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Kada pomisliš da si najbolji,
znaj da si tada sigurno najgori.
Tadija Čaluković

Ovaj citat jednog mladog i nadarenog čoveka, inače savremenika naše kolektivne paranoje, samo je pokazatelj da pamet i znanje ne mogu „iščiliti" iz svesti hrabrih i slobodoumnih, bez obzira na strah kao dominantnu emociju naše svakodnevice.

Iako je aktuelni trenutak u svakom segmentu života, pa i u nauci, umetnosti i kulturi, lišen elementarnih moralnih, etičkih, pravnih ali i ostalih vrednosti, mudrost i sloboda pojedinaca ipak mogu okrenuti „točak bezizlaza" u suprotnom smeru. A samo sloboda, znanje i kreativnost rađaju prave vrednosti i mogu nas odvesti u bolju budućnost kojoj težimo.

To, naravno, danas nije ni lako ni jednostavno, jer našom „scenom" caruju laž, primitivizam, bahatost i prostakluk, dok su moralne i etičke norme „izopštene" iz sfere elementarnih kulturnih i društvenih vrednosti. U društvenom životu je danas potpuno „isključen" stepen elementarne pristojnosti u svim sferama života i apsolutno legalizovana glorifikacija svakodnevnih laži i falsifikovanja činjenica. Promocija agresije i netolerancije na svakom koraku, legalizacija nepoštovanja zakona i svakodnevica u kojoj neprestano „živimo" prošlost siguran je put u bezizlaz. Beščašće i laži onih koji se aкtivno bave nevažnim stvarima, oblikujući „kolektivni društveni rijaliti", osnovni su razlog zbog kojeg u brojnim sferama društvenog života tavorimo na dnu svakojakih lestvica. Kada se „nezdravo hranimo" i kada ne možemo da vidimo sopstveni udeo u nesrećama koje proizvodimo, „normalnost" nam je udaljena svetlosnim godinama. Zato su na početku 21. veka „svekoliki" napredak u svemu i „zlatno doba" u dubokom nesaglasju sa kolonama mladih koji odlaze. Činjenica je takođe, ali i istorijsko licemerstvo, da piromani ne mogu u isto vreme biti i jedini vatrogasci.

Zato su hrabrost i mudrost mladih ljudi, njihova slobodoumnost, znanje i neprestana borba za istinu jedina ispravna formula za rešavanje „jednačine" sa mnogo laži, primitivizma, bahatosti, straha i izopačene kulture, i jedinom konstantom vezanom za agresiju i netoleranciju u svakom segmentu života.

Jedan prijatelj mi je rekao da ovaj problem rešava tako što u aktuelnu svakodnevicu uvodi „sopstveni" paralelni svet u kome je dobro uvek dobro, u kome je pravda dostupna i dostižna, u kome je zlo zlo, u kome su moralne i etičke norme vrlo važni vrednosni kriterijumi, što je, u stvari, sušta suprotnost svega onoga što trenutno živimo.

I ovaj komentar ću završiti slično kako sam i započeo, ali citatom Martina Lutera Kinga: „Ćutanje i ravnodušnost dobrih ljudi su mnogo veći problem nego zlo loših ljudi", jer to najbolje oslikava problem koji može nastati ukoliko se zanemari aktuelni obrazac življenja i izostane hrabrost onih koji slobodno misle.

Prof. dr Slavoljub Živković

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Efficiency of root canal cleaning using a single instrument with reciprocating motion systems and full rotation systems: a SEM study

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SUMMARY

Introduction Efficient cleaning and canal design present a basic precondition for successful endodontic treatment. The aim of this study was to evaluate, using SEM analysis, the efficacy of cleaning the apical third of the canal with one instrument in both reciprocating motion and full rotation systems.

Materials and methods Thirty (30) single-rooted teeth (upper incisors), extracted due to periodontal reasons, were used and selected in 2 groups. In the group 1, instrumentation was done with a single instrument UNICONE with reciprocal movements (MEDIN, Czech Republic), while in the group 2, a single instrument XPS endo SHAPER (XPS) (FKG, Dentaire, Swiss) with full rotation was used. 2% solution of NaOCl and 17% EDTA solution were used as irrigation agents in both groups. SEM analysis of smear layer in the apical third was done at standard magnification of 1000x and presented on a scale of 1-5.

Results showed mostly clean canal walls and no smear layer in the apical region in both tested groups. Canal walls were somewhat cleaner after using UNICONE with reciprocal movements (80%) compared to the canals instrumented with XPS ENDO SHAPER (76%), but this difference was not statistically significant.

Conclusion Single-file NiTi systems did not remove smear layer completely, but provided efficient cleaning in the apical part of the canal. UNICONE file with reciprocal movements was somewhat more efficient in removing smear layer compared to the full rotation XPS ENDO SHAPER file.

Keywords: smear layer; reciprocal movements; NiTi rotating instruments; SEM

INTRODUCTION

Chemomechanical instrumentation and quality cleaning and shaping of the root canal system are basic preconditions for successful endodontic treatment. NiTi instruments are highly flexible and elastic, and they provide efficient and safe preparation [1, 2]. However, due to their frequent fracture, their application in curved root canals can be challenging [3, 4, 5]. In order to eliminate the risk during their use, dental technology has developed several new strategies in designing these instruments that include new cross section design of the working part of the instrument, special thermo-mechanical procedure in the preparation of the alloy, and different kinetics of instrument motion in the canal [2, 6, 7, 8].

Introduction of new root canal preparation system, based on the use of reciprocating motion instruments, has opened new possibilities for cleaning and shaping [9, 10]. Problems related to deformation and fractures of instruments with reciprocal movements are significantly less common compared to instruments with full rotation [9, 10, 11]. In addition, reciprocal movements are based on the technique of balanced forces and imply instrument rotation in the counterclockwise (cutting) direction and much shorter movement in the clockwise direction (relaxation of the instrument), significantly reducing the contact surface between instrument and dentin, and thus torsion stress and cyclical fatigue [7, 9, 12, 13].

Reducing the number of instruments necessary for preparation is also one of the current requirements in chemo-mechanical instrumentation. Instruments with reciprocal movements are most often represented with only one instrument [9, 10, 14, 15]. They are made of special NiTi alloys with specific thermal treatment, which significantly increases their flexibility and resistance to cyclic fatigue [8, 9, 15, 16]. The results of the research on reciprocal systems have confirmed the benefits of these instruments associated with shorter preparation time [9, 12], increased resistance to cyclic fatigue [9, 13, 15], and similar shaping efficiency to full-rotation systems that use multiple files [9, 10, 16, 17].

Complete cleaning of the root canal system is difficult to achieve (especially in the apical third), however data about the efficacy of files with reciprocal movements are very scarce. Numerous studies have confirmed that effective dentin cutting is largely dependent on instrument

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itself: material it is made of, design of the working part of the instrument, thread number, top design, special treatment of the surface of the file, and dynamics and kinetics of motion within the canal [9, 10, 12, 16, 18].

The aim of this study was to evaluate, using SEM analysis, the efficacy of cleaning the apical third of the canal using two different NiTi rotation systems: one instrument with reciprocating motion and one instrument with full rotation.

The following hypotheses were tested:

1. Reciprocal single-file systems produce less smear layer than rotary single-file systems.
2. Reciprocal single-file systems produce quantities of smear layer that are similar to those produced by single-file rotary systems.

MATERIALS AND METHODS

Thirty (30) single-rooted teeth (upper incisors), extracted due to periodontal reasons, were used and randomly selected into the two groups. Working length (1 mm shorter than the length when instrument appears on the apex) was determined after the formation of the access cavity. A single researcher carried out all canal instrumentation.

In the group 1, instrumentation was done using single instrument UNICONE (25/06) with reciprocal movements (MEDIN, the Czech Republic). After checking the canal patency, the canal was filled with 2% NaOCl solution (CHLORAKSID 2%, Cercamed, Poland) and the instrument placed to the working length (3-5 times) with gentle pulling movements. The canal was washed with 2 ml solution before and after instrumentation. A 17% EDTA solution (Calcinase, EDTA solution, Lege artis, Pharma GmbH) (1 ml) was used for irrigation after sodium hypochlorite. Final rinsing was done with additional 2 ml of sodium hypochlorite solution.

In the group 2, the canal preparation was done with a full rotation single instrument XPS (30/04) (FKG, Dentaire, Swiss). In the canal filled with a 2% sodium hypochlorite solution instrument was placed to the working length with gentle pulling movements for 1 minute (10-12 movements). The canal was also washed with a 2 ml solution before and after the instrumentation. The same procedure was repeated after inserting 17% EDTA solution (1 ml). Final rinsing was here also carried out with a 2% NaOCl solution (2 ml).

Canal instrumentation in both groups was performed with ENDO A CLASS endo motor (Medin, Czech Republic), which has both reciprocal and full rotation options.

Coronal parts of the teeth were cut into the enamel cement junction area, so that each root segment was 12 mm long. Using a diamond disc, two grooves on the vestibular and oral sides of the teeth were made, and then the root was split with a chisel into two halves. SEM analysis was done only in the apical third (3 mm from the end of instrumentation) (JOEL, JSM, 6460 LV, Japan), and 5 standardized microphotographs (1,000 X magnification) were created for each sample, which were then stored, analyzed and evaluated by 2 independent researchers. The presence of smear layer on the canal walls was evaluated according to the criteria of Hilsman et al. [19].

Score 1 – no smear layer, dentinal tubules are open;

Score 2 – some smear layer, several tubules are open;

Score 3 – homogeneous smear layer covers the wall, a few tubules are open;

Score 4 – the entire wall of the canal is covered with a smear layer, there are no open tubules;

Score 5 – non-homogenous smear layer covers the entire wall of the canal.

SEM photographs of the samples (300 images) were examined by two independent researchers. The obtained results were processed in the SPSS 20 program (IBM "Chicago"). The methods of descriptive statistics were used in the statistical analysis, and Hi square test was used for checking the frequency of the findings between the tested parameters.

RESULTS

The obtained results are shown in Tables 1 and 2 and Figure 1 (a, b).

