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Marginal microleakage of newly synthesized nanostructured materials based on calcium aluminate after application in interradicular perforations

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SUMMARY

Introduction Marginal seal or adequate marginal adaptation of material along the cavity walls should be able to prevent leakage of tissue fluid and consequently bacterial microleakage, terefore, it is considered as significant factor for long term success of endodontic treatment. The aim of this work was to test the marginal microleakage of newly synthesized nanostructured biomaterials based on calcium aluminate, calcium silicate and MTA with a dye penetration test after application the material to the interradicular perforations of extracted teeth.

Material and method The study included 48 extracted human maxillary and mandibular molars. Newly synthesized nanostructured materials were tested: material based on calcium aluminate, calcium silicate. Commercial calcium silicate cement (MTA Angelus, Londrina, Brazil) was used as a control material. Marginal microleakage was examined with a dye penetration test six months after application of the material in experimentally prepared interradicular perforations on extracted human molars. Penetration depth measurement was analyzed with a binocular magnifier (Leica DM 500, Leica Byosistems). Results are expressed in millimeters and statistically processed by applying the analysis of variance for repeated measurements with the Sidak test.

Results The lowest average penetration (mm) was for MTA (1.40 ± 0.63 mm), and the highest for Ca aluminates (2.10 ± 0.63 mm), while for calcium silicates an average color penetration of 1.73 ± 0, 67mm was recorded. By testing intergroup differences in color penetration between groups, a statistically significant difference between MTA and Ca aluminate was obtained using Sidak's test ($t = -0.693$; $p = 0.036$). No statistically significant difference was recorded between MTA and Ca silicate, nor was there a statistically significant difference between Ca aluminate and Ca silicate.

Conclusion The lowest marginal microleakage, the best marginal sealing, was recorded with the MTA material. The microleakage of calcium aluminate-based materials was significantly higher compared to calcium silicate and MTA.

Keywords: marginal micropermeability; calcium aluminates; calcium silicates

INTRODUCTION

Perforation means mechanical or pathological communication between the root canal system and the outer surface of the tooth caused by caries, resorption or iatrogenic factors [1]. According to localization, root perforations can be interradicular, lateral and apical. Untreated perforations cause inflammation, resorption of bone, dentin and cementum. This is especially pronounced in perforations in the furcation of multi-rooted teeth. The main goal in the treatment of perforations is to prevent inflammatory process and consequent loss of tissue attachment at the site of the perforation, i.e. the establishment of a new tissue attachment, if the lesion already exists [2].

Many materials have been used in the therapy of root canal perforations: zinc oxide-eugenol, amalgam, calcium hydroxide, glas ionomer, cavity, gutta-percha, composite materials, hydroxyapatite, calcium phosphate cements, tricalcium phosphate cements, IRM (Intermediate Restorative Material), glas ionomer cements, Portland cement, MTA. But none of them completely satisfy all the requirements [3, 4].

The properties that an ideal material for closing perforations should have are: biocompatibility, short bonding time, ability to have a good edge closure, as well as an appropriate antibacterial effect, that it is insoluble in tissue fluids and that it has adequate mechanical properties. The material of choice in the treatment of root perforations is MTA, presented as early as 1993 by Loma Linda University [5, 6].

MTA has good physical, chemical and biological characteristics, and one of its indications is root canal

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perforations. Due to its antimicrobial effect and very high pH, it enables the regeneration of periodontal ligaments around the site of injury, perforation. In their study, Main et al. concluded that MTA provides effective marginal closure in root canal perforations. Placement of the material in an appropriate period of time can prevent bacterial contamination from the pulp chamber into the periodontal tissue [7]. However, the time it takes to bind MTA is 2 hours and 30 minutes. The ability to seal the edge of MTA in an aqueous environment may be compromised during the first 72 hours due to the solubility of the material. Mohan et al. claimed that it takes 3 days for MTAs to show good marginal closure.

Contemporary research is focused on the synthesis and testing of the possible application of nanostructured biomaterials in endodontic indications. In recent years, at the Institute for Nuclear Research in Vinča, according to the recipe of V. Jokanović, a new nanostructured material based on the calcium-aluminate system obtained by the hydrothermal sol-gel method and a self-propagating combustion reaction was synthesized. This method of synthesis provides high activity of particles, faster hydration and short bonding time [9]. Calcium aluminate-based cements provide great potential in the field of biomaterials due to the reduced setting time and associated microstructure.

The aim of this work was to test the marginal microleakage of newly synthesized nanostructured biomaterials based on calcium aluminate, calcium silicate and MTA with a dye penetration test after applying the material to the interradicular perforations of extracted teeth.

MATERIAL AND METHOD

In this research, materials based on calcium aluminate systems (CA), white Mineral Trioxide Aggregate MTA (White MTA, Angelus® Soluções odontológicas Londrina, Brazil) and materials based on calcium silicate (CS) were tested. The study included 48 extracted human maxillary and mandibular molars with fully developed roots. The access cavities were prepared with a high-speed handpiece, after which the working length of the root canal was determined with hand files (Kfiles, VDW Gmbh, Germany). The working length was determined up to the physiological foramen, 1 mm shorter than the anatomical foramen. All root canals were prepared using Crown-Down technique. During the preparation of the root canal, copious irrigation with 0.5% NaOCl was performed. The canals were dried with paper points and obturated using the monocone technique with sealer AH PlusTM (Dentsply, Germany).

After incubation of the samples, perforation of the floor of pulp chamber was performed between the roots (interradicular perforation). Perforation was done with a high speed handpiece and a size #4 round drill bit. The width of the perforations corresponded to the diameter of the burr, while the depth depended on the thickness of the floor of the pulp chamber. Interradicular perforations were washed with distilled water and divided into the three groups, after which they were closed with the tested materials.

