especially when used after mixing with 0, 12% clorhexidine. [6]

It is a real challenge to design and test in laboratory a reliable experimental procedure that can be easily repeated and be clearly in explaining the results. Therefore, as an attempt to solve this problem, different methods, such as dye bacterial leakage or microscopy analysis, were suggested in order to assess the sealing ability of different root-end filling materials. [3] Our study aims a comparative evaluation of dye leakage and apical sealing ability for a polycarboxilate cement (Adhesor Carbofine® - Spofa Dental), a glass ionomer cement (Kavitan Plus® - Spofa Dental), a composite resin (Core-It® - SpiDent) and a MTA based cement (MTA Fillapex® - Angelus).

RESULTS AND DISCUSSION

For the dye-leakage evaluation, linear dye penetration was measured independently by two observers at two different times under same conditions; the mean value of the recorded measurements was chosen as the extent of dye penetration into each specimen. For each image, two measures were made, annotated with “Dim L” and “Dim R”; the annotation reveals the side of the root-canal where the measures were made, respectively left and right. All the four sealers produced apical leakage to a certain extent. The teeth in the control group showed maximum penetration. There was no statistically significant difference between groups (Dim.R) regarding apical leakage as determined by one-way ANOVA ($F(4, 40) = 2.009$, $p = .115$). Also, there was no statistically significant difference between groups (Dim.L) regarding apical leakage as determined by one-way ANOVA ($F(4, 40) = 1.58$, $p = .201$). (Table 1)

For the SEM evaluation the measures were made on the calibrated images obtained. The measures were made by two observers at two different times under same conditions; the mean value of the recorded measurements was chosen as the extent of the gap size for each specimen. There were chosen four points to measurement for each root, two different points for each side (left and right) of the root-canal filling (first point at the bottom and the second point at the top of the root-end filling). (Figure 1)

Table 1. Results of micro-leakage assessment of the five experimental groups

	Group	Dye penetration length Mean (µm) ±SD	Nr. Teeth	F	p	p1	p2	p3	p4	p5
Dim.R	CG	1.58±0.77	8			-	.579	.885	.983	.921
	PC	1.07±0.96	8			.579	-	.136	.877	.166
	GI	1.37±0.46	8	2.009	.115	.885	.136	-	.591	1.00
	MTA	1,32±0.46	8			.983	.877	.591	-	.653
	CR	1.94±0.82	8			.921	.166	1.00	.653	-

	Group	Dye penetration length Mean (μm) ±SD	Nr. teeth	F	p	p1	p2	p3	p4	p5
Dim.L	CG	1.52±0.77	8			-	.730	.993	.980	.771
	PC	1.07±0.96	8			.730	-	.924	.960	.138
	GI	1.37±0.46	8	1.58	.201	.993	.924	-	1.00	.516
	MTA	1.32±0.46	8			.980	.960	1.00	-	.431
	CR	1.94±0.82	8			.771	.138	.516	.431	-

Dim.R and Dim.L – the places where the measures were made for the apical leakage test, SD – standard deviation; CG – control group, PC – polycarboxylate cement, GI – glass ionomer cement, MTA – MTA based cement, CR – composite resin; F and P – values by one-way ANOVA; p1 – compared with CG; p2 – compared with PC; p3 – compared with GI; p4 – compared with MTA; p5 – compared with CR