Table 1. Evaluation of smear layer in the apical third
Tabela 1. Evaluacija razmaznog sloja u apikalnoj trećini kanala

		Score of smear layer Vrednost razmaznog sloja					
		N	$\bar{x}$	SD	Med	Min.	Max.
Group Grupa	UNICONE	150	1.95	1.00	2.00	1.00	5.00
	XPS	150	1.95	1.00	2.00	1.00	5.00
	Total Ukupno	300	1.95	0.99	2.00	1.00	5.00

Table 2. Evaluation of cleaning quality in the apical third
Tabela 2. Procena kvaliteta instrumentacije apikalne trećine kanala

			Evaluation of smear layer Procena razmaznog sloja		Total Ukupno
			Clean canal Očišćen kanal	Smear layer Razmazni sloj	
Group Grupa	UNICONE	N	120	30	150
		%	80%	20%	100%
	XPS	N	114	36	150
		%	76%	24%	100%
Total Ukupno		N	234	66	300
		%	78%	22%	100%

clean canal (score 1, 2)
čist kanal (vrednost 1, 2)
smear layer (score 3, 4, 5)
razmazni sloj (vrednost 3, 4, 5)

SEM sample analysis found mostly clean canal walls in the apical region and an identical average rating of the smear layer in both tested groups (Table 1). Clean canal (scores 1 and 2) in the apical third was registered in 80% of samples where the canal instrumentation was done with the UNICONE instrument with reciprocal movement, and in 76% of the samples where the XPS instrument with full rotation was used. This difference was not statistically significant (Table 2). Clean walls with a little smear layer in the apical third were most often reported in both groups (score 2): instrumentation with reciprocal

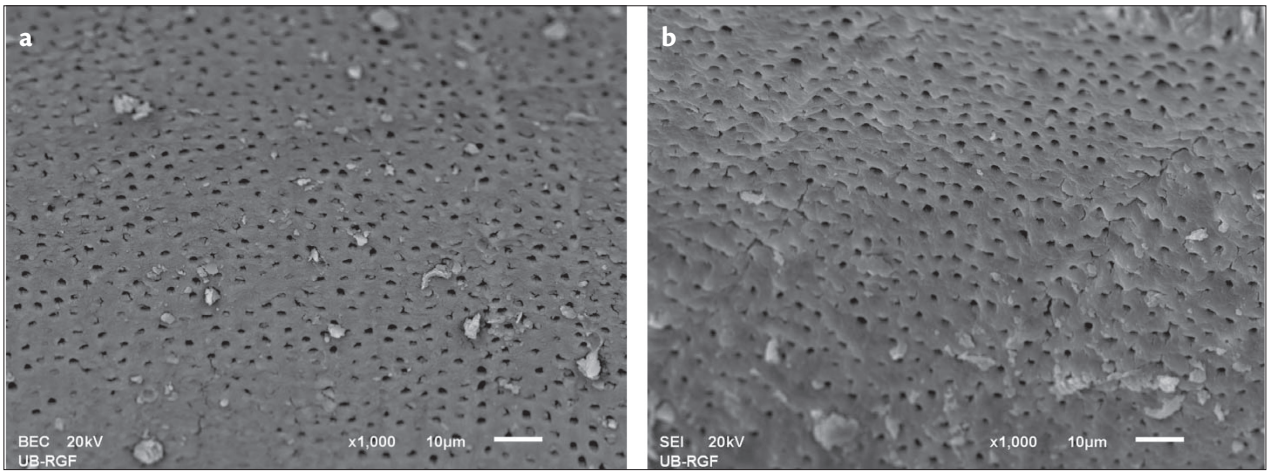

Figure 1. Representative microphotography of smear layer in the apical third
Slika 1. Reprezentativne mikrografije razmaznog sloja u apikalnoj trećini kanala
a) UNICONE file preparation with reciprocal movements (score 2). SEM 1000×
a) instrumentacija je vršena instrumentom UNICONE sa recipročnim pokretima (vrednost 2). SEM 1000×
b) XP-endo SHAPER preparation with full rotation (score 2). SEM 1000×
b) insrumentacija je vršena instrumentom SHAPER sa pokretima pune rotacije (vrednost 2). SEM 1000×

movements of instrument (Figure 1a) and full rotation instrumentation (Figure 1b).

DISCUSSION

Clean root canal without smear layer is one of the basic preconditions for achieving successful endodontic treatment [12, 16, 17]. The fact is that effective cleaning of the canal system is hardly feasible, especially in the apical third, due to inaccessibility, small diameter of the canal where the amount, concentration and time of exposure of this part of the canal to irrigation solutions is limited [12, 16, 17, 20, 21, 22].

The main purpose of our study was to compare the effects of two files with different kinetics (reciprocal and full rotation) on the quality of the canal cleaning. An endodontic procedure was performed by a single practitioner on single-rooted teeth (simple canal systems), using an identical irrigation protocol (with an identical amount of solution) to make the preparation process as close as possible to clinical conditions. The results did not show significant differences in the amount of residual smear layer in the apical third between the reciprocal motion file (UNICONE) and the full-rotation file (XPS), so the first hypothesis was rejected. Another hypothesis was accepted because both instruments produced similar amounts of smear layer during the preparation.

Slightly cleaner canal and smaller amount of smear layer in the apical third using the file with reciprocating movements could be explained by design of the working part of the instrument, and its cutting efficiency with increased agitation of the irrigation solution [9, 16, 17]. However, the influence of the file motion dynamics in a small number of realized studies has not been confirmed [20, 23, 24]. There are few studies in the literature that examined the efficacy of reciprocating files regarding the removal of the smear layer from the canal walls [12, 16, 17]. In our study, a new NiTi file (UNICONE), with specific working part design and different helix angle that provides exceptional flexibility and better elimination of dentin debris during instrumentation, was used as an instrument with reciprocal movements. The convex triangular cross section and inactive peak with special thermal treatment of the alloy ensured efficient dentin cutting and rapid and safe preparation with significantly increased bending and cyclic fatigue resistance [7, 9, 25]. More efficient cleaning and less smear layer in the apical region, with the application of UNICONE file, could be explained by good cutting efficiency of the instrument [18] and enhanced agitation of irrigant caused by reciprocal movements in the canal [26]. The results of the current study are consistent with studies that have confirmed the efficacy of reciprocal files in the removal of smear layer and better cleaning of the apical part compared to full rotation instruments [12, 26].

However, in the Poggio et al. study, instruments with reciprocal movements produced larger amount of smear layer in the canal system of single-rooted teeth [16]. They explained this finding by the fact that these movements produce "well-packed" smear layer in the canal. Reciprocal movements provide similar effects on the shaping canals just like systems with full rotation [27], but the effect of cutting dentin can be reduced due to prolonged (repeated) clinical use, which may result in larger amount of smear layer on the canal walls.

The efficiency of cleaning the apical region in the full rotation system was also checked after the application of a new instrument with unique XPS geometry. The specific design of the working part with extreme flexibility of the instrument allows changing the shape during rotation in the canal (by its contracting and expanding) and reaching inaccessible parts of the canal [30]. A snakelike XPS shape with its super elasticity and slightly faster rotation speed ensure increased "turbulence" of the irrigation solution, dissolving and removing debris from the canal walls [30].

In our study, small amount of smear layer in the apical region, after the application of XPS, can be explained

by an adequate dimension of apical preparation (30/04), which provides better effect of the irrigation solution and more efficient removal of smear layer from the canal walls [17, 29, 30]. These results are consistent with studies that assessed the amount of apically extruded debris after the preparation with different instruments, especially XP endo SHAPER [31]. Although the working part of the XPS does not have a significant cut effect, its alternative expansion and contraction in the canal enhance the solubility of irrigants and therefore cleaning of difficult to reach canal zones [29].

CONCLUSION

Within the limitations of this study, the obtained results showed that single-file NiTi systems (with reciprocal or full-rotation motions) did not remove smear layer completely, but provided efficient cleaning of the apical part of the canal. The file with reciprocal movements UNICONE was somewhat more efficient in removing smear layer from the apical third.

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Efikasnost čišćenja kanala jednim instrumentom kod sistema sa recipročnim pokretima i sistema sa punom rotacijom: SEM studija

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KRATAK SADRŽAJ

Uvod Efikasno čišćenje i oblikovanje kanala je osnovni preduslov za uspeh endodontskog tretmana. Cilj ovog rada je bio da se SEM analizom proveri efikasnost čišćenja apikalne trećine kanala jednim instrumentom kod sistema sa recipročnim pokretima i sistema sa punom rotacijom.

Materijal i metode Korišćeno je 30 jednokorenih zuba (gornji sekutići) ekstrahovanih zbog parodontalnih problema i podeljenih u dve grupe. U prvoj grupi preparacija kanala je realizovana instrumentom sa recipročnim pokretima – UNICONE (MEDIN, Czech Republic), a u drugoj instrumentom sa punom rotacijom – XP-endo Shaper (XPS) (FKG, DENTAIRE, Swiss).

Kao sredstvo za irigaciju u obe grupe korišćen je 2% rastvor NaOCl i 17% rastvor EDTA. SEM analiza razmaznog sloja apikalne trećine je urađena na standardnom uvećanju od 1000× i procenjivana prema skali od 1 do 5.

Rezultati Istraživanje je ukazalo na uglavnom čiste zidove kanala i bez razmaznog sloja u apikalnoj regiji u obe testirane grupe. Zidovi kanala su bili nešto čistiji posle primene instrumenta sa recipročnim pokretima – UNICONE (80%) u odnosu na kanale druge grupe gde je korišćen instrument sa punom rotacijom – XPS (76%), ali bez statistički značajnih razlika.

Zaključak NiTi sistemi sa samo jednom turpijom ne uklanjaju potpuno razmazni sloj, ali obezbeđuju efikasno čišćenje apikalnog dela kanala. Turpija sa recipročnim pokretima UNICONE je bila nešto efikasnija u uklanjanju razmaznog sloja u odnosu na XPS turpiju sa punom rotacijom.

Ključne reči: razmazni sloj; recipročni pokreti; NiTi rotirajući instrumenti; SEM

UVOD

Mašinska instrumentacija danas predstavlja standard u hemomehaničkoj preparaciji, a kvalitetno čišćenje i oblikovanje kanalskog sistema je osnovni preduslov za uspešnost endodontskog tretmana. NiTi instrumenti poseduju izrazitu fleksibilnost i elastičnost, koja obezbeđuje efikasnu i bezbednu preparaciju [1, 2], ali zbog čestog loma njihova primena u povijenim kanalima predstavlja značajan rizik [3, 4, 5]. Da bi se eliminisao rizik tokom njihovog korišćenja, dentalna tehnologija je razvila nekoliko novih strategija u dizajniranju ovih instrumenata koje uključuju novi poprečni presek i dizajn radnog dela instrumenta, posebne termomehaničke procedure u pripremi legure, odnosno različitu kinetiku kretanja instrumenta u kanalu [2, 6, 7, 8].

Uvođenje novog sistema preparacije kanala koji se bazira na primeni instrumenata sa recipročnim pokretima ukazalo je na nove mogućnosti u čišćenju i oblikovanju [9, 10]. Problemi vezani za deformaciju i lom kod instrumenata sa recipročnim pokretima su značajno manji nego kod instrumenata sa punom rotacijom [9, 10, 11]. Naime, recipročni pokreti su bazirani na tehnici balansiranih sila i podrazumevaju rotaciju instrumenta u smeru suprotnom kretanju kazaljke na satu (sekući smer) i mnogo kraćeg pokreta u smeru kazaljke (opuštanje instrumenta), čime se značajno smanjuje površina kontakta instrumenta sa dentinom, a time i torzioni stres i ciklični zamor [7, 9, 12, 13].

Smanjivanje broja instrumenata neophodnih za preparaciju takođe je jedan od aktuelnih zahteva hemomehaničke preparacije, pa su i instrumenti sa recipročnim pokretima najčešće predstavljeni sa samo jednim instrumentom [9, 10, 14, 15]. Turpije sa recipročnim pokretima su izrađene od specijalne NiTi legure i sa specifičnim termičkim tretmanom, čime su im značajno povećane fleksibilnost i otpornost na ciklični zamor [8, 9, 15, 16].

Rezultati istraživanja o recipročnim sistemima potvrđuju prednosti ovih instrumenata vezane za kraće vreme preparacije [9, 12], povećanu otpornost na ciklični zamor [9, 13, 15] i slične efekte vezane za efikasnost oblikovanja u poređenju sa sistemima pune rotacije koji koriste više turpija [9, 10, 16, 17].

Iako je potvrđeno da je čišćenje kanalskog sistema zuba teško ostvarivo (pogotovo u apikalnoj trećini), podaci o efektima turpija sa recipročnim pokretima su vrlo oskudni. Brojne studije su potvrdile da efikasno sečenje dentina ipak najviše zavisi od samog instrumenta: materijala od koga je izrađen, dizajna radnog dela, broja navoja, dizajna vrha, posebnog tretmana površine turpije, odnosno dinamike i kinetike kretanja unutar kanala [9, 10, 12, 16, 18].

Cilj ovog rada je bio da se SEM analizom proveri efikasnost čišćenja apikalne trećine kanala primenom dva različita NiTi rotaciona sistema: jednog instrumenta sa recipročnim pokretima i jednog instrumenta sa punom rotacijom.

Testirane su sledeće hipoteze:

1. Recipročni sistemi sa jednom turpijom produkuju manje razmaznog sloja nego rotirajući sistemi sa jednom turpijom.
2. Recipročni sistemi sa jednom turpijom produkuju slične količine razmaznog sloja kao i rotirajući sistemi sa jednom turpijom.