Group 1: calcium silicate was used to close the perforations - 12 samples.

Group 2: MTA was used to close the perforations - 12 samples.

Group 3: calcium aluminate-based material was used to close the perforations - 24 samples.

Before the application of tested materials, the teeth were placed in a sponge soaked in distilled and deionized water up to the level of the enamel-cementum border. The calcium aluminate-based material was mixed with distilled water in a ratio of 3:1 and control materials were mixed according to the manufacturer's instructions, then placed in the furcal perforations with the help of a reamer. Wet cotton pellet was placed over the material, and then the teeth were incubated at 37°C for 24 h. After bonding the material, the access cavities were definitely closed with the composite. The teeth were stored in a sponge soaked with distilled and deionized water and incubated at 37°C for the next 6 months.

After 6 months, the marginal microleakage was measured. The teeth were coated with two layers of nail polish, except in the area around furcation (1 mm around the furcation). After that, all samples were immersed in a 50% solution of silver nitrate (AgNO3) for 2 h, and then washed in photographic developer for the next 6 h. Then longitudinal sectioning of the teeth was performed through the perforation itself with a 0.7 mm thick diamond disc, linear precision saw with water cooling (Isomet saw 4000, Buehler, Lake Bluff, IL, USA). Penetration depth was measured with a binocular magnifier (Leica DM 500, Leica Byosistems). The obtained values were expressed in millimeters, and results were statistically analyzed using analysis of variance for repeated measurements with the Sidak test.

RESULTS

The lowest average penetration (mm) was for MTA (1.40 ± 0.63mm), and the highest for Ca aluminates (2.10 ± 0.63mm), while for calcium silicates an average color penetration of 1.73 ± 0.67 mm was recorded.

By testing intergroup differences in color penetration between groups, a statistically significant difference between MTA and Ca aluminate was obtained using Sidak's test ($t=-0.693$; $p=0.036$). No statistically significant difference was recorded between MTA and Ca silicate, nor was there a statistically significant difference between Ca aluminate and Ca silicate.

DISCUSSION

Many methods of dye penetration [10], liquid filtration [11], bacterial penetration [12] and protein penetration [13] have been used to investigate microleakage.

In this research, linear dye penetration method was used to assess microleakage. This method was also most commonly used method for evaluating the quality of edge closure due to its simplicity of application. Despite the

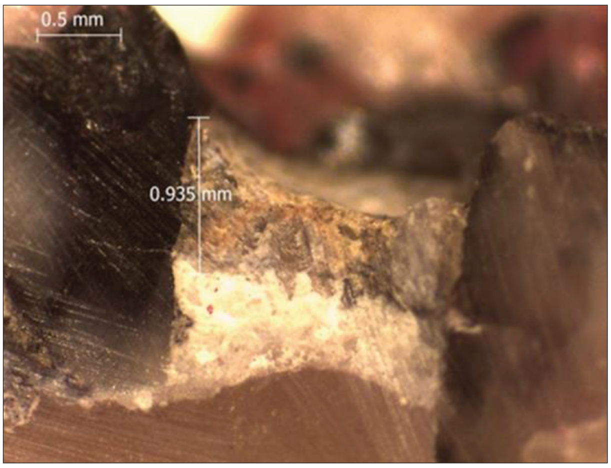

Figure 1. Inter-radicular perforation filled with material based on calcium aluminate. There is partial dissolution of the material, that is, disintegration with absorption of color and partial color change of the material (×30).
Slika 1. Interardiksna perforacija ispunjena materijalom na bazi kalcijum-aluminata. Uočava se delimično rastvaranje materijala, odnosno dezintegracija sa apsorpcijom boje i delimičnim prebojavanjem materijala (30×).

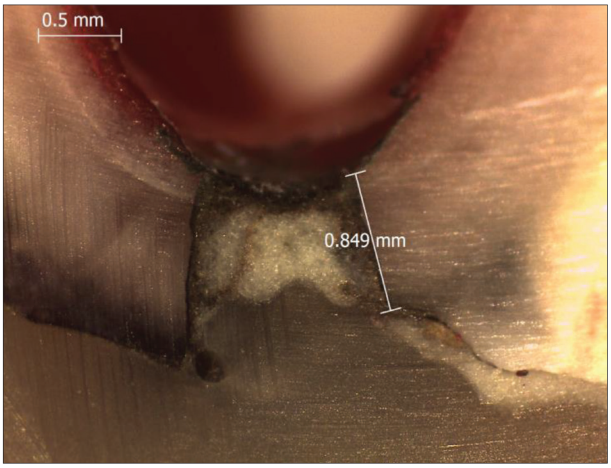

Figure 2. Inter-radicular perforation filled with MTA. There is dye absorption with most of the material having changed color (×30).
Slika 2. Interradiksna perforacija ispunjena sa MTA. Uočava se marginalni prodor boje sa prebojavanjem većeg dela materijala (30×).