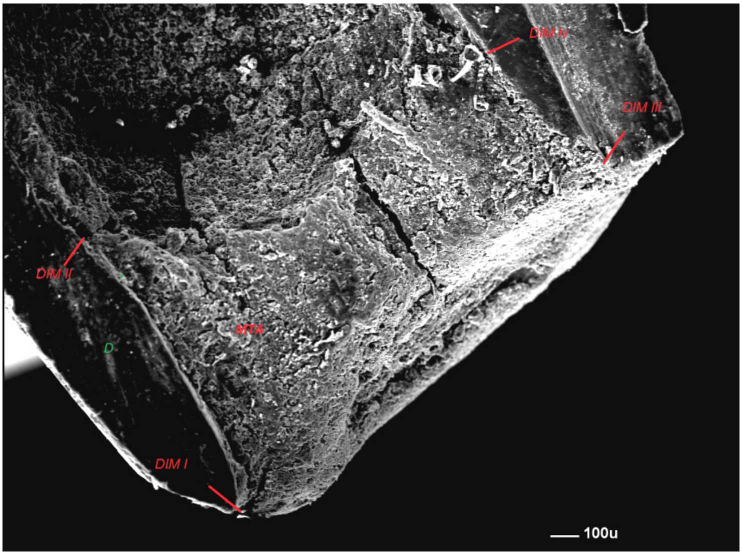

Figure 1. - SEM image in sagittal view, showing retrofilling material/dentin interface. D - dentin, MTA – MTA based cement; DIM. I to IV – the points where the measures were made for each sample SEM – Scanning Electron Microscopy (magnification 45x)

There was a statistically significant difference between groups as determined by one-way ANOVA ($F (4, 40) = 3.28$, $p = .015$). A Tukey post-hoc test revealed that dim.I was statistically significantly lower in CG (13.45±5.99μm, p= .006) comparing to PC group (51.90±25.41μm), as well as the dim.II was statistically significantly lower in CG (9.75±4.27μm, p= .020) comparing to PC group (41.86±24.53μm). A Tukey post-hoc test revealed that dim.III was statistically significantly lower in CG (9.99±3.98μm, p= .041) comparing to PC group (39.02±29.23μm). Also dim. IV was statistically significantly lower in CG (7.33±5.31μm, p= .019) comparing to PC group (47.98±41.11μm).

There was also a statistically significant difference in CR group (9.23±6.61μm, p=.028) comparing to PC group (47.98±41.11μm) regarding just dim.IV. There were no statistically significant differences between GI and MTA groups (p = .538) and between PC and MTA groups (p=.466). (Table 2)

Table 2. Results of SEM evaluation of sealing ability for the five experimental groups

	Group	The gap size Mean(μm)±SD	Nr. teeth	F	P	p1	p2	p3	p4	p5
Dim.I	CG	13.45±5.99	8	4.61	.004	-	.006	.096	.653	.979
	PC	51.90±25.41	8			.006	-	.773	.146	.024
	GI	39.97±31.73	8			.096	.773	-	.742	.279
	MTA	27.45±18.94	8			.653	.146	.742	-	.928
	CR	19.21±8.25	8			.979	.024	.279	.928	-
		The gap size Mean(μm)±SD	**Nr. teeth**	**F**	**P**	**p1**	**p2**	**p3**	**p4**	**p5**
Dim.II	CG	9.75±4.27	8	4.76	.004	-	.020	.057	.603	1.00
	PC	41.86±24.53	8			.020	-	.993	.388	.017
	GI	37.63±23.86	8			.057	.993	-	.647	.048
	MTA	24.04±26.97	8			.603	.388	.647	-	.559
	CR	9.06±5.90	8			1.00	.017	.048	.559	-
		The gap size Mean(μm)±SD	**Nr. teeth**	**F**	**P**	**p1**	**p2**	**p3**	**p4**	**p5**
Dim.III	CG	9.99±3.98	8	3.36	.020	-	.041	.052	.858	.859
	PC	39.02±29.23	8			.041	-	1.00	.302	.302
	GI	38.08±28.72	8			.052	1.00	-	.350	.350
	MTA	19.70±12.52	8			.858	.302	.350	-	1.00
	CR	19.70±8.58	8			.859	.302	.350	1.00	-
		The gap size Mean(μm)±SD	**Nr. teeth**	**F**	**P**	**p1**	**p2**	**p3**	**p4**	**p5**
Dim.IV	CG	7.33±5.31	8	3.28	.015	-	.019	.433	.503	1.00
	PC	47.98±41.11	8			.019	-	.538	.466	.028
	GI	28.73±17.02	8			.433	.538	-	1.00	.525
	MTA	27.28±32.09	8			.503	.466	1.00	-	.598
	CR	9.23±6.61	8			1.00	.028	.525	.598	-