MATERIJAL I METOD

Korišćeno je 30 jednokorenih zuba (gornji sekutići) ekstrahovanih zbog parodontalnih problema, koji su metodom slučajnog izbora podeljeni u dve grupe.

Kod svih zuba je posle formiranja pristupnog kaviteta određena radna dužina (1 mm kraće od dužine na kojoj se vrh instrumenta pojavljuje na apeksu) i potom na vrhu korena po-

stavljena kuglica roze voska. Preparaciju svih kanala realizovao je jedan istraživač.

U grupi 1 preparacija je realizovana jednim instrumentom sa recipročnim pokretima UNICONE (25/06) (MEDIN, Czech Republic). Posle provere prohodnosti kanal je ispunjen 2% rastvorom NaOCl (CHLORAXID 2%, Cercamed, Poland) i potom instrument nežnim pokretima uvlačenja i izvlačenja plasiran do radne dužine (3–5 puta). Kanal je ispiran sa 2 ml rastvora pre i posle instrumenta. Potom je u kanal ubačen 17% rastvor EDTA (Calcinase, EDTA solution, Lege artis, Pharma GmbH) i istim pokretima (3–5 puta) plasiran do radne dužine (1 ml). Finalno ispiranje je realizovano sa dodatnih 2 ml rastvora NaOCl.

U grupi 2 preparacija kanala je urađena jednim instrumentom sa potpunom rotacijom XPS (30/04)(FKG, Dentaire, Swiss). U kanal ispunjen 2% rastvorom NaOCl instrument je nežnim pokretima uvlačenja i izvlačenja tokom jednog minuta (10–12 pokreta) plasiran do radne dužine. Kanal je i ovde ispiran sa 2 ml rastvora pre i posle instrumenta. Isti postupak je ponovljen i posle ubacivanja 17% rastvora EDTA (1 ml). Finalno ispiranje je i ovde urađeno 2% rastvorom NaOCl (2 ml).

Preparacija u obe grupe je realizovana endomotorom ENDO A CLASS (Medin, Czech Republic) koji poseduje i opcije sa recipročnom i opcije sa punom rotacijom.

Krunice zuba su zatim presecane u predelu gleđno-cementne granice, tako da je svaki segment korena imao dužinu 12 mm. Uz pomoć dijamantskog diska napravljena su dva žleba na vestibularnoj i oralnoj strani zuba i potom koren dletom razdvojen na polovine. SEM analiza je urađena samo u apeksnoj trećini (region 3 mm od granice preparacije) (JOEL, JSM, 6460 LV, Japan), a za svaki uzorak je napravljeno pet standardizovanih mikrofotografija (uvećanja 1000 puta), koje su sačuvane i potom analizirane i ocenjivane od strane dva nezavisna istraživača. Prisustvo razmaznog sloja na zidovima kanala procenjivano je prema kriterijumima Hilsmana i sar. [19].

Ocena 1 – nema razmaznog sloja i dentinski tubuli otvoreni;

Ocena 2 – malo razmaznog sloja i nekoliko tubula otvoreno;

Ocena 3 – homogeni razmazni sloj pokriva zid i malo je tubula otvoreno;

Ocena 4 – ceo zid kanala je pokriven razmaznim slojem i nema otvorenih tubula;

Ocena 5 – nehomogen razmazni sloj pokriva celukupan zid kanala.

SEM fotografije dobijenih uzoraka (300 slika) proučavala su dva nezavisna istraživača. Dobijeni rezultati su obrađeni u programu SPSS 20 (IBM „Chicago"). U statističkoj analizi su korišćeni metodi deskriptivne statistike, a za proveru učestalosti nalaza između ispitivanih parametara korišćen je hi-kvadrat test.

REZULTATI

Dobijeni rezultati istraživanja prikazani su u tabelama 1 i 2 i na Slici 1 (a, b).

SEM analiza uzoraka je ukazala na uglavnom čiste zidove kanala u apikalnoj regiji i identičnu prosečnu ocenu razmaznog sloja u obe testirane grupe (Tabela 1).

Čist kanal (ocena 1 i 2) u apikalnoj trećini je registrovan u 80% uzoraka gde je preparacija kanala realizovana instrumentom sa recipročnim pokretima UNICONE, odnosno u 76% uzoraka gde je korišćen instrument sa punom rotacijom XPS. Ova razlika nije bila statistički značajna (Tabela 2).

Čisti zidovi sa malo razmaznog sloja u apikalnoj trećini najčešće su ocenjivani ocenom 2 i u grupi gde je preparacija realizovana instrumentom sa recipročnim pokretima (Slika 1a) i u grupi sa punom rotacijom (Slika 1b).

DISKUSIJA

Čist kanal korena bez razmaznog sloja je jedan od osnovnih preduslova za uspeh endodontskog lečenja [12, 16, 17]. Činjenica je da je efikasno čišćenje kanalskog sistema teško ostvarivo i da je problem apikalne trećine, zbog nepristupačnosti, neadekvatnog dijametra apeksne preparacije, odnosno količine, koncentracije i vremena izlaganja ovog dela kanala rastvorima za irigaciju, dodatno otežan [12, 16, 17, 20, 21, 22].

Osnovna svrha ovog istraživanja je bila da se uporede efekti dve turpije sa različitom kinetikom kretanja u kanalu tokom preparacije (recipročni i pokreti sa punom rotacijom) i analizira njihov uticaj na kvalitet čišćenja kanala. Endodontsku proceduru je realizovao jedan praktičar na jednokorenim zubima (jednostavni kanalski sistemi) po identičnom protokolu irigacije (i sa identičnom količinom rastvora) kako bi postupak preparacije bio što bliži kliničkim uslovima.

Rezultati ovih istraživanja nisu pokazali značajne razlike u količini zaostalog razmaznog sloja u apikalnoj trećini između turpije sa recipročnim pokretima (UNICONE) i turpije koja koristi punu rotaciju (XPS), pa je zato prva hipoteza odbačena. Prihvaćena je druga hipoteza jer su oba instrumenta tokom preparacije produkovala slične količine razmaznog sloja.

Iako neznatno, nešto čistiji kanal i manja količina razmaznog sloja u apikalnom delu posle primene turpije sa recipročnim pokretima mogli bi se objasniti, pre svega, dizajnom radnog dela instrumenta i njegovom sečivnom efikasnošću uz pojačanu agitaciju rastvora za irigaciju [9, 16, 17], s obzirom na to da uticaj dinamike kretanja turpije u malom broju realizovanih istraživanja nije potvrđen [20, 23, 24].

U literaturi je malo studija koje su ispitivale efektivnost recipročnih turpija u uklanjanju razmaznog sloja sa zidova kanala [12, 16, 17]. U ovom istraživanju je kao instrument sa recipročnim pokretima korišćena nova NiTi turpija (UNICONE) sa specifičnim dizajnom radnog dela i različitim heliksnim uglom, koji obezbeđuje izuzetnu fleksibilnost i bolju eliminaciju dentinskog debrisa tokom preparacije. Konveksni trouglasti poprečni presek i neaktivni vrh uz posebnu termičku obradu legure obezbeđuju efikasno sečenje dentina i brzu i bezbednu preparaciju uz značajno povećanu otpornost na savijanje i ciklični zamor [7, 9, 25].

Nešto efikasnije čišćenje i manje razmaznog sloja u apikalnoj regiji primenom UNICONE trupije moglo bi se objasniti pre svega dobrom sečivnom efikasnošću instrumenta [18] odnosno pojačanom agitacijom rastvora za irigaciju uzrokovanom recipročnim pokretima u kanalu [26]. Rezultati ovog istraživanja su u saglasnosti sa studijama koje su potvrdile efikasnost recipročnih turpija u uklanjanju razmaznog sloja i bolje čišćenje apikalnog dela u poređenju sa instrumentima sa punom rotacijom [12, 26].

Međutim, u studiji Poggioa i sar. instrumenti sa recipročnim pokretima su u kanalskom sistemu jednokorenih zuba produ-

kovali veću količinu razmaznog sloja [16]. Oni ovo objašnjavaju činjenicom da ovi pokreti u kanalu produkuju „dobro upakovan“ razmazni sloj.

Turpije sa recipročnim pokretima obezbeđuju slične efekte oblikovanja kanala korena kao i sistemi sa punom rotacijom [27], ali im efekat sečenja dentina može biti redukovan zbog produžene (višekratne) kliničke upotrebe, što za posledicu može imati i veću količinu razmaznog sloja na zidovima kanala.

Efikasnost čišćenja apikalne regije kod sistema sa punom rotacijom proveravana je i posle primene novog instrumenta sa jedinstvenom geometrijom XPS-a. Specifičan dizajn radnog dela uz ekstremnu fleksibilnost instrumenta omogućava menjanje oblika tokom rotacije u kanalu (kontrahuje se i širi) i dosezanje do nepristupačnih delova kanala do kojih većina turpija ne dospeva [30]. Zmijoliki oblik XPS-a uz superelastičnost i nešto veću brzinu rotacije obezbeđuje povećanu „turbulenciju“ rastvora za irigaciju, što omogućava da se debris održava u tečnom stanju i manje zadržava na zidovima kanala [30].

Manje razmaznog sloja u apikalnoj regiji posle primene XPS-a u ovom istraživanju moglo bi se objasniti i adekvatnom dimenzijom apikalne preparacije (30/04), čime se obezbeđuju bolji efekat rastvora za irigaciju i efikasnija elimincija razmaznog sloja sa zidova kanala [17, 29, 30]. Ovi rezultati su u saglasnosti sa nalazima studije koja se bavila količinom apikalno ekstrudiranog debrisa posle preparacije različitim instrumentima, ali je između ostalog ukazala i na značajno manju količinu debrisa u apeksnom delu kanala posle primene XP-endo Shapera [31]. Iako radni deo XPS-a nema izraženi sečivni efekat, svojim naizmeničnim širenjem i skupljanjem u kanalu pojačava rastvarački efekat irigansa i time može značajno uticati na čišćenje teško dostupnih zona kanala [29].

ZAKLJUČAK

U okviru ograničenja ove studije dobijeni rezultati su pokazali da NiTi sistemi sa samo jednom turpijom (uz recipročne odnosno pokrete pune rotacije) ne uklanjaju potpuno razmazni sloj, ali obezbeđuju efikasno čišćenje apikalnog dela kanala. Turpija sa recipročnim pokretima UNICONE je bila nešto efikasnija u uklanjanju razmaznog sloja i ukazala na čistije zidove u apikalnoj trećini.

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Examination of postextraction space closure speed using elastic chains and NiTi closed coil springs

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SUMMARY

Introduction In everyday clinical practice, we often encounter a lack of space for placing all the teeth present into dental arch; therefore it is often recommended to extract teeth within orthodontic treatment. In clinical practice, the most commonly used methods of closing space after are elastic chains and NiTi closed spiral springs.

The aim of this paper was to compare postextraction space closure speed using two different sliding mechanisms, NiTi closed coil springs and elastic chains within treatment with fixed orthodontic appliances.

Material and Method The total sample in this study consisted of 46 postexstraction spaces in 23 patients indicated for the extraction of first premolars using treatment with fixed orthodontic appliances. Two sliding mechanisms, NiTi closed coil spring and elastic chains were applied to postextraction space closure. Postextraction spaces were monitored for 4 months with appointments every 4 weeks. During appointments mechanisms were activated and digital caliper was used to measure the width of the postextraction space.

Results The results showed that NiTi closed coil springs method achieved greater reduction in postexstraction space (3.94 mm) while with elastic chain method the closure of 3.10 mm was achieved. The total difference between these two methods in the observed period was 0.84mm and no statistically significant difference was found ($p > 0.05$). The lowest value for NiTi closed springs was 2.19 mm, while for the elastic chain it was 1.29 mm. The best postextraction space closure was 5.70 mm and it was completed using NiTi closed springs while for elastic chain the best value was 4.80 mm.