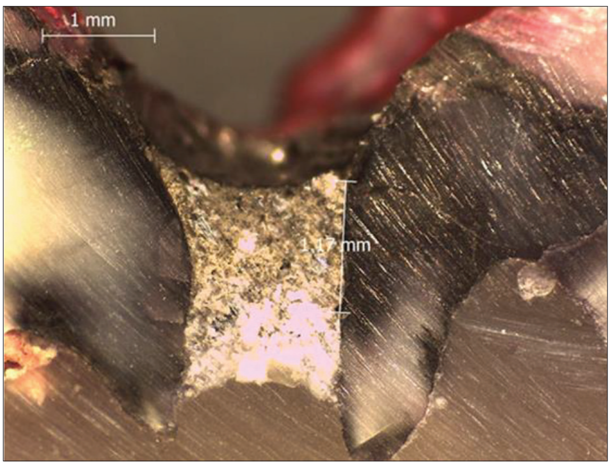

Figure 3. Perforation filled with calcium silicate material. There is marginal dye leakage with color change of material (×30).
Slika 3. Perforacija ispunjena materijalom kalcijum-silikatom. Uočava se izražen prodor boje sa prebojavanjem dela materijala (30×).

Table 1. Mean values and standard deviations of color penetration for the tested materials
Tabela 1. Srednje vrednosti i standardne devijacije prodora boje ispitivanih materijala

Material N	$\bar{x}$	SD	Med	Min	Max
Ca aluminates Ca-aluminati 24	2.10	0.63	2.19	0.83	3.25
Ca silicates Ca-silikati 12	1.73	0.67	1.78	0.00	2.97
MTA 12	1.40	0.63	1.47	0.00	2.24
Total Ukupno 48	1.74	0.68	1.76	0.00	3.25

mentioned advantages, dye penetration method has several disadvantages: molecular size of most dye particles is larger than the size of bacteria, and the dye penetration is observed in one plane, i.e. point [14]. Dye penetration method is static, it does not reflect the dynamic interaction with the periradicular tissue, and it is not possible to determine the volume of dye between hard tooth tissue and dental materials. In the analysis of marginal closure of dental materials, many types of dyes were used (methylene blue, silver nitrate, basic fuchsin, rhodamine B...). Methylene blue is a commonly used dye due to its affordable price [15]. Silver nitrate solution was used in this research due to its stability in materials with high pH values. Silver nitrate particles possess a large molecular mass similar to the size of bacteria. Silver nitrate is not a hydrophilic solution, like basic fuchsin [16].

Torabinejad et al. indicated that a material capable of preventing the penetration of small dye molecules could also prevent the passage of bacteria and their by-products [17].

An ideal material for closing interradicular perforations should have dimensional stability, be easy to place, non-toxic, non-carcinogenic, have X-ray contrast and be biocompatible [18], as well as the ability to induce osteogenesis and cementogenesis [19].

In 1993, Torabinejad presented MTA as a biomaterial, which can lead to deposition of cement when used in the treatement of interradicular perforations [17]. This material has superior marginal sealing capabilities compared to other restorative materials in the indication of tooth root perforations. [20]. Aldayri et al. found that MTA, when placed in interradicular perforations, can induce the formation of cementum tissue [21].

In 2009, Jacobovitz M et al. analyzed bacterial microleakage of MTA and an experimental material based on calcium aluminate (EndoBinder). Both materials showed effective microleaking of *Enterococcus faecalis in vitro* after 30 days. The authors attribute this result to the addition of a dispersant, that is, additive that enables the reduction of the water content when mixing the material, which results in a denser structure [22].

In this research, linear penetration of the dye was measured, expressed in millimeters, to the point that dye penetrated after longitudinal sectioning of the tooth. The disadvantage of this method is that dye penetration could not be determined at the deepest point of penetration. The diameter of the experimental preparations corresponded to the diameter of the drill (size #4), while the depth, that is, the dimension of the perforation, depended on the thickness of the floor of pulp chamber. Camps and Pashley found that dye penetration relies on randomly cutting the root in two without knowing whether the section goes through the deepest dye penetration [23].

The results of our research showed that best marginal seal was achieved in interradicular perforations filled with MTA. Color penetration values for calcium silicate were slightly higher, but without a statistically significant difference. The highest color penetration was recorded in furcations that were filled with calcium aluminate cements. The obtained results of marginal micropermeability correspond to the results of solubility and porosity of the tested materials. The reason for such results may be the structure of the cement itself, built of numerous pores and capillaries that could lead to a certain permeability. Sarkar et al. in 2005 attributed good marginal seal of MTA to its ability to form hydroxyapatite and its deposition on the surface of the material in contact with tissue [24]. Jeffries et al. detected the ability to form surface apatite with cements based on calcium aluminate when closing marginal microcracks in simulated physiological conditions [25]. According to the results of previous research, MTA showed better marginal closure, less color penetration compared to other tested dental materials, which is in accordance with the results of our study [26]. This may be due to different consistency of dental materials. After mixing, MTA has a paste consistency, while when mixing calcium aluminate, it had a grainy consistency, which made it impossible to completely close the edges.

Pace et al. found that the use of biocompatible materials in the repair of furcal perforations leads to reduction in the inflammatory response of the surrounding tissues. With the use of MTA for furcal perforations in dog teeth, cement formation occurs over the material without inflammatory cell infiltrates. Therefore, MTA represents an ideal material in the treatment of furcal perforations, it is non-toxic and insoluble in a moist environment [27].

Comparing marginal sealing ability of MTA, calcium phosphate cement and bone cement in the repair of furcal perforations in an *in vitro* study on 70 human extracted molars, Chordiya et al. indicated the lowest micropermeability of MTA. They stated that good marginal sealing of MTA is a consequence of excellent adaptation of the material to the outer edges of the perforation due to the expansion of the material [28].

Examining bacterial microleakage of calcium aluminate-based materials in combination with glas ionomer cements, Pameijer et al. in an observation period of 60 days on 30 extracted premolars, showed that calcium aluminate-based material has good marginal closure and is bioactive, has the ability to form hydroxyapatite, providing the possibility of tissue remineralization. The authors attribute this result to the chemical composition of the material [29].