Dim. I to IV – the points where the measures were made for each sample; SD – standard deviation; CG – control group, PC – polycarboxylate cement, GI – glass ionomer cement, MTA – MTA based cement, CR – composite resin; F and P – values by one-way ANOVA; p1 – compared with CG; p2 – compared with PC; p3 – compared with GI; p4 – compared with MTA; p5 – compared with CR

The present study compared the sealing ability of four root-end filling materials (Adhesor Carbofine®, Kavitan Plus®, MTA Fillapex® and Core-It®). This research compared these four different materials under the same conditions, which represents a novelty for the experimental studies regarding root-end fillings. Also, the materials were compared using two methods of evaluation in order to determine more accurate results.

For the evaluation of apical leakage, the results failed to demonstrate any significant difference between the four root-end fillings materials used. This method, using dye penetration, has been chosen in order to assess microleakage. We used methylene blue because it is not expensive, has a high degree of staining and has a lower molecular weight than bacterial toxins. The limitation of dye leakage studies is that they measure the degree of leakage in only one dimension, which makes it impossible to evaluate the total amount of leakage. [14, 15, 16, 17]

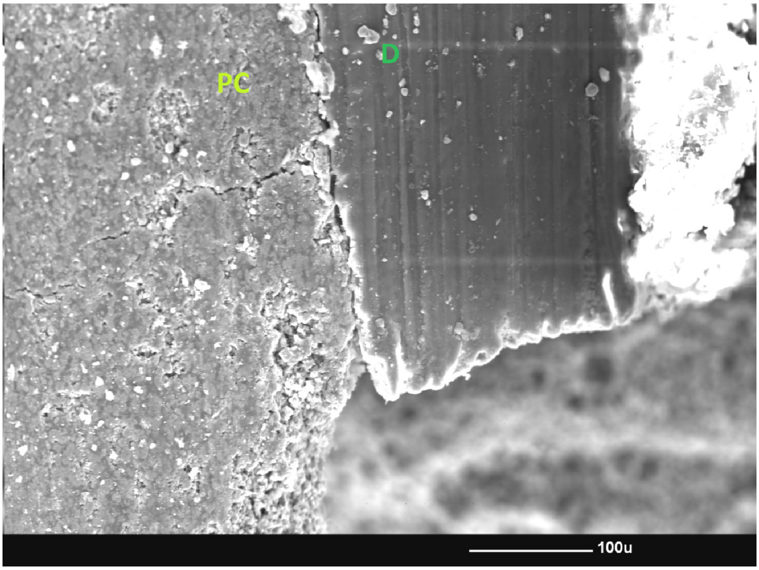

Figure 2. - SEM image in sagittal view, showing retrofilling material/dentin interface. D- dentin, PC – polycarboxylate cement; SEM – Scanning Electron Microscopy (magnification 200x)

For Scanning Electron Microscopy (SEM) evaluation the results demonstrate a significant difference in sealing abilities between the control group and the first group (Adhesor Carbofine®). Polycarboxylate cement has not a specific indication when used as a root-end filling material, studies showing that it leaks at level significantly greater than amalgam or gutta-percha.

Significant differences were found between the control group and poycarboxylate cement, meaning that policarboxilate cement showed a lower sealing ability than the sealer used in control group. (Image 2) [18]

In our study, MTA Fillapex® was used as a MTA based cement. This material was developed as a paste/paste sealer in a formulation that allows its appropriate insertion into the root canal. [22] MTA contains tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide and other mineral oxides forming a hydrophilic powder which sets in presence of water. Several studies have indicated that MTA exhibits significantly lesser leakage than other materials. [5, 18, 20, 21] The results found in this research showed a good sealing ability for MTA Fillapex, but not significantly different when compared with the other materials studied. (Image 3)

Figure 3. - SEM image in sagittal view, showing retrofilling material/dentin interface. D- dentin, MTA – MTA based cement; SEM – Scanning Electron Microscopy (magnification 45x)

Glass ionomer cements can be used for repairing perforated root canals or as retrograde root fillings. [19] Sealing ability of glass ionomer cements was adversely affected when the root end cavities were contaminated with moisture at the time of placement of cement. [18] Although in our study the root end cavities were completely dry before applying the glass ionomer cement, the sealing ability of Kavitan Plus® was poor. (Image 4) In our study, there was no statistically significant difference between the sealing ability of glass ionomer cement and the other three materials used.