Conclusion: NiTi closed coil springs lead to faster closure of postextraction spaces in relation to elastic chain. Since this difference is minimal, in practice, both methods can be used equally.

Keywords: NiTi closed coil springs; elastic chains; postextraction space closure

INTRODUCTION

In everyday clinical practice, a lack of space for alligning all present teeth in dental arch is common problem. There are various clinical procedures that can create additional space in dental arch like expansion, molar distalization, interproximal reduction, protrusion of teeth [1]. Dental arch expansion can be dentoalveolar and skeletal, but for every 4 mm expansion, only 1 mm of space is gained along the arch. Molar distalisation requires an additional intra or extraoral anchorage for their movement. Interproximal reduction or stripping is done by grinding of enamel from the aproximal surfaces of the tooth, which must not be more than half of its total thickness. These clinical procedures are carried out in cases when lack of space is not more than 2.5 mm on one side of dental arch [2, 3]. However, in some cases, where there is bigger lack of space, teeth extraction is used [3, 4].

The teeth that are most often extracted in clinical procedure are first premolars. Postextraction space closure is done by bringing adjacent teeth to the place of extracted first premolar in one phase (*en-masse*) or in two phases. *En-masse* retraction of teeth means simultaneously moving all frontal teeth (4 incisors and 2 canines), while the two-phase closure first moves canine into the postextraction space and then incisors. The method to be applied depends on the type of orthodontic maloclusion and therapist skills [5, 6, 7].

There are various techniques for carrying out this clinical procedure, and proper selection can affect the outcome of the treatment. Two basic techniques that differ in biomechanics are closing loops and sliding mechanics [8].Closing loops are incorporated into the archwire design and transmit force over it to close the space without any friction [9]. Sanjay et al. stated that the closure of postextraction spaces without friction, while producing continuous forces, was the biggest advantage of a loop [10]. On the other hand, Chaudhari et al. stated that large bendings of wire that require precision and orthodontic skills may cause great loss of time, difficulties in determining the strength of applied force, as well patient discomfort as major weaknesses of closing loops [11]. Unlike the loops, force must be applied to sliding mechanism, which can overcome archwire resistance through the bracket system and move the teeth along the archwire, with friction occurring [12].

In clinical practice, the most commonly used mechanisms are elastic chains and NiTi closed coil springs [13]. Mc Laughlin and Benett give advantage to elastic chains

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because they are not expensive, easy to use and can be applied to different clinical cases. However, they must be changed every 4 to 6 weeks due to plaque retention that affects their mechanical properties and leads to force decrease [14]. NiTi closed coil springs have ability to memorize shape and superelasticitiy and therefore retain constant force through the time, which makes them highly reliable [15]. Although difficult to maintain oral hygiene, Wichelhaus found that the presence of plaque does not diminish their mechanical properties. Their disadvantage is that they are expensive, but they do not need to be changed, just reactivated on the control checkups [16].

The aim of this study was to compare postextraction space closure speed using two different sliding mechanisms, NiTi closed coil springs and elastic chains within treatment using fixed orthodontic appliances.

MATERIAL AND METHODS

The study was carried out at the Faculty of Medicine - study program Dentistry in Banja Luka, with the approval of the Ethic Committee of the Institute of Dentistry. The total sample in this study was 46 postextraction spaces in 23 patients who had first premolar extracted for the purpose of orthodontic treatment. Respondents were required to meet the following inclusion criteria: 12–18 years of age at the beginning of treatment, no contraindications for orthodontic treatment, no other extractions (except premolar) and written consent of the examinee or parent. Exclusion criteria for this study were: one or more teeth missing (apart from the third molars), cleft palate or some of the craniofacial syndromes, poor oral hygiene, teeth development anomalies, patients who do not come for checkups regularly, and patients previously treated with fixed orthodontic appliances.

Methodology by McLaughlin et al. was used in the research [14]. After the first premolar extractions, fixed orthodontic appliance (Dentaurum, Discovery, Roth prescription, slot 0.022 in) was placed, and then initial leveling was performed with NiTi round and rectangular archwires before placing stainless steel rectangular arches. Rectangular stainless steel archwire of 0.019x0.025 in was in the bracket slots for at least 4 weeks to become passive and then an appropriate sliding mechanisms were applied to close the postextraction space according to methodology of Balhoff et al. [17].

Patients were randomly assigned to the two groups according to the type of mechanism used to close the postextraction space:

Group 1: Elastic Chains (American Orthodontics, USA) - 24 post extraction spaces

Elastic chain was placed from the hook of canine bracket to the first molar hook, stretching it to approximately double the initial length. On each subsequent visit it was replaced by a new one.

Group 2: NiTi closed coil spring (American orthodontics, USA) - 22 post extraction spaces

NiTi closed coil spring was placed from the hook of canine bracket to the first molar hook, with the springs not stretched more than 9 mm. It was activated during each appointment.

Measurements

Postextraction spaces were monitored for 4 months from the beginning of their closure. Control appointments were performed every 4 weeks and it was verified whether the applied mechanisms have been damaged and their activation was performed. Digital vernier caliper (precision 0.01 mm) was used to mesure maximum distance from the distal surface of canine to mesial surface of the second premolar at each appointment:

- T_0 – at the beginning of the postextraction space closure
- T_1 – after 4 weeks
- T_2 – after 8 weeks
- T_3 – after 12 weeks
- T_4 – after 16 weeks

RESULTS

Post-extraction areas after four months (T_0-T_4) in the application of the NiTi closed coil spring were at average 3.94 mm while this value with the elastic chain method was 3.10 mm. The total difference between these two methods in the observed period was 0.84 mm. The lowest value for NiTi closed springs was 2.19 mm, while it was 1.29 mm for the elastic chain. The maximum movement of tooth into postextraction space after application NiTi closed coil spring was 5.70 mm, elastic chain 4.80 mm (Table 1).

Table 1. Descriptive statistics for total sample after four months (T_0–T_3)
Tabela 1. Pokazatelji deskriptivne statistike na ukupnom uzorku posle četiri meseca (T_0–T_3)

	N	M	SD	Min	Max
NiTi closed coil spring NiTi zatvorena opruga	22	3.94	1.06	2.19	5.70
Elastic chain Elastični lanac	24	3.10	1.00	1.29	4.80

Table 2. Number of closed postextraction spaces on monthly basis depending on the choice of method
Tabela 2. Broj zatvorenih postekstrakcionih prostora na mesečnom nivou zavisno od izbora metode

	NiTi closed coil spring NiTi zatvorena opruga		Elastic chain Elastični lanac	
	f	%	f	%
1.	0	0.00	0	0.00
2.	0	0.00	1	4.17
3.	7	31.81	10	41.67
4.	13	59.09	20	83.33

In the first three months, there was a difference in the speed of space closure in favor of NiTi closed springs, but no statistically significant differences were found between them ($p > 0.05$). In the first month the difference was 0.20 mm, in second 0.10 mm and in third 0.18 mm. Unlike to

the first three months, statistically significant differences in mean reduction of the postextraction space ($p < 0.05$) was found in the fourth month in favor of NiTi closed coil springs, giving a difference of 0.29 mm.

After 4 months, using elastic chains, 20 (83.33%) postextraction spaces were completely closed, while in the NiTi closed coil springs group only 13 (59.09%) (Table 2).

DISCUSSION

Effectiveness of postextraction space closure depends on a number of factors, such as the type of brackets, size of archwire and applied mechanism [18]. Also, an important role is played by individual differences, the different structure of periodontal fibers and bone activity in adult patients and children, as well as the resistance of the alveolar bone to pressure and its elastic capacity. Space closing speed depends on when the force begins to apply, as the regenerative bone tissue fills the tooth socket of the extracted tooth for 3 weeks and becomes resistant and solid in 3 months. As the bone tissue becomes tighter, the rate of tooth movement is reduced [19]. More efficient and faster closing of the postextraction space is also affected by the force of the applied mechanism over time. Only mild continuous forces provide an optimal system for the movement of tooth in a biologically acceptable way without adverse effects. Orthodontic tooth movement requires the application of continuous force over a certain period of time, whereby the efficiency increases if the force is maintained for as long as possible [20, 21].

Although there are numerous ways to close post-extraction spaces, many authors recommend sliding mechanisms as very effective method using fixed orthodontic appliances [22]. Minimal bending of the wire, quick and simple reactivation and time saving are the advantages that make the sliding mechanism method of choice for most orthodontists [11]. Monini et al. found that 63.8% of the orthodontists use sliding mechanism for postextraction space closure due to simple use [13]. In a study conducted by Banks et al. in United Kingdom, they found that loops are almost never used while sliding mechanisms are applied in 98% of cases. Two most commonly used methods are elastic chains and NiTi closed coil springs, and that is why they were subject of our research [23].

In our study, we used methodology of McLaughlin's et al. who used rectangular stainless steel archwires of 0.019 x 0.025 in diameter in brackets with slots 0.022 in. These archwires have maximum rigidity and at the same time provide sufficient free sliding [14]. At the begining, 50 postextraction spaces were observed in 25 patients, however, 4 spaces closed in the leveling phase of the treatment, so they could not be included in the study and the final number was 46. Control appointments were performed once a month, or every 4 weeks and applied sliding mechanics were activated. In clinical practice, it is common to do control checkups every 4 to 6 weeks. If the appliance produces mild continuous forces and only leads to frontal resorption, no additional activation is required. Frequent activation does not allow proper reparative process to occur and can lead to teeth damage. This can be prevented or decreased with not so frequent activation [20, 21, 24].

In the current study, at 24 postexstraction spaces elastic chains were applied according to the method of Balhoff et al., based on comparative study using different techniques of placing elastic chain. They came to conclusion that direct placement from molar hook and hook on canine bracket or front hooks on the wire is the most effective, and that is why this method was applied [17]. The results of our study showed that in the observed period of 4 months using elastic chains postextraction space closure was 3.10mm. In the first three months, spaces were closing faster, while the fourth month showed reduced speed of closure compared to the previous 3 months. These results can be explained by the fact that control appointments and measurements were performed every month, and in the last month there was great number of postextraction spaces already closed (20), where the time between T_3 to T_4 measurement was also smaller.

Nightingale and Jones observed closure of 20 postextraction spaces on a weekly basis using elastic chains and came to similar results with value of 0.21mm [25]. Bokas and Woods conducted study on 22 postexstraction spaces closure using elastic chains within fixed orthodontic treatment with approximately 200g of initial force. Their research suggests faster closure of postextraction spaces when using this method (1.68 mm per month) [26]. Unlike previous authors, Chaudhari and Tarvade came to results with significantly lower values, on average 0.62 mm per month [11]. The results of Fang et al. research are in agreement with the previous one, with somewhat smaller value of 0.52mm [27]. In these researches, *en-masse* method of tooth movement was used, where the mechanisms were placed at attached hooks on posted stainless steel archwire and molar hook on tube, that resulted in somewhat slower closure of postextraction spaces.

In the current study, NiTi closed coil springs were applied to 22 post exstraction spaces in the same way as elastic chains, except in cases of larger distances when they were placed indirectly. Nightingale et al. recommend the method of indirect placement through steel ligatures in situations where they cannot be placed directly or due to excessive stretching that would result in mechanism damage and undesired effects of tooth movement [25].

The results of our study showed that in the observed period of 4 months, post-extraction space closure of 3.91 mm was achieved using NiTi closed coil springs. We found that in the first three months the spaces were closing faster, while in the fourth month there was reduced speed compared to the first 3 months. Studies using similar methodology showed actually different results. Nightingale and Jones established 0.26 mm closure in 20 post extraction spaces on a weekly basis, and that is consistent with our research [25], while the results of Bokas and Woods reported significantly higher values of 1.85 mm [26]. In studies of Chaudharia and Tarvade, as well as Fang et al. using the *en-masse* method, the average measured values were 0.87 mm and 1.06 mm [11, 27].