Haghgoo et al. analyzed microleakage in iatrogenic furcal perforations for MTA and calcium-enriched mixture. Color penetration was recorded with both materials, without statistically significant differences. The authors attribute this result to the hydrophilicity of both materials, that allowed good adaptation of the material to the perforation walls [15].

One of the possible reasons for good marginal seal of MTA is expansion of the material after setting, which increased the quality of seal. Another possibility is related to crystal deposition in MTA. In general, by reviewing the literature, it can be concluded that an ideal material that would prevent dye penetration, liquids, bacteria and microorganisms, still does not exist.

CONCLUSION

The lowest marginal micropermeability, i.e. the best marginal seal was recorded with MTA. Micropermeability of calcium aluminate-based materials was significantly higher compared to calcium silicate and MTA.

REFERENCES

1. da Silva GF, Guerreiro-Tanomaru JM, Sasso-Cerri E, Tanomaru-Filho M, Cerri PS. Histological and histomorphometrical evaluation of furcation perforations filled with MTA, CPM and ZOE. Int Endod J. 2011;44(2):100–10. [DOI: 10.1111/j.1365-2591.2010.01803.x] [PMID: 21039627]
2. Kakani AK, Veeramachaneni C. Sealing ability of three different root repair materials for furcation perforation repair: An in vitro study. J Conserv Dent. 2020;23(1):62–5. [DOI: 10.4103/JCD.JCD_371_19] [PMID: 33223644]
3. Krupalini KS, Udayakumar, Jayalakshmi KB. A comparative evaluation of medicated calcium sulphate, hydroxylapatite, mineral trioxide aggregate (MTA) as barrier and their effect on the sealing ability of furcation perforation repair material--an in vitro study. Indian J Dent Res. 2003;14(3):156–61. [PMID: 15164658]
4. Koç C, Aslan B, Ulusoy Z, Oruçoğlu H. Sealing ability of three different materials to repair furcation perforations using computerized fluid filtration method. J Dent Res Dent Clin Dent Prospects. 2021;15(3):183–7. [DOI: 10.34172/joddd.2021.031] [PMID: 34712409]
5. Alazrag MA, Abu-Seida AM, El-Batouty KM, El Ashry SH. Marginal adaptation, solubility and biocompatibility of TheraCal LC compared with MTA-angelus and biodentine as a furcation perforation repair material. BMC Oral Health. 2020;20(1):298. [DOI: 10.1186/s12903-020-01289-y] [PMID: 33121465]
6. Alghamdi FT, Aljahdali EA. Comparison of Mineral Trioxide Aggregate, EndoSequence Root Repair Material, and Biodentine Used for Repairing Root Perforations: A Systematic Review. Cumhuriyet Dent J. 2019;22(4):469–76. [DOI: 10.7126/cumudj.589413]
7. Main C, Mirzayan N, Shabahang S, Torabinejad M. Repair of root perforations using mineral trioxide aggregate: a long-term study. J Endod. 2004;30(2):80–3. [DOI: 10.1097/00004770-200402000-00004] [PMID: 14977301]
8. Mohan D, Singh AK, Kuriakose F, Malik R, Joy J, John D. Evaluation of Sealing Potential of Different Repair Materials in Furcation Perforations Using Dye Penetration: An In Vitro Study. J Contemp Dent Pract. 2021;22(1):80–3. [PMID: 34002714]
9. Jokanovic V, Colovic B, Mitric M, Markovic D, Cetenovic B. Synthesis and properties of a new endodontic material based on nanostructured highly active calcium-silicates and calcium carbonates. Int J Applied Ceram Tech. 2014;11:57–64. [DOI: 10.1111/ijac.12070]