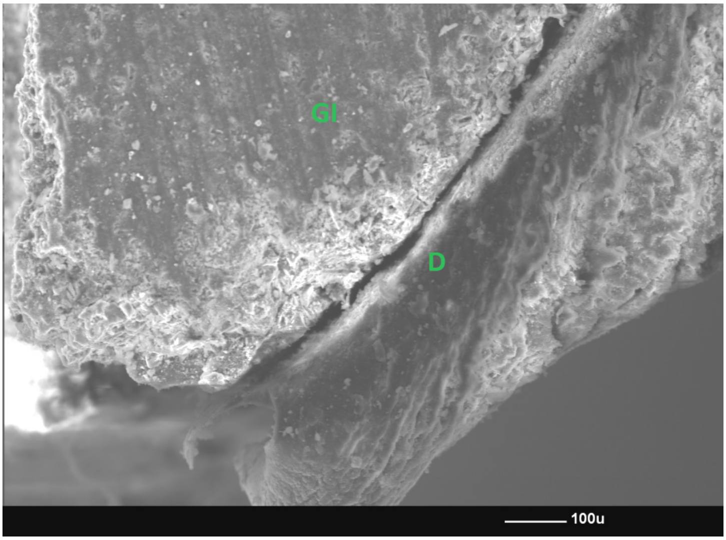

Figure 4. - SEM image in sagittal view, showing retro-filling material/dentin interface. D- dentin, GI– glass ionomer cement; SEM – Scanning Electron Microscopy (magnification 100X)

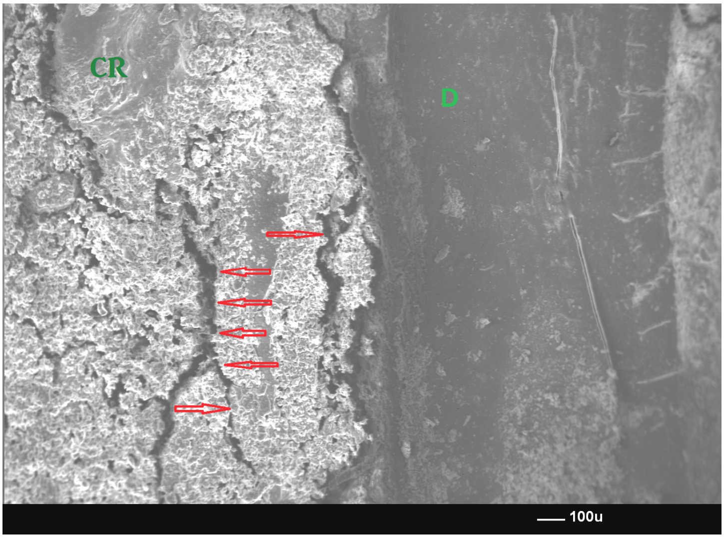

Figure 5. - SEM image in sagittal view, showing retrofilling material/dentin interface. D- dentin, CR – composite resin; red arrows showing the cracks in the material; SEM – Scanning Electron Microscopy (magnification 45X)

The composite resin has a good sealing ability, which was proven also in this study. In clinics, the composite resin presents difficulties in endodontic surgery, because the presence of a dry field during placement is mandatory. [6,18] In our study, composite resin (Core-It®) showed a good sealing ability, in association with a bonding agent, but the SEM images revealed cracks in the material. (Image 5)

CONCLUSIONS

Within the limitations of this study the results in the present study revealed no difference in microleakage between all four materials used as root-end fillers.