Comparing the rate of post-extraction spaces closure in observed period, our study found the value greater

than 0.84 mm using NiTi closed coil springs. There are numerous studies that compared these two methods and confirmed these results [22, 28, 29]. In addition to these, Dixon et al. also compared active ligatures ie. sliding mechanism consisting of a steel ligature and elastic ring. In their research, NiTi closed coil springs proved to be the fastest mechanism (0.81 mm per month), then elastic chains (0.58 mm per month) and the slowest active ligature (0.35 mm per month). The authors also showed the number of post extraction space closure that was completed in 4 months, which was significantly different from the results obtained in the current study. The highest number of space closures were found in the application of active ligatures (38%), followed by NiTi closed coil springs (32%), and at least elastic chains (30%), while in our study, after 4 months the method of elastic chains and NiTi closed coil springs closed 33% and 59.09% of post extraction spaces, respectively. Considering that in both studies most spaces were closed slowly, the conclusion would be that these methods were applied to those spaces that had lower values after the leveling stage of orthodontic treatment [30].

One of the most important factors that affects the speed of tooth movement is the strength and type of applied force. The force produced by elastic chains decreases rapidly in the first 24 hours and then continues to decline until the next control appointment, therefore it can be called intermittent rather than continuous [31]. Nickel titanium alloys have the properties of superelasticity and shape memory, that makes NiTi closed coil springs produce mild continuous forces over a longer period of time. In several *in vivo* studies, NiTi springs have been shown to provide faster and more reliable space closure, and that is consistent with our research. It is believed that retaining a constant force over a certain period of time contributes most to their efficiency [16, 32].

CONCLUSION

NiTi closed coil springs lead to faster post extraction spaces closure compared to elastic chain. Considering that the difference was minimal with no great clinical significance, in practice, both methods can be used equally to clinician choice.

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Ispitivanje brzine zatvaranja postekstrakcionog prostora elastičnim lancem i NiTi zatvorenim spiralnim oprugama

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KRATAK SADRŽAJ

Uvod U svakodnevnoj kliničkoj praksi se često susrećemo sa nedostatkom prostora za postavljanje svih prisutnih zuba u zubni niz, pa se zato u okviru ortodontske terapije veoma često preporučuje ekstrakcija zuba. U kliničkoj praksi među najčešće korišćenim metodama su elastični lanci i NiTi zatvorene spiralne opruge.

Cilj ovog rada je bio da se uporedi brzina zatvaranja postekstrakcionog prostora primenom dva različita klizna mehanizma, NiTi zatvorenim spiralnim oprugama i elastičnim lancima u okviru terapije fiksnim ortodontskim aparatima.

Materijal i metode rada Ukupan uzorak u istraživanju činilo je 46 postekstrakcionih prostora kod 23 pacijenta kojima je indikovana ekstrakcija prvih premolara i primena fiksnog ortodontskog aparata u cilju sprovođenja terapije. Za zatvaranje postekstrakcionih prostora primenjena su dva klizna mehanizma – NiTi zatvorene spiralne opruge i elastični lanci. Postektrakcioni prostori su praćeni tokom četiri meseca sa kontrolnim pregledima svake četiri sedmice. Na kontrolnim pregledima su aktivirani mehanizmi, a digitalnim nonijusom merene širine postektrakcionih prostora.

Rezultati Rezultati istraživanja pokazuju da posle četiri meseca kod primene NiTi zatvorene opruge dolazi do nešto većeg smanjenja postekstrakcionog prostora, pri čemu je prosečna vrednost kod ove metode iznosila 3,94 mm, u odnosu vrednosti metode elastičnog lanca od 3,10 mm. Ukupna razlika između ove dve metode u posmatranom periodu je bila 0,84 mm i nije utvrđena statistički značajna razlika ($p > 0,05$). Najmanja vrednost za NiTi zatvorene opruge je iznosila 2,19 mm, dok je za elastični lanac iznosila 1,29 mm. Najveće pomeranje zuba u postekstrakcioni prostor posle primene NiTi zatvorene opruge je iznosilo 5,70 mm, a kod elastičnog lanca 4,80 mm.

Zaključak NiTi zatvorene spiralne opruge dovode do bržeg zatvaranja postekstrakcionih prostora u odnosu na elastični lanac. S obzirom na to da je ova razlika minimalna, u praksi se mogu ravnopravno koristiti obe metode.

Ključne reči: NiTi zatvorene spiralne opruge; elastični lanac; postekstrakcioni prostor

UVOD

U svakodnevnoj kliničkoj praksi kod pacijenata se susrećemo sa nedostatkom prostora za postavljanje svih prisutnih zuba u zubni niz. Postoje razne kliničke procedure kojima se može stvoriti dodatni prostor u zubnom luku koji bi omogućio uslove za postizanje pravilne okluzije. Dodatni prostor u zubnim lukovima se može dobiti njihovim širenjem, distalizacijom molara, interproksimalnom redukcijom, protruzijom zuba [1]. Širenje ili ekspanzija zubnih lukova može biti dentoalveolarna i skeletna, ali na svaka 4 mm ekspanzije oslobađa se samo 1 mm prostora duž luka. Distalizacija molara zahteva dodatno intraoralno ili ekstraoralno uporište za njihovo pomeranje. Interproksimalnom redukcijom ili stripovanjem se vrši brušenje gleđi sa aproksimalnih površina zuba koje ne sme da iznosi više od polovine njene ukupne debljine. Ove kliničke procedure se sprovode u slučajevima gde postoji manji nedostatak prostora, odnosno gde nam je potrebno najviše 2,5 mm prostora sa jedne strane zubnog niza [2, 3]. Međutim, u nekim slučajevima gde je veći nedostatak prostora primenjuje se ekstrakcija zuba, odnosno smanjenje broja zuba u zubnom nizu [3, 4].

Ortodontska terapija kod koje se primenjuje ekstrakcija zuba ima za cilj postizanje pravilne okluzije, sklada u izgledu lica i stabilnih rezultata lečenja [3, 4]. U kliničkoj proceduri, u svrhu ortodontske terapije, zubi koji se najčešće ekstrahuju su prvi premolari. Zatvaranje postekstrakcionog prostora se vrši dovođenjem susednih zuba na mesto ekstrahovanog prvog premolara u jednoj fazi (*en-masse*) ili u dve faze. Pomeranje ili retrakcija zuba *en-masse* podrazumeva istovremeno pomeranje svih frontanih zuba (četiri sekutića i dva očnjaka), dok se dvofaznim zatvaranjem prvo pomera očnjak u postekstrakcioni prostor, a zatim sekutići. Koja će se metoda primeniti zavisi od vrste ortodontske nepravilnosti i veštine i umeća terapeuta [5, 6, 7].

Postoje različite tehnike za sprovođenje ove kliničke procedure, a pravilan izbor odgovarajuće može da utiče na ishod terapije. Dve osnovne tehnike koje se razlikuju po biomehanici su mehanizam omči za zatvaranje prostora i klizni mehanizam [8]. Omče su inkorporirane u dizajn žičanog luka i imaju ulogu da preko njega prenesu silu koja dovodi do zatvaranja prostora bez pojave trenja [9]. Sanjay i saradnici navode da zatvaranje postekstrakcionih prostora bez pojave trenja, pri čemu se proizvode kontinuirane sile, predstavlja najveću prednost omči [10]. S druge strane, Chaudhari i saradanci kao glavne nedostatke omči navode velika savijanja žice koja zahtevaju preciznost i veštinu ortodonta, što uzrokuje veliki gubitak vremena, zatim poteškoće u određivanju jačine primenjene sile, kao i nelagodnost koju izazivaju kod pacijenta [11]. Za razliku od omči, kod kliznog mehanizma se mora primeniti sila koja može savladati otpor klizanja žičanog luka kroz sistem bravica i pomeriti zube duž žičanog luka, pri čemu se javlja trenje [12].

U kliničkoj praksi među najčešće korišćenim mehanizmima su elastični lanci i NiTi zatvorene spiralne opruge [13]. Mc Laughlin i Benett daju prednost elastičnim lanacima zato su što nisu skupi, lako se koriste i mogu se primeniti na različite kliničke slučajeve. Međutim, moraju se menjati svakih četiri do šest sedmica zbog zadržavanja plaka, koji utiče na njihova mehanička svojstva i dovodi do očekivanog opadanja sile [14]. NiTi zatvorene spiralne opruge imaju sposobnost memorisanja oblika i superelastičnosti, zbog čega zadržavaju konstantnu silu kroz vreme i zbog čega ih Mangazini i saradnici smatraju veoma pouzdanim [15]. Iako otežavaju održavanje oralne higijene, Wichelhaus je utvrdio da prisustvo plaka ne umanjuje njihove mehaničke osobine. Nedostatak je što su skupi, ali se na kontrolnim pregledima ne moraju menjati, već ponovo aktivirati [16].

Cilj ovog rada je bio da se uporedi brzina zatvaranja postekstrakcionog prostora sa dva različita klizna mehanizma,

elastičnim lancima i NiTi zatvorenim spiralnim oprugama u okviru terapije fiksnim ortodontskim aparatima.

MATERIJAL I METODE

Istraživanje je sprovedeno na Medicinskom fakultetu na studijskom programu stomatologija u Banjaluci, uz saglasnost Etičkog komiteta Zavoda za stomatologiju. Ukupan uzorak u istraživanju činilo je 46 postekstrakcionih prostora kod 23 pacijenta kojima je indikovana ekstrakcija prvih premolara u cilju sprovođenja ortodontske terapije. Ispitanici su morali ispunjavati sledeće inkluzione kriterijume: uzrast od 12 do 18 godina na početku terapije, da nema kontraindikacija za ortodontsku terapiju, da nema drugih ekstrakcija (osim premolara) i pismeni pristanak ispitanika ili roditelja. Ekskluzioni kriterijumi za ovu studiju su bili: nedostatak jednog ili više zuba (osim trećih molara), rascep nepca ili neki od kraniofacijalnih sindroma, loša oralna higijena, razvojne anomalije zuba, pacijenti koji neredovno dolaze na kontrole i pacijenti koji su prethodno bili u terapiji fiksnim ortodontskim aparatima.

U istraživanju je korišćena metodologija koju su ustanovili McLaughlin i saradnici [14]. Posle ekastrakcije prvih premolara ispitanicima je postavljen fiksni ortodontski aparat (*Dentaurum, Discovery, Roth preskripcija, slot 0,022 in*), nakon čega je izvršena početna nivelacija sa NiTi lukovima okruglog i četvrtastog preseka, pre postavljanja čeličnih četvrtastih lukova. Četvrtasti čelični luk preseka 0,019 × 0,025 in je stajao u slotovima bravica najmanje četiri sedmice, da bi postao pasivan, posle čega su aplicirani odgovarajući klizni mehanizmi za zatvaranje postekstrakcionih prostora prema metodologiji Balhoffa i saradnika [17].

Ispitanici su metodom slučajnog izbora raspoređeni u dve grupe prema vrsti mehanizma koji se primenjivao za zatvaranje postekstrakcionog prostora:

Grupa 1: Elastični lanci (*American Orthodontics, USA*) – 24 postekstrakciona prostora

Elastični lanac je postavljan tako da povezuje kukicu na tubi prvog molara i kukicu bravice na očnjaku, pri čemu je rastegnut na približno dvostruku početnu dužinu. Na svakoj narednoj kontroli je bio zamenjen novim.