10. Balachandran J, Gurucharan. Comparison of sealing ability of bioactive bone cement, mineral trioxide aggregate and Super EBA as furcation repair materials: A dye extraction study. J Conserv Dent. 2013;16(3):247–51. [DOI: 10.4103/0972-0707.111326] [PMID: 23833460]
11. Hardy I, Liewehr FR, Joyce AP, Agee K, Pashley DH. Sealing ability of One-Up Bond and MTA with and without a secondary seal as furcation perforation repair materials. J Endod. 2004;30(9):658–61. [DOI: 10.1097/01.don.0000121619.33952.9a] [PMID: 15329572]
12. Jafari F, Sobhani E, Samadi-Kafil H, Pirzadeh A, Jafari S. In vitro evaluation of the sealing ability of three newly developed root canal sealers: A bacterial microleakage study. J Clin Exp Dent. 2016;8(5):e561–e565. [DOI: 10.4317/jced.52992] [PMID: 27957271]
13. Shahi S, Rahimi S, Hasan M, Shiezadeh V, Abdolrahimi M. Sealing ability of mineral trioxide aggregate and Portland cement for furcal perforation repair: a protein leakage study. J Oral Sci. 2009;51(4):601–6. [DOI: 10.2334/josnusd.51.601] [PMID: 20032614]
14. Grover R, Sadana G, Gupta S, Gupta T, Mehra M, Kaur B. Comparative evaluation of sealing ability of two different biocompatible materials in repair of furcal perforation: An in vitro study. Dent Med Res. 2020;8(2):70–4. [DOI: 10.4103/dmr.dmr_32_20]
15. Haghgoo R, Arfa S, Asgary S. Microleakage of CEM Cement and ProRoot MTA as Furcal Perforation Repair Materials in Primary Teeth. Iran Endod J. 2013;8(4):187–90. [PMID: 24171027]
16. Nabeel M, Tawfik HM, Abu-Seida AMA, Elgendy AA. Sealing ability of Biodentine versus ProRoot mineral trioxide aggregate as root-end filling materials. Saudi Dent J. 2019;31(1):16–22. [DOI: 10.1016/j.sdentj.2018.08.001] [PMID: 30723363]
17. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod. 1993;19(12):591–5. [DOI: 10.1016/S0099-2399(06)80271-2] [PMID: 8151252]
18. Mohan D, Singh AK, Kuriakose F, Malik R, Joy J, John D. Evaluation of Sealing Potential of Different Repair Materials in Furcation Perforations Using Dye Penetration: An In Vitro Study. J Contemp Dent Pract. 2021;22(1):80–3. [PMID: 34002714]
19. Francis T, Joshi SB, Pai AV, Sakkir N, Thaha KA. Comparison of the sealing ability of MTA-angelus, biodentine and CEM cement in the repair of large furcal perforations - a bacterial leakage study. J Clin Diagn Res. 2019;13(1):32–5. [DOI: 10.7860/JCDR/2019/38476.12503]
20. Abualhasan HM, Alhussain BS. Clinical Properties and Efficacy of MTA VS Biodentine VS GIC in Repairing Root Perforations. Arch Pharm Pract. 2022;13(1):53–7. [DOI: 10.51847/46MIz4Calq]
21. Aldayri B, Nourallah AW, Badr F. Clinical and Radiographic Evaluation of the Healing After MTA Application on Mechanical Furcal Perforations in Primary Molars-part2. Preprints. 2019;2019050322.
22. Jacobovitz M, Vianna ME, Pandolfelli VC, Oliveira IR, Rossetto HL, Gomes BP. Root canal filling with cements based on mineral aggregates: an in vitro analysis of bacterial microleakage. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;108(1):140–4. [DOI: 10.1016/j.tripleo.2009.03.013] [PMID: 19540451]
23. Camps J, Pashley D. Reliability of the dye penetration studies. J Endod. 2003;29(9):592–4. [DOI: 10.1097/00004770-200309000-00012] [PMID: 14503834]
24. Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I. Physicochemical basis of the biological properties of mineral trioxide aggregate. J Endod. 2005;31(2):97–100. [DOI: 10.1097/01.don.0000133155.04468.41] [PMID: 15671817]
25. Jefferies SR, Fuller AE, Boston DW. Preliminary Evidence That Bioactive Cements Occlude Artificial Marginal Gaps. J Esthet Restor Dent. 2015;27(3):155–66. [DOI: 10.1111/jerd.12133] [PMID: 25640821]
26. Lanker F, Patel N. Evaluating sealing ability of three different furcation perforation repair materials. J Adv Med Dent Sci Res. 2020;8(10):189–91. [DOI: 10.21276/jamdsr]
27. Pace R, Giuliani V, Pagavino G. Mineral trioxide aggregate as repair material for furcal perforation: case series. J Endod. 2008;34(9):1130–3. [DOI: 10.1016/j.joen.2008.05.019] [PMID: 18718380]
28. Chordiya R, Hiremath H, Metgud S, Heda A. Evaluation of the sealing ability of bone cement as furcation perforation repair material when compared with mineral trioxide aggregate and calcium phosphate cement: An in-vitro study. Journal of the International Clinical Dental Research Organization. 2010;2(2):75–81. [DOI: 10.4103/2231-0754.95264]
29. Pameijer CH, Zmener O, Alvarez Serrano S, Garcia-Godoy F. Sealing properties of a calcium aluminate luting agent. Am J Dent. 2010;23(2):121–4. [PMID: 20608305]

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Ispitivanje marginalne mikropropustljivosti novosintetisanih nanostrukturnih materijala na bazi kalcijum-aluminata posle aplikacije u interradiksne perforacije

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KRATAK SADRŽAJ

Uvod Kvalitetno rubno zaptivanje, odnosno odgovarajuća marginalna adaptacija materijala uz zidove kaviteta, treba da onemogući protok tkivnih tečnosti i posledično bakterijsko mikrocurenje, zbog čega se smatra značajnim faktorom za dugoročan uspeh endodontskog lečenja. Cilj ovog rada bio je da se testom prodora boje ispita marginalna mikropropustljivost novosintetisanih nanostrukturnih biomaterijala na bazi kalcujum-aluminata, kalcijum-silikata i MTA a nakon primene materijala u interradiksne perforacije ekstrahovanih zuba.

Materijal i metode rada Ispitivanje je vršeno na 48 ekstrahovanih humanih maksilarnih i mandibularnih molara. Testirani su novosintetisani nanostrukturni materijali – materijal na bazi kalcijum-aluminata, kalcijum-silikata. Kao kontrolni materijal korišćen je komercijalni kalcijumsilikatni cement (MTA Angelus, Londrina, Brazil). Marginalna mikropropustljivost je ispitivana testom prodora boje šest meseci posle primene materijala u eksperimentalno preparisane interradiksne perforacije na ekstrahovanim humanim molarima. Merenje dubine prodora analizirano je binokularnom lupom (Leica DM 500, Leica Byosistems). Dobijene vrednosti su izražene u milimetrima, a dobijeni rezultati su statistički obrađeni primenjivanjem analize varijanse za ponovljena merenja Sidakovim testom.

Rezultati Najmanji prosečan prodor (mm) bio je za MTA (1,40 ± 0,63 mm), a najveći za kalcijum-aluminate (2,10 ± 0,63 mm), dok je za kalcijum-silikate zabeležen prosečan prodor boje od 1,73 ± 0,67 mm. Testiranjem međugrupnih razlika prodora boje između grupa Sidakovim testom dobijena je statistički značajna razlika između MTA i kalcijum-aluminata (t = -0,693; p = 0,036). Između MTA i kalcijum-silikata nije zabeležena statistički značajna razlika, kao ni između kalcijum-aluminata i kalcijum-silikata.