Scanning Electron Microscopy (SEM) evaluation showed a significant difference between the sealing ability of polycarboxylate cement versus the control group. No statistically significant difference was observed between the four materials used in this experimental study. However, further *in vitro* and *in vivo* investigations should be conducted to assess the sealing ability of each material used in this study.

EXPERIMENTAL SECTION

Forty, extracted, human teeth with single, straight root canals were selected for this *in vitro* experimental study. The teeth were extracted for different periodontal problems and stored in distilled water and thymol (0, 2%) until use. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Hațieganu", Cluj Napoca (Protocol No. 805/ 25.06.2013).

Teeth surfaces were scaled in order to remove calculus and were immersed in NaOCl 5,25% in order to remove organic tissue. After 1 h of immersion in NaOCl, an orifice was created inside each tooth's crown, as an access for the root canal, using a high speed hand piece under continuous water spray.

A #15 K-file (Dentsply®) was used to establish the working length for each root canal. Crown-down root canal preparation was performed using a 0.06 ProTaper (Dentsply®) rotary instruments to the size of #40. During mechanical preparation, 5ml of 5,25% NaOCl was used for irrigation and at the end of the chemo-mechanical preparation, as the final flush, 5 ml of sterile saline were used. Root canals were dried with paper points (Gapadent®) and obturated with gutta-percha (Gapadent®) and sealer AH26 sealer (Dentsply, DeTrey, Konstanz, Germany) by the lateral condensation method. IRM cement was used to fill the coronal cavities. Afterwards, the sealer set completely for 24h.

From each root, a 3mm apical region was removed perpendicular to the long axis of root, under continuous water irrigation. The teeth were then divided into five groups, first group being the control group. In the control group we included 8 teeth with the apicoectomy made and for the other 4 groups of teeth, using a 008 diamond root bur; the root-end cavities were prepared, to a depth of 3 mm. For the second group the root-end cavity was filled with Adhesor Carbofine®, for the third group the cavities were filled with Kavitan Plus®, for the teeth in the forth group it was used MTA Fillapex® and for the fifth group a composite resin, Core-It® it was used, after applying etching gel and a bonding. For the last group, the material was immediately light cured for 40 seconds. All materials were prepared according to the manufacturer's directions. After the set of the materials, all specimens were stored in distilled water at 37^0C at 100% humidity for 72h.

Study A: Evaluation of apical leakage

All the external surfaces were coated with two layers of nail polish except the sectioned apical region. [8] All the teeth were afterwards immersed in methylene blue 1% for 24h. The samples were then taken out of the methylene blue and sectioned longitudinally by grooving the roots with a diamond disk in the bucco-lingual direction and splitting them with a chisel. All sections were photographed under a light microscope at 10X magnification using a digital camera and the images were analyzed using Cell D (Olympus) program. Afterwards, for each sample it was measured the length of dye penetration. Linear dye penetration was measured independently by two observers at two different times under same conditions; the mean value of the recorded measurements was chosen as the extent of dye penetration into each specimen. [9]

Study B: SEM evaluation

The sections obtained from the teeth were immersed in 6 mol LHCl for 30s, for acid dissolution (inorganic part), and 1% NaOCl for 30 min (organic part) and dried for 24h. Afterwards, the samples were mounted on aluminium stubs, gold sputtered in a Polaron E-5100 plasma-magnetron sputter coater (Polaron Equipment Ltd., Watford, Hertfordshire, UK) in argon atmosphere [10] of about 20 nm and then examined under Scanning Electron Microscopy (SEM) (*Jeol JSM 25S - Jeol, Japan*) at different magnifications (45X, 70x, 100x, 200x, 300x and 700x) for adaptation of each root-end material into the canal walls and the findings were measured, for each sample choosing 4 points to measure. All the images were captured using image processor Deben Pixie–3000 (Deben UK Ltd., Debenham, Suffolk, UK) [11] and then calibrated for measuring using a Cell^*D* software (Olympus Imaging Software Solutions, Germany) [12] Sagital examination was performed, as the roots were sectioned longitudinaly. The Image tool programe includes functions as dimensional (distance, angle, perimeter, area) and gray scale measurement. The gap size was measured at four points in the longitudinal section. [13]