Grupa 2: NiTi zatvorena spiralna opruga (*American orthodontics USA*) – 22 postekstrakciona prostora

NiTi zatvorena spiralna opruga je postavljana od kukice na tubi prvog molara do kukice bravice na očnjaku, pri čemu opruge nisu bile istegnute više od 9 mm. Za vreme terapije opruga je aktivirana na kontrolnim pregledima.

Merenja

Postekstrakcioni prostori su praćeni četiri meseca od početka njihovog zatvaranja. Kontrolni pregledi su obavljani svake četiri sedmice i na njima je proveravano da li je došlo do oštećenja apliciranih mehanizama i izvršena njihova aktivacija. Digitalnim nonijusom (preciznosti 0,01 mm) na kontrolnim pregledima (T_0–T_4) mereno je maksimalno rastojanje od distalne površine očnjaka do mezijalne površine drugog premolara:

- T_0 – na početku zatvaranja postekstrakcionog prostora
- T_1 – posle četiri sedmice
- T_2 – posle osam sedmica
- T_3 – posle 12 sedmica
- T_4 – posle 16 sedmica

REZULTATI

Rezultati istraživanja pokazuju da na ukupnom uzorku od 46 postekstrakcionih prostora posle četiri meseca (T_0–T_4) kod primene NiTi zatvorene opruge dolazi do nešto većeg smanjenja postekstrakcionog prostora, pri čemu je prosečna vrednost kod ove metode iznosila 3,94 mm, u odnosu na vrednost metode elastičnog lanca – 3,10 mm. Ukupna razlika između ove dve metode u posmatranom periodu je bila 0,84 mm. Najmanja vrednost za NiTi zatvorene opruge je iznosila 2,19 mm, dok je za elastični lanac iznosila 1,29 mm. Najveće pomeranje zuba u postekstrakcioni prostor posle primene NiTi zatvorene opruge je iznosilo 5,70 mm, a kod elastičnog lanca 4,80 mm (Tabela 1).

U prva tri meseca postoji razlika u brzini zatvaranja prostora u korist NiTi zatvorenih opruga, ali nisu utvrđene statistički značajne razlike zavisno od izbora metode ($p > 0,05$). U prvom mesecu je razlika iznosila 0,20 mm, u drugom 0,10 mm i u trećem 0,18 mm. Za razliku od prva tri meseca, u četvrtom mesecu su utvrđene statistički značajne razlike u prosečnom smanjenju postekstrakcionog prostora ($p < 0,05$) u prilog NiTi zatvorenih opruga, pri čemu je dobijena razlika iznosila 0,29 mm.

Posmatrajući promene na mesečnom nivou, utvrđen je veći broj slučajeva zatvaranja postekstrakcionog prostora posle primene elastičnih lanaca u odnosu na NiTi zatvorene opruge. Četiri meseca posle primene elastičnih lanaca došlo je do zatvaranja 20 (83,33%) postekstrakcionih prostora, dok je pomoću NiTi zatvorenih opruga zatvoreno 13 postekstrakcionih prostora (59,09%) (Tabela 2).

DISKUSIJA

Efikasnost zatvaranja postekstrakcionih prostora zavisi od brojnih faktora, kao što su vrsta bravica, veličina žice i primenjenog mehanizma [18]. Takođe, važnu ulogu imaju individualne razlike, različita struktura periodontalnih vlakana i koštane aktivnosti kod odraslih pacijenata i dece, kao i otpornost alveolarne kosti na pritisak i njen elastični kapacitet. Brzina zatvaranja prostora zavisi i od toga kada se počinje sa primenom sile, pošto regenerativno koštano tkivo popunjava alveolarnu čašicu ekstrahovanog zuba za tri sedmice i postaje otporno i čvrsto za tri meseca. Kako koštano tkivo postaje čvršće, smanjuje se brzina pomeranja zuba [19]. Na efikasnije i brže zatvaranje postekstrakcionog prostora utiče i opadanje sile primenjenog mehanizma kroz vreme. Samo blage kontinuirane sile obezbeđuju optimalan sistem sila za pomeranje zuba na biološki prihvatljiv način bez neželjenih efekata. Ortodontsko pomeranje zuba zahteva primenu kontinuirane sile kroz određeni period, pri čemu se efikasnost povećava ukoliko se jačina sile održi što duže [20, 21].

Iako postoje brojni načini za zatvaranje postekstrakcionih prostora, mnogi autori preporučuju klizne mehanizme kao veoma efikasnu metodu u okviru terapije fiksnim ortodontskim aparatima [22]. Minimalno savijanje žice, brza i jednostavna reaktivacija i ušteda vremena su prednosti zbog kojih klizni mehanizam predstavljaju metodu izbora kod većine ortodonata [11]. Monini i saradnici su ustanovili da 63,8% ortodonata koristi klizni mehanizam prilikom zatvaranja postekstrakcionog prostora u okviru terapije fiksnim ortodontskim aparatima zbog jednostavnosti i lakoće primene [13]. U studiji koju su sproveli Banks i saradnici u Velikoj Britaniji ustanovljeno je da se opruge

gotovo i ne koriste, dok se klizni mehanizmi primenjuju u 98% slučajeva. Kao dve najčešće korišćene metode navode se elastični lanci i NiTi zatvorene spiralne opruge, zbog čega su one predmet ovog istraživanja [23].

U ovom istraživanju je primenjena metodologija McLaughlina i saradnika, koji su utvrdili da su četvrtasti čelični lukovi promera 0,019 × 0,025 in u bravicama slota 0,022 in najefikasniji za primenu kliznih mehanizama zato što imaju maksimalnu rigidnost, a istovremeno obezbeđuju dovoljno slobode za klizanje [14]. Na početku je posmatrano 50 postekstrakcionih prostora kod 25 pacijenata, međutim četiri prostora su se zatvorila u nivelacionoj fazi terapije, zbog čega nisu mogla biti uključena u istraživanje, tako da je konačan broj iznosio 46. Kontrolni pregledi, na kojima su se primenjeni klizni mehanizmi aktivirali, obavljani su jednom mesečno, odnosno svake četiri sedmice. U kliničkoj praksi je uobičajeno da se kontrolni pregledi zakazuju na četiri do šest sedmica. Ukoliko aparat proizvodi blage kontinuirane sile i dovodi samo do frontalne resorpcije, nije potrebna dodatna aktivacija. Učestale aktivacije aparata ne dozvoljavaju da se desi odgovarajući reparativni proces, što može dovesti do oštećenja zuba. Ovo se može sprečiti ili umanjiti sa ne tako čestim aktiviranjem [20, 21, 24].

U ovom istraživanju su posmatrana 24 postekstrakciona prostora kod kojih su primenjeni elastični lanci po metodologiji Balhoffa i saradnika. Na osnovu studije u kojoj su poredili različite tehnike postavljanja elastičnih lanaca došli su do zaključka da je direktno povezivanje kukice na molaru i bravice na očnjaku ili prednje kukice na žici najefikasnije, zbog čega je ovaj metod primenjen i u ovom istraživanju [17]. Rezultati ovog istraživanja pokazuju da su se u posmatranom periodu od četiri meseca elastičnim lancima postekstrakcioni prostori zatvorili za 3,10 mm. U prva tri meseca prostori se više zatvaraju, dok četvrti mesec pokazuje smanjenu brzinu zatvaranja postektrakcionog prostora u odnosu na prethodna tri meseca. Ovi rezultati se mogu objasniti činjenicom da su kontrole i merenja obavljani svakih mesec dana, a da je u poslednjem mesecu došlo do najvećeg broja zatvaranja postekstrakcionih prostora (20), gde su i rastojanja pri merenju od T_3–T_4 bila manja.

Do sličnih rezultata su došli i Nightingale i Jones, koji su posmatrali zatvaranje 20 postekstrakcionih prostora primenom elastičnih lanaca na sedmičnom nivou, koje je iznosilo 0,21 mm [25]. Bokas i Woods su sproveli istraživanje u kojem su isitivali zatvaranje 22 postekstrakciona prostora pomoću elastičnih lanaca u okviru terapije fiksnim ortodontskim aparatom sa inicijalnom silom približno 200 g. Njihovo istraživanje govori u prilog bržeg zatvaranja postekstrakcionih prostora prilikom korišćenja ove metode (1,68 mm mesečno) [26]. Za razliku od prethodnih autora, Chaudhari i Tarvade su došli do rezultata sa značajno manjim vrednostima, koja u proseku iznose 0,62 mm mesečno [11]. Rezultati istraživanja Fanga i saradnika su u saglasnosti sa prethodnim, pri čemu je ta vrednost nešto manja i iznosila je 0,52 mm [27]. U ovim istraživanjima korišćena je metoda *en-masse* pomeranja zuba, gde su se mehanizmi postavljali na kukice koje su lotovane na čeličnu žicu i za kukicu na tubi, što je rezultiralo nešto sporijim zatvaranjem postekstrakcionih prostora.

NiTi zatvorene opruge su u ovom istraživanju primenjene na 22 postekstrakciona prostora na isti način kao i elastični lanci, osim u slučajevima gde su veća rastojanja, zbog čega su postavljani indirektno. Nightingale i saradnici preporučuju metodu indirektnog povezivanja preko čeličnih ligatura u situacijama gde ih nije moguće postaviti direktno ili zbog prevelikog istezanja, što bi za posledicu imalo oštećenje mehanizma i neželjene efekte na pomeranje zuba [25].

Rezultati ovog istraživanja pokazuju da su se u posmatranom periodu od četiri meseca postekstrakcioni prostori pomoću NiTi zatvorenih opruga zatvorili za 3,94 mm. Utvrđeno je da se u prva tri meseca prostori više zatvaraju, dok četvrti mesec pokazuje smanjenu brzinu u odnosu na prethodna tri meseca. Istraživanja u kojima je korišćena slična metodologija pokazuju različite rezultate. Nightingale i Jones su na sedmičnom nivou ustanovili zatvaranje za 0,26 mm kod 20 postekstrakcionih prostora, što je u skladu sa rezultatima ovog istraživanja [25], dok rezultati Bokasa i Woodsa pokazuju značajno veće vrednosti, koje iznose 1,85 mm [26]. U istraživanjima Chaudharia i Tarvade, kao i Fanga i saradnika u kojima je korišćena *en-masse* metoda, prosečne izmerene vrednosti su iznosile 0,87 mm i 1,06 mm [11, 27].

Prilikom poređenja brzine zatvaranja postekstrakcionih prostora u posmatranom periodu, ovim istraživanjem je ustanovljeno da je primenom NiTi zatvorenih spiralnih opruga vrednost veća za 0,84 mm u odnosu na elastične lance. Postoji veliki broj istraživanja u kojima su se poredile ove dve metode i koja potvrđuju ove rezultate [22, 28, 29]. Dixon i saradnici, pored navedenih, poredili su i aktivne ligature, odnosno klizni mehanizam koji se sastoji od čelične ligature i elastičnog prstena. I u njihovom istraživanju su se NiTi zatvorene spiralne opruge pokazale kao najbrži mehanizam (0,81 mm mesečno), zatim elastični lanci (0,58 mm mesečno) i najsporije – aktivne ligature (0,35 mm mesečno). Autori su takođe prikazali i broj zatvaranja postekstrakcionih prostora koji su završeni za četiri meseca, što se značajno razlikuje od rezultata dobijenih u ovoj studiji. Najveći broj zatvorenih prostora su ustanovili kod primene aktivnih ligatura (38%), zatim NiTi zatvorenih spiralnih opruga (32%), a najmanje kod elastičnih lanaca (30%), dok se u ovom istraživanju posle četiri meseca kod metode elastičnih lanaca zatvorilo čak 83,33%, a kod NiTi zatvorenih spiralnih opruga 59,09% postekstrakcionih prostora. S obzirom na to da su se i u jednom i u drugom istraživanju najviše zatvarali prostori kod primene mehanizama koji su ga najsporije zatvarali, zaključak je da su ove metode primenjene kod onih prostora koji su imali manje vrednosti posle nivelacione faze ortodontske terapije [30].