Zaključak Najmanja marginalna mikropropustljivost, odnosno najbolje rubno zaptivanje zabeleženo je kod materijala MTA. Mikropropustljivost materijala na bazi kalcijum-aluminata bila je značajno veća u odnosu na kalcijum-silikat i MTA.

Ključne reči: marginalna mikropropustljivost; kalcijum-aluminati, kalcijum-silikati

UVOD

Perforacija podrazumeva mehaničku ili patološku komunikaciju između sistema kanala korena i spoljašne površine zuba uzrokovanu karijesom, resorpcijom ili jatrogenim faktorima [1]. Prema lokalizaciji, perforacije korena mogu biti interradiksne, lateralne i apikalne. Nelečene perforacije uzrokuju inflamaciju, resorpciju kosti, dentina i cementa. Ovo je naročito izraženo kod perforacija u furkaciji višekorenih zuba. Glavni cilj u tretmanu perforacija je sprečavanje zapaljenskog procesa i posledičnog gubitka tkivnog pripoja na mestu perforacije, odnosno uspostavljanje novog tkivnog pripoja, ukoliko lezija već postoji [2].

Mnogi materijali su korišćeni u terapiji perforacija kanala korena: cink-oksid eugenol, amalgam, kalcijum-hidroksid, glasjonomer, kavit, gutaperka, kompozitni materijali, hidroksiapatit, kalcijum-fosfatni cementi, trikalcijum-fosfatni cementi, IRM (Intermediate Restorative Material), glas-jonomerni cementi, Portland cement, MTA. Ali nijedan u potpunosti ne zadovoljava sve tražene zahteve [3, 4].

Osobine koje bi trebalo da poseduje idealni materijal za zatvaranje perforacija kanala korena zuba su: biokompatibilnost, kratko vreme vezivanja, sposobnost dobrog rubnog zatvaranja, kao i odgovarajući antibakterijski efekat, da je nerastvorljiv u tkivnim tečnostima i da poseduje adekvatna mehanička svojstva. Materijal izbora u terapiji korenskih perforacija je MTA, predstavljen još 1993. godine od strane Univerziteta Loma Linda [5, 6].

MTA poseduje dobre fizičke, hemijske i biološke karakteristike, te je jedna od njegovih indikacija i terapija perforacija kanala korena. S obzirom na antimikrobno dejstvo i veoma visok MTA, omogućava regeneraciju periodontalnih ligamenata oko mesta povrede, odnosno perforacije. Main i saradnici su u svojoj studiji zaključili da MTA obezbeđuje efektivno rubno zatvaranje kod perforacija kanala korena. Postavka materijala u odgovarajućem vremenskom periodu može da spreči bakterijsku kontaminaciju iz pulpne komore u periodontalno tkivo [7]. Međutim, vreme koje je potrebno za vezivanje MTA iznosi dva sata i 30 minuta. Sposobnost rubnog zatvaranja MTA u vodenoj sredini može biti ugroženo tokom prva 72 sata zbog rastvorljivosti materijala. Mohan i saradnici tvrde da je potrebno tri dana da MTA pokaže dobro rubno zatvaranje, kada se koristi kao materijal za zatvaranje kod perforacija kanala korena [8].

Savremena istraživanja su fokusirana na sintezu i ispitivanja moguće primene nanostrukturnih biomaterijala u endodontskim indikacijama. Poslednjih godina na Institutu za nuklearna istraživanja u Vinči prema recepturi V. Jokanovića sintetisan je novi nanostrukturni materijal na bazi kalcijum-aluminatnog sistema dobijen hidrotermalnom sol-gel metodom i samoširećom reakcijom sagorevanja. Ovakav način sinteze obezbeđuje visoku aktivnost čestica, bržu hidrataciju i kratko vreme vezivanja [9]. Cementi na bazi kalcijum-aluminata zbog smanjenog vremena vezivanja i povezane mikrostrukture obezbeđuju veliki potencijal na polju biomaterijala.

Cilj ovog rada bio je da se testom prodora boje ispita marginalna mikropropustljivost novosintetisanih nanostrukturnih biomaterijala na bazi kalcujum-aluminata, kalcijum-silikata i MTA a nakon primene materijala u interradiksne perforacije ekstrahovanih zuba.

MATERIJAL I METODE RADA

U ovom istraživanju testirani su materijali na bazi kalcijum-aluminatnih sistema, beli mineralni trioksidni agregat – MTA (White MTA, Angelus® Soluções odontológicas Londrina, Brazil) i materijali na bazi kalcijum-silikata. Istraživanje je realizovano na 48 ekstrahovanih humanih maksilarnih i mandibularnih molara sa potpuno razvijenim korenovima. Pristupni kaviteti su preparisani visokoturažnom bušilicom, odnosno turbinom, nakon čega je ručnim turpijama (Kfiles, VDW Gmbh, Germany) utvrđena radna dužina kanala korena. Radna dužina je određena do fiziološkog foramena, na 1 mm kraće od anatomskog foramena. Svi kanali korena su preparisani tehnikom Crown-Down. U toku preparacije kanala korena vršena je obilna irigacija sa 0,5% NaOCl-om. Kanali su posušeni papirnim poenima i opturisani monokonom tehnikom uz siler AH PlusTM (Dentsply, Germany), a zatim čuvani u inkubatoru sedam dana, u suvoj sredini na 37°C.