Statistical analysis was performed by SPSS software package, Version 21.0 for Windows (SPSS Inc. Chicago, IL, USA). Quantitative values are presented as mean ± standard deviation (SD). Kolgorow-smirnow z test was used to determine the type of data distribution. One-way analysis of variance (ANOVA) followed by a Tukey test were used to determine the statistical difference between groups, with $P < 0.05$ set as significant.

ACKNOWLEDGMENTS

This research was funded by POSDRU grant no.159/1.5/S/138776 grant with title: “Model colaborativ institutional pentru translatarea cercetarii stiintifice biomedicale in practica clinica– TRANSCENT”.

REFERENCES

1. Ashraf H., Faramarzi F., Paymanpour P., *Iranian Endodontic Journal*, **2013**, *8*, 177.
2. Moradi S., Ghoddusi J., Forghani M., *Journal of Endodontics*, **2009**, *31*, 1563.
3. Kazem M., Mahjour F., Dianat O., Fallahi S., Jahankhah M., Root-end filling with cement-based materials: *Dental Research Journal* (*Isfahan*), **2013**, *10*, 46.
4. Friedman S., *Journal of Endodontics*, **2011**, *37*, 577.
5. Torabinejad M., Ford P., Rooi T.R., *Endodontics and Dental Traumatology*, **1996**, *12*, 161.
6. Priyanka S.R., *Journal of Medical and Dental Sciences*, **2013**, *9*, 20.
7. Castelucci A., Endodontics, Second edition. Il Tridente, **2003**, 548.
8. Kumar N.S., Palanivelu A., Narayanan L.L., *Journal of Conservative Dentistry*, **2013**, *16*, 449.
9. Shahi S., Yavari H.R., Rahimi S., Eskandarinezhad M., Shakouei S., Unchi M., *Journal of Oral Sciences*, **2011**, *53,* 517.
10. G. Nicula, St. Balici, A. Florea, E. Mironescu, R. Munteanu, P. Murea, Gh. Benga, *Annals of the Romanian Society for Cell Biology*, **2010**, *15,* 22.
11. Flegler S.L., Heckman J.W., Komparens K.L., Scanning and transmission electron microscopy: an introduction, W.H. Freeman and Comp., New York, **1993**, 151.
12. Dudea D., Florea A., Mihu C., Câmpeanu R., Nicola C., Benga Gh., *Romanian Journal of Morphology and Embryology,* **2009**, *50*, 435.
13. Khalid, Al-Garawi Z., Al-Hezaimi K., Javed F., Al-Shalan T., Rotstein I., *International Journal of Oral Sciences*, **2012**, 202.

14. Ahlberg K.M., Assavanop P., Tay W.M., *International Endodontic Journal*, **1995**, *28*, 30.
15. Tamse A., Katz A., Kablan F., *International Endodontic Journal*, **1998**, *31*, 333.
16. Wu M.K., Wesselink P.R., *International Endodontic Journal*, **1993**, *26*, 37.
17. Ozata F., Erdilek N., Tezel H., *International Endodontic Journal*, **1993**, *26*, 241.
18. Vasudev S.K., Goel B.R. TS, ABSTRACT. *Endodontology*. **2003**, *15,* 12.
19. Kamel F.M., *Saudi Dental Journal*, **1994**, *6*, 107.
20. Torabinejad M., Higa R.K., McKendry D.J., Pitt Ford T.R., *Journal of Endodontics*, **1994**, *20*, 159.
21. Tang H.M., Torabinejad M., Kettering J.D., *Journal of Endodontics*, **2002**, *28*, 5.
22. Yoshino P., Nishiyama C.K., Modena K.C.D.S., Santos C.F., Sipert C.R., *Brazilian Dental Journal*, **2013**, *24,* 111.