Jedan od najvažnijih faktora koji utiče na brzinu pomeranja zuba su jačina i vrsta sile koja se primenjuje. Sila koju proizvode elastični lanci opada ubrzano u prva 24 sata i zatim nastavlja da opada i dalje sve do sledećeg kontrolnog pregleda, zbog čega se ova sila pre može nazvati intermitentna nego kontinuirana [31]. Nikl-titanijumske legure imaju osobine superelastičnosti i memorisanja oblika, zbog čega NiTi zatvorene opruge proizvode blage kontinuirane sile kroz duži period. U nekoliko *in vivo* studija NiTi opruge su pokazale da obezbeđuju brže i pouzdanije zatvaranje prostora, što je u skladu sa ovim istraživanjem. Smatra se da zadržavanje konstantne sile kroz određeni period najviše doprinosi njihovoj efikasnosti [16, 32].

ZAKLJUČAK

NiTi zatvorene spiralne opruge dovode do bržeg zatvaranja postekstrakcionih prostora u odnosu na elastični lanac. S obzirom na to da je ova razlika minimalna i da nema veliki klinički značaj, u praksi se mogu ravnopravno koristiti obe metode prema izboru kliničara.

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Fluoride release from conventional, resin-modified and hybrid glass ionomer cements

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SUMMARY

Introduction The aim of the study was to quantify and compare fluoride release from four different glass ionomer cement products (GICs).

Materials and Methods Standardized disk-shaped samples (5x2mm; n=5/group) of GIC restorative materials: conventional (Fuji IX, GC Corp., Japan), resin-modified (Fuji II LC, GC) and hybrid glass ionomer cement (Equia Forte, GC) and a conventional GIC liner/base material (Alfagal, Galenika, Serbia) were tested for fluoride release up to 21 days post-setting. Each sample was immersed in 5 mL of fresh deionized water during each time interval. Universal microhybrid composite (Filtek Z250, 3M EPSE, USA) and adhesive (Adper Single Bond, 3M ESPE) were used as negative controls. Fluoride release was measured using an F-selective electrode (Cole-Parmer, USA) and an ion meter (Oakton 700, Cole-Parmer, USA). Data were statistically analyzed using one-way ANOVA, regression and correlation analysis at 0.05.

Results The highest total fluoride release was measured from Alfagal (386±61 ppm/g), and significantly less from Equia (188±29 ppm/g), Fuji IX (143±11 ppm/g) and Fuji II LC (104±14 ppm/g) ($p < 0.05$). All GICs showed the highest fluoride release during the first 24 hours post-setting. After 3 days, fluoride release slowed down reaching a plateau for all materials. Regression and Pearson correlation analysis showed significant inverse relationship between fluoride release and sample mass and density ($p<0.001$).

Conclusion Of the three GICs indicated for use as restorative materials, Equia Forte released the highest fluoride concentration. Fluoride release was material and density dependent, with higher release occurring from lower density GICs.

Keywords: fluoride; glass ionomer cements; glass hybrid; resin-modified glass ionomer

INTRODUCTION

One of the main reasons for restoration failure is secondary caries [1, 2, 3] which results in further tooth tissue loss, weakened remaining tooth structure or even premature tooth loss. Any mechanism that inhibits acid production by bacteria, increases resistance to demineralization or facilitates remineralization could be considered clinically significant. A recent study showed that the most commonly used restorative materials in contemporary restorative dentistry, resin-based composites, do not exhibit buffering potential, thus being more susceptible to the formation of secondary caries around such restorations [4].

Fluoride from fluoride-containing materials has the capacity to chemically interact with hydroxyapatite of enamel and dentin adjacent to the restoration resulting in the formation of fluorapatite [5, 6] which increases resistance to acid demineralization and prevents secondary caries [7, 8]. Fluoride released from fluoride-containing restorative materials, such as glass ionomer cements (GICs), may improve resistance to demineralization [9, 10], facilitate remineralization [11, 12] or even directly affect cariogenic bacteria by inhibiting their metabolic enzymes [13]. Fluoride was found in dentin, released from the bottom parts of GIC restorations (Fuji IX and Fuji II LC improved) in artificially demineralized monkey`s teeth after three days [14]. Fluoride-releasing materials show cariostatic properties and may affect bacterial metabolism under simulated cariogenic conditions *in vitro* [15].

GICs have been substantially improved over the years, especially their mechanical properties, thereby expanding indications for use. These improvements include the introduction of photopolimerizable resins (light-cured GIC), or ultrafine, highly reactive glass particles, dispersed within the conventional glass ionomer structure and a higher molecular weight polyacrylic acid in hybrid GIC. The latter has led to the recently launched hybrid GIC, Equia Forte, currently the only material in this 'class' of GICs.

It has been widely accepted that during the setting action of GICs a variety of ionic constituents is released from the glass phase, including fluoride. Fluoride is released in short-term by rapid dissolution from the outer surface of the set material into solution. Sustained ion release is the consequence of ion diffusion through the bulk cement. Despite the latest improvements, it is imperative that GICs maintain abundant fluoride release in order to support their antiocariogenic potential.

The aim of the present study was to quantify and compare fluoride release from the conventional, resin-

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modified and the new glass hybrid GICs into deionized water 1, 6, 24 h, 3, 7, 14 and 21 days post-setting. The null hypotheses were: (1) there is no significant difference in fluoride release from different GICs and (2) there is no significant relationship between fluoride release and sample mass/density.

MATERIALS AND METHODS

Details on the materials used in this study are given in the Table 1. All materials were used according to manufacturers' instructions. Capsules of Fuji IX, Fuji II and Equia were mixed in an auto-mixer for 10 s. Alfagal was prepared by hand mixing 1 scoop of powder and 2 drops of liquid for 30 s using a plastic spatula on the paper pad. Z250 and Adper were used directly from the tube or bottle, respectively.

Standardized plastic molds, 5 mm in diameter and 2 mm deep, were placed on a Mylar strip and a glass pad, filled with GIC, composite or adhesive, covered with another strip, and pressed with a glass slide to extrude excess material and form smooth surfaces. Five samples were prepared per group, except the adhesive control group (Adper) with one prepared sample to avoid wasting material. Alfagal and Equia were allowed to set for 6 min and 2 min 30 s, respectively. Fuji II, Z250 and Adper were light-cured through the Mylar strip and 1 mm thick glass slide using a conventional LED light-curing unit (LEDition, Ivoclar Vivadent, Schaan, Liechtenstein) operating at intensity of 800 mW/cm^2. Adper and Z250 were light-cured for 40 s, and Fuji II for 20 s each from the top and bottom side. Each sample was weighed on an analytical balance with an accuracy of 0.1 mg (ACCULAB ALC-110.4, Sartorius group, Goettingen, Germany).

All samples were stored dry for 24 h at 37°C. Following storage, each sample was immersed in 5 mL of deionised water in a sterile glass vial and kept at 37°C. Fluoride concentrations were measured after 1 h, 6 h, 24 h, 3, 7, 14 and 21 days using a F-selective electrode (Cole-Parmer, Bunker CT, Vernon Hills, Illinois) and an ion meter (Oakton pH/Ion 700 Bench Meter, Cole-Parmer, Bunker CT, Vernon Hills, Illinois). The electrode was first calibrated using 0.1, 1 and 10 ppm F. Ionic Strength Adjuster solution (0.5 mL) was added to all tested solutions just before measuring. Between measurements the electrode was rinsed with deionized water.

Statistical analysis was performed in Minitab 16 (Minitab Inc., State College, PA, USA). The data were analyzed using one-way ANOVA with Tukey's post-hoc test for multiple comparisons at the level of significance alpha=0.05. Regression analysis and Pearson correlation were performed to determine the relationship between fluoride release and sample mass/density.

Table 1. Materials used in the study
Tabela 1. Materijali korišćeni u ovom istraživanju

Material / Materijal (Code / Oznaka)	Type / Tip Manufacturer / Proizvođač	Indications for use / Indikacije za upotrebu	Composition / Sastav
ALFAGAL ® bejz (Alfagal)	Conventional glass ionomer cement / Konvencionalni GJC Galenika a.d. Belgrade, Serbia	Base material under composite or amalgam restorations For cementation of prosthodontic restorations and orthodontic rings For atraumatic restorative treatment / Baza ispod kompozitnih ili amalgamskih ispuna, cementiranje protetskih nadoknada i ortodontskih bravica, atraumatski restaurativni tretman	Liquid / Tečnost: 55% water solution of acrylic and itaconic acidcopolymers Powder / Prah: calcium-aluminium-barium-fluoro-silicate glass
GC Fuji IX GP CAPSULE (Fuji IX)	Conventional reinforced glass ionomer cement / Konvencionalni ojačani GJC GC Corporation, Tokyo, Japan	Final restorations (non-stress areas), Intermediate Restorative (IRM), core material and long-term, temporary restorations / Definitivni ispuni (zone van opterećenja), privremeni ispuni, dentinski zamenik	Liquid / Tečnost: Polyacrylic acid, water, polybasic carboxylic acid Powder / Prah: Aluminofluorosilicate glass, polyacrylic acid powder
GC Fuji II LC (Fuji II)	Light-cured resin-modified glass ionomer cement / Svetlosno-polimerizujući smolom modifikovani GJC GC Corporation, Tokyo, Japan	Class III and V Restorations, cervical erosions/abfraction lesions and root surface caries liner/base / Ispuni III i V klase, cervikalne erozije/abfrakcije i karijes korena, lajner/baza	Liquid / Tečnost: water, polyacrilic acid, HEMA Powder / Prah: fluoroaluminosilicate glass, polyacrilic acid
EQUIA® Forte (Equia)	Conventional hybrid glass-ionomer cement / Konvencionalni hibridni GJC GC Corporation, Tokyo, Japan	Bulk fill glass hybrid restorative for Class I, II and V restorations / „Bulk fill" staklo-hibridni materijal za ispune I, II i V klase	Liquid / Tečnost: water, polybasic carboxylic acid, polyacrylic acid Powder / Prah: fluoro-alumino-silicate glass, iron(III)-oxide
FiltekTM Z250 Universal Restorative (Z250)	Microhybrid composite / Mikrohibridni kompozit 3M ESPE, St. Paul, MN, USA	All classes of restorations / Ispuni svih klasa	BisEMA6, UDMA, BisGMA, TEGDMA, silane-treated ceramic (75-85 wt%), benzotriazol, EDMAB
Adper Single Bond (Adper)	One-step self-etch adhesive system / Jednofazni samonagrizajući adhezivni sistem 3M ESPE, St. Paul, MN, USA	Adhesive for composites / Adheziv za kompozite	Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiators, water and ethanol

HEMA – 2-hydroxyethyl methacrylate; Bis-EMA – ethoxylated bisphenol A glycol dimethacrylate; UDMA – urethane dimethacrylate; Bis-GMA – bisphenol A diglycidyl ether dimethacrylate; TEGDMA – triethylene glycol dimethacrylate; EDMAB - ethyl-4-dimethylamino benzoate

RESULTS

Aside from the control composite Z250, the mass and density of GIC samples varied significantly, with the conventional GIC Alfagal showing lower mass and density than other tested GICs (p<0.001) (Table 2).

Table 2. Mass and density of the tested materials per sample. Mean (standard deviation)
Tabela 2. Masa i gustina ispitivanih materijala po uzorku. Srednja vrednost (standardna devijacija)

Material / Materijal	Mass / Masa (mg)	Density / Gustina (mg/mm³)
Z250	123.4 (4.9)^{A}	3.14 (0.13)^{A}
Fuji II	117.2 (3.2)^{AB}	2.98 (0.08)^{AB}
Fuji IX	113. 0 (1.7)^{BC}	2.88 (0.04)^{BC}
Equia	109.1 (2.8)^{C}	2.78 (0.07)^{C}
Alfagal	94.7 (3.2)^{D}	2.41 (0.08)^{D}

In each column, the same upper-case letters indicate no statistically significant difference between groups (p > 0.05).
U svakoj koloni ista velika slova ukazuju da nema statistički značajne razlike između grupa (p > 0,05).