Nakon inkubacije uzoraka, izvršena je perforacija poda komore pulpe između korenova (interradiksna perforacija). Perforacija je urađena kolenjakom i okruglim borerom veličine #4. Širina perforacija je odgovarala promeru borera, dok je dubina zavisila od debljine poda pulpne komore. Zatim su interradiksne perforacije isprane destilovanom vodom i podeljene u tri grupe, nakon čega su zatvorene testiranim materijalima.

Grupa 1: za zatvaranje perforacija je korišćen kalcijum-silikat – po 12 uzoraka

Grupa 2: za zatvaranje perforacija je korišćen MTA – po 12 uzoraka

Grupa 3: za zatvaranje perforacija je korišćen materijal na bazi kalcijum-aluminata – po 24 uzorka

Pre aplikacije testiranih materijala zubi su postavljeni u sunđer natopljen destilovanom i dejonizujućom vodom do nivoa gleđno-cementne granice. Materijal na bazi kalcijum-aluminata je zamešan sa destilovanom vodom u odnosu 3 : 1 i kontrolni materijali su zamešani prema uputstvu proizvođača, zatim su plasirani u furkalne perforacije uz pomoć nabijača. Preko materijala je postavljena vlažna vatica, a zatim su zubi inkubirani na 37°C tokom 24 h. Po vezivanju materijala pristupni kaviteti su definitivno zatvoreni kompozitom. Zubi su čuvani u sunđeru natopljenom destilovanom i dejonizujućom vodom i inkubirani na 37°C, narednih šest meseci.

Posle šest meseci izvršeno je merenje marginalne mikropropustljivosti. Zubi su premazani sa dva sloja laka za nokte, osim u predelu oko same furkacije (1 mm oko furkacije). Nakon toga su svi uzorci potopljeni u 50% rastvor srebro-nitrata ($AgNO3$) tokom 2 h, a potom isprani u fotografskom razvijaču tokom narednih 6 h. Zatim je izvršeno longitudinalno presecanje zuba kroz samu perforaciju sa dijamantskim diskom debljine 0,7 mm, linearnom preciznom testerom sa vodenim hlađenjem (Isomet testera 4000, Buehler, Lake Bluff, IL, USA). Merenje dubine prodora je analizirano binokularnom lupom (Leica DM 500, Leica Byosistems). Dobijene vrednosti su izražene u milimetrima, a dobijeni rezultati su statistički obrađeni primenjivanjem analize varijanse za ponovljena merenja Sidakovim testom.

REZULTATI

Najmanji prosečan prodor (mm) bio je za MTA (1,40 ± 0,63 mm), a najveći za kalcijum-aluminate (2,10 ± 0,63 mm), dok je za kalcijum-silikate zabeležen prosečan prodor boje od 1,73 ± 0,67 mm.

Testiranjem međugrupnih razlika prodora boje između grupa Sidakovim testom dobijena je statistički značajna razlika između MTA i kalcijum-aluminata ($t = -0,693$; $p = 0,036$). Između MTA i kalcijum-silikata nije zabeležena statistički značajna razlika, kao ni između kalcijum-aluminata i kalcijum-silikata.

DISKUSIJA

Za ispitivanje mikropropuštanja korištene su mnoge metode prodora boje [10], filtracija tečnosti [11], bakterijsko propuštanje [12] i proteinsko propuštanje [13].

U ovom istraživanju za procenu mikropropustljivosti je primenjena metoda linearnog prodora boje. Ova metoda je ujedno i najčešće korišćena metoda za procenu kvaliteta rubnog zatvaranja zbog jednostavnosti primene. Uprkos navedenim prednostima, metoda prodora boje ima i nekoliko nedostataka: molekularna veličina većine čestica boje je veća od veličine bakterija, a prodor boje se uočava u jednoj ravni, odnosno tački [14]. Metod prodora boje je statički, ne odražava dinamičku interakciju sa periradikularnim tkivom, te nije moguće odrediti zapreminu boje između tvrdih zubnih tkiva i dentalnih materijala. U analizi rubnog zatvaranja dentalnih materijala korišćeni su mnogi tipovi boje (metilensko plavo, srebro-nitrat, bazični fuksin, rodamin B...). Metilensko plavo je boja koja se obično koristi zbog svoje pristupačne cene [15]. Rastvor srebro-nitrata je korišćen u ovom istraživanju zbog svoje stabilnosti kod materijala sa visokim pH vrednostima. Čestice srebro-nitrata poseduju veliku molekularnu masu slične veličine kao i same bakterije. Srebro-nitrat nije hidrofilan rastvor, kao što je bazni fuksin [16].

Torabinejad i sardanici su ukazali da bi materijal koji je sposoban da spreči penetraciju malih molekula boje mogao sprečiti i prolazak bakterija i njihovih nusprodukata [17].

Idealan materijala za zatvaranje interradiksnih perforacija trebalo bi da poseduje dimenzionalnu stabilnost, da bude lagan za plasiranje, netoksičan, nekancerogen, da ima rendgensku kontrastnost i da je biokompatibilan [18], odnosno da poseduje sposobnost da izazove osteogenezu i cementogenezu [19].

Torabinejad je 1993. godine predstavio MTA kao biomaterijal koji može da dovede do depozicije cementa kada se koristi u terapiji furkalnih perforacija korena zuba [17]. Ovaj materijal ima superiornije sposobnosti rubnog zatvaranja u poređenju sa drugim restaurativnim materijalima u indikaciji perforacija korena zuba. [20]. Aldayri i saradnici tvrde da MTA prilikom plasiranja u interradiksne perforacije može indukovati formiranje cementnog tkiva [21].

Jacobovitz M. i saradnici su 2009. godine analizirali bakterijsko mikropropuštanje MTA i eksperimentalnog materijala na bazi kalcijum-aluminata (EndoBinder). Oba materijala su se pokazala efikasnima kod mikropropuštanja Enterococcus faecalis in vitro nakon 30 dana. Autori ovaj rezultat pripisuju dodatku disperzantnog sredstva, odnosno aditiva, koji omogućavaju smanjenje udela vode prilikom mešanja materijala, čime se dobija gušća struktura [22].

U ovom istraživanju meren je linearni prodor boje, izražen u milimetrima, odnosno tačka do koje je boja prodrla nakon longitudinalnog presecanja zuba. Nedostatak ove metode je taj što se prodor boje nije mogao odrediti u najdubljoj tački prolaska boje u zubno tkivo. Prečnik eksperimentalnih preparacija odgovarao je prečniku borera (veličina #4), dok je dubina, odnosno dimenzija perforacije zavisila od debljine poda komore pulpe. Camps i Pashley tvrde da se prodor boje oslanja na nasumično presecanje korena na dva dela ne znajući da li presek prolazi kroz najdublji prodor boje [23].

Rezultati sprovedenog istraživanja pokazuju da je najbolje rubno zaptivanje ostvareno kod furkalnih perforacija koje su ispunjene MTA-om. Vrednosti prodora boje kod kalcijum-silikata bile su nešto veće ali bez statistički značajne razlike. Najveći prodor boje zabeležen je kod furkacija koje su ispunjene kalcijum-aluminatnim cementima. Dobijeni rezultati marginalne mikropropustljivosti odgovaraju rezultatima rastvorljivosti i poroznosti testiranih materijala. Razlog ovakvih rezultata može biti i sama struktura cementa izgrađena od brojnih pora i kapilara koji su mogli dovesti do određene propustljivosti. Sarkar i saradnici 2005. godine dobro rubno zaptivanje MTA pripisuju sposobnosti stvaranja hidroksiapatita i njegovoj depoziciji na površini materijala u kontaktu sa tkivom [24]. Jeffrries i saradnici ukazuju na sposobnost formiranja površinskog apatita cemenata na bazi kalcijum-aluminata kod zatvaranja marginalnih mikropukotina u simuliranim fiziološkim uslovima [25].

Prema rezultatima dosadašnjih istraživanja, MTA pokazuje bolje rubno zatvaranje, odnosno manji prodor boje u odnosu na ostale testirane dentalne materijale, što je u skladu sa rezultatima ove studije [26]. Ovo je možda posledica različite konzistencije dentalnih materijala. Nakon mešanja, MTA ima konzistenciju paste, dok je prilikom mešanja kalcijum-aluminata bilo zrnaste konzistencije, što je onemogućilo rubno zatvaranje kompletnim.

Pace i saradnici smatraju da korištenjem biokompatibilnih materijala u reparaciji kod furkalnih perforacija dolazi do smanjenja inflamatornog odgovora okolnih tkiva. Upotrebom MTA za furkalne perforacije kod zuba pasa dolazi do formiranja cementa preko materijala bez infiltrata inflamatornih ćelija. Stoga, navedeni istraživači smatraju da MTA predstavlja idealni materijal u lečenju furkalnih perforacija, jer je netoksičan i nerastvorljiv u vlažnoj sredini [27].

Ispitujući marginalnu mikropropustljivost, odnosno upoređujući sposobnost rubnog zatvaranja MTA, kalcijum-fosfatnog cementa i koštanog cementa u reparaciji furkalnih perforacija u studiji in vitro na 70 humanih ekstrahovanih molara, Chordiya i saradnici su ukazali na najmanju mikropropustljivost MTA. Oni navode da je dobro rubno zaptivanje MTA posledica izvrsne adaptacije materijala na spoljašnje rubove perforacionog otvora zbog ekspanzije materijala [28].

Ispitujući bakterijsko mikropropuštanje materijala na bazi kalcijum-aluminata u kombinaciji sa glas-jonomernim cementima, Pameijer i saradnici u opservacionom periodu od 60 dana na 30 ekstrahovanih premolara pokazuju da materijal na bazi kalcijum-aluminata poseduje dobro rubno zatvaranje i da je bioaktivan, odnosno da poseduje sposobnost stvaranja hidroksiapatita, pružajući mogućnost remineralizacije tkiva. Autori ovakav rezultat pripisuju hemijskoj kompoziciji materijala [29].

Haghgoo i saradnici su analizirali mikropropuštanje kod jatrogenih furkalnih perforacija za MTA i miksturu obogaćenu kalcijumom. Kod oba materijala zabeležen je prodor boje, bez statistički značajnih razlika. Ovaj rezultat autori pripisuju hidrofilnosti oba materijala, odnosno dobroj adaptaciji materijala za zidove perforacije [15].

Jedan od mogućih razloga dobrog rubnog zatvaranja MTA je ekspanzija materijala posle vezivanja, što povećava kvalitet zaptivanja. Druga mogućnost je povezana sa depozicijom kristala kod MTA. Generalno, pregledom literature može se zaključiti da idealan materijal koji bi sprečio prodor boje, odnosno tečnosti, bakterija i mikroorganizama još uvek ne postoji.

ZAKLJUČAK

Najmanja marginalna mikropropustljivost, odnosno najbolje rubno zaptivanje zabeleženo je kod materijala MTA. Mikropropustljivost materijala na bazi kalcijum-aluminata je bila značajno veća u odnosu na kalcijum-silikat i MTA.