Fluoride release varied in concentration between materials and time periods (Figure 1). All GICs had the highest fluoride release during the first 24 h post-setting. Relatively high fluoride release occurred from GICs except Fuji II over the next 48 h (the first 3 days in total) as indicated by steep slopes in Figure 1. Further release was notable until the end of the experiment (21 days) with base/liner GIC Alfagal showing the highest fluoride release in all tested times (more than double the values of other materials) (p<0.05). Equia released more fluoride than Fuji IX and Fuji II with Fuji II releasing the least fluoride concentration of the tested GICs (p<0.05). Fluoride release in negative control groups, both composite Z250 and Adper adhesive, was 10 or more times lower compared to GICs (11.46±2.45 and 14.81 ppm per gram composite and adhesive, respectively, over 21 days).

Regression and Pearson correlation analysis showed significant inverse relationship between fluoride release (F) and sample mass and density (p<0.001) (Figure 2). The regression equations may be expressed as Equations 1 and 2:

$$F = 132.00 - 1020.43\ x\ Mass \qquad \text{Equation 1.}$$
$$F = 132.00 - 40.072\ x\ Density \qquad \text{Equation 2.}$$

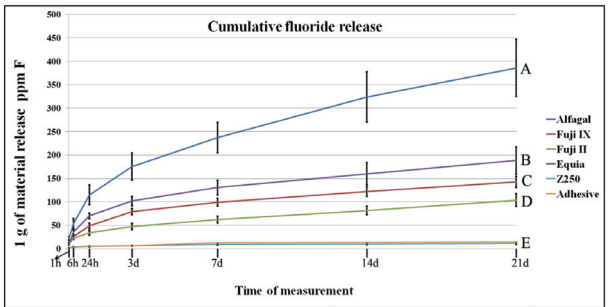
with R-sq = 86.52% and R-sq(adj.) = 85.77%

Pearson correlation coefficient r=-0.930 and p<0.001 indicate strong negative correlation between fluoride release and mass/density *i.e.* higher fluoride release occurred from lower density GICs.

DISCUSSION

Both null hypotheses were rejected as the results confirmed significant differences in fluoride release between GICs as well as an inverse relationship between fluoride release and sample mass and density.

In the present study, a range of GICs with different composition was tested, from the 'classical' GIC formulated Alfagal to reinforced conventional Fuji IX, resin-modified and light-curable Fuji II to the latest glass hybrid Equia. This choice was made to cover a wide range of different GIC compositions so as to ascertain a range of potential fluoride concentrations released over a period of 21 days.



	1h	6h	24h	3d	7d	14d	21d	Mean release
Alfagal	22.03 (4.11)	53.09 (11.30)	114.69 (21.03)	175.41 (29.44)	237.04 (32.62)	323.75 (53.56)	385.77 (60.88)	55.11 (21.74)
Fuji IX	10.99 (2.50)	25.71 (4.81)	48.57 (6.19)	79.02 (6.82)	99.39 (8.56)	122.26 (10.37)	142.64 (11.47)	20.38 (6.25)
Fuji II	10.41 (1.40)	22.40 (3.05)	33.69 (4.55)	47.37 (6.73)	62.07 (7.41)	81.55 (9.41)	103.67 (13.69)	14.81 (4.40)
Equia	9.02 (0.95)	35.47 (5.28)	69.87 (6.26)	101.66 (10.12)	130.57 (15.84)	159.81 (23.9)	188.17 (28.97)	26.88 (8.28)
Z250	2.01 (0.33)	3.36 (0.85)	5.01 (1.03)	6.11 (1.24)	9.38 (1.62)	10.32 (1.86)	11.46 (2.45)	1.64 (0.81)

Figure 1. Fluoride release from the tested materials in different time periods. Cumulative fluoride release normalized per 1 g of material over the period of 21 days. For clarity, mean (standard deviation) values in ppm are presented in the accompanying table. "Mean release" indicates the amount of fluoride released between two test measurements *i.e.* per test period.
Grafikon 1. Otpuštanje fluorida iz ispitivanih materijala u različitim periodima. Kumulativno oslobađanje fluorida normalizovano na 1 g materijala tokom perioda od 21 dana. Zbog jasnog prikazivanja, srednja vrednost (standardna devijacija) u ppm je prikazana su u pratećoj tabeli. „Srednja vrednost oslobađanja" predstavlja količinu oslobođenog fluorida između dva merenja tj. testirang perioda.

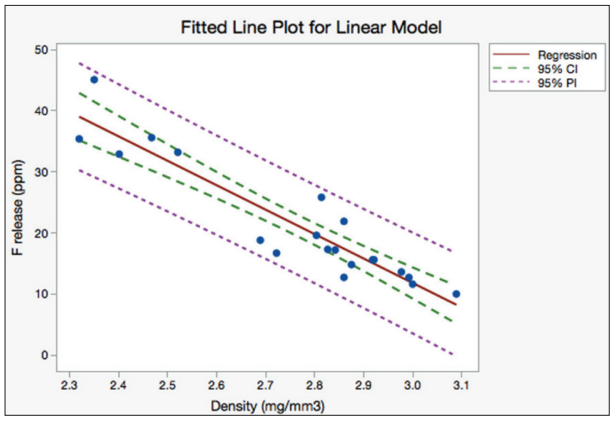


Figure 2. Regression analysis for 'F release' versus 'Mass' or 'Density' of the tested GICs. The fitted line plot for a linear model shows points adequately covering the entire range of mass/density values without any outliers or curvature in the data.
CI – confidence interval; PI – prediction interval
Grafikon 2. Regresiona analiza za F otpuštanje nasuprot masi ili gustini ispitivanih GJC. Linearni model pokazuje tačke koje adekvatno pokrivaju čitav raspon vrednosti mase/gustine bez značajnih odstupanja u podacima.
CI – interval pouzdanosti; PI – predikcioni interval

There is a lack of standardization vis-à-vis sample size, shape, the type and quantity of immersion media as well as data presentation in the current literature. Data comparison is often difficult due to these differences. The present results are expressed as the amount of fluoride released from 1 gram of material. Deionized water was used as the immersion medium as it is most often used in other studies and has been shown to facilitate more fluoride release than artificial saliva [16].

Short- and long- term fluoride release from restorative materials is related to their matrices, setting mechanisms and fluoride content and as well as environmental conditions [15]. The present results showed the highest fluoride release from the conventional GIC Alfagal. Alfagal has the most 'classical' GIC composition of all tested GICs, based on a water solution of acrylic and itaconic acid copolymers. It is of lower density (lower viscosity) than other tested GICs and is specifically indicated for use as a liner/base under direct and indirect restorations. Alfagal is not indicated as a filling (restorative) material, not even in non-load-bearing areas, in contrast to other tested GICs. It is well known that early, conventional GICs exhibit inferior mechanical properties than other filling materials and profound sensitivity to water imbalance, especially during the first 24 h [17]. Resin modification of the conventional formula led to somewhat improved mechanical properties but still below those of resin-based composites [18]. Also, GICs with higher powder-to-liquid ratio exhibit better mechanical properties [19]. Higher water uptake and solubility as well as less complicated internal structure compared to GICs containing high molecular polyacrylic and polybasic carboxylic acid and/or resin monomers may have led to more pronounced fluoride release from Alfagal than other tested GICs.

Equia had higher fluoride release compared to Fuji IX and Fuji II, but the difference was not statistically significant between Equia and Fuji IX, probably due to relatively high SD values. However, the results are indicative of a tendency of higher fluoride release from Equia than Fuji IX. These two GICs share a similar composition, and Equia is considered a successor of Fuji IX. Higher fluoride content, slightly lower density or other compositional modification undisclosed by the manufacturer could be the reason(s) for somewhat higher fluoride release from Equia than Fuji IX. In clinical practice it is recommended to cover the surface of Equia restorations with Equia Forte Coat, a light-curable resin-based liquid. This coat would probably act as a semi-permeable membrane, allowing partial fluoride release into the oral environment. However, low wear resistance of unfilled or very low filled resin liquid indicates that protective coat would be worn during function leaving Equia exposed for unrestricted fluoride release. As the longevity of the coat layer in clinical conditions is unpredictable and individual, the present study design without any protective layer allowed measuring maximum fluoride release for the given sample size and shape.

Previous studies reported different findings related to fluoride release from resin-modified and conventional GICs. Several studies showed no significant difference in fluoride release between light-cured and conventional GICs [20, 21, 22]. The present study detected less fluoride release from light-cured Fuji II compared to other tested GICs. Fluoride release from resin-modified GICs could be hampered by the polymer network intertwined with polyalkeenoate chains.

Following the highest fluoride release during the first 24h post-setting, fluoride release from each material decreased sharply over the first week and continued to decrease steadily over the 3 weeks period which is in agreement with other studies reporting the maximum release during the first 24-48 h [23–27]. In all GICs a tendency for fluoride release was observable based on the increasing slope in Figure 1. This indicated that new formulations of GICs also act as a pool of fluoride with potential continuous release over a long time, especially in environment with acidic pH. Earlier studies have shown a steady fluoride release over the period of 2 years from conventional GICs [28].

Continuous fluoride release was also detected in a rare *in vivo* study by Koch et al. [29] who showed that fluoride concentration in saliva immediately after placement of GIC restorations increased from 0.04 ppm to 0.8-1.2 ppm, but slowly decreased about 35 % after 3 weeks and additional 30 % after 6 weeks.

Although clinical importance of fluoride as an anticariogenic agent is nowadays generally accepted, the evidence to corroborate this statement comes from *in vitro* and in situ studies. Randomized clinical trials offer inconclusive evidence of greater caries protection by GICs [30]. *Ex vivo* studies showed the potential of fluoride ions to migrate from GIC restorations into the surrounding enamel and dentin of primary molars [31, 32]. Also, GICs were shown to inhibit secondary caries formation *in vitro* in artificial biofilm models [33, 34]. Until clinical evidence becomes definitive regarding the anticariogenic efficiency of fluoride-containing materials, primarily GICs, it is important that new and improved formulations of these materials maintain high levels of fluoride release.

CONCLUSION

Conventional, glass hybrid and resin-modified GICs showed continuous release of fluoride ions over 21 days after setting. Concentrations of released fluoride differed and were affected by material composition and density. The addition of resin into GIC formulation decreased its ability to release fluoride. Higher viscosity GICs could be also linked to lower fluoride release. Of the three GICs indicated for use as restorative materials, Fuji II LC, Fuji IX and Equia Forte, the highest fluoride release occurred from Equia Forte.

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Otpuštanje fluorida iz konvencionalnih, smolom modifikovanih i hibridnih glas-jonomer cemenata

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KRATAK SADRŽAJ

Uvod Cilj ovog istraživanja je bio da se kvantifikuje i uporedi otpuštanje fluorida iz četiri glas-jonomer cementa (GJC).

Materijal i metode Napravljeni su standardizovani diskovi (5 × 2 mm; n = 5/grupa) od sledećih GJC: konvencionalnih (Fuji IX, GC Corp., Japan), smolom modifikovani (Fuji II LC, GC), hibridni GJC (Equia Forte, GC) i konvencionalni GJC lajner/baza (Alfagal, Galenika, Srbija). Ispitivano je otpuštanje fluorida tokom 21 dana tako što je svaki uzorak potapan u 5 ml sveže destilovane vode posle svakog mernog intervala. Univerzalni mikrohibridni kompozit (Filtek Z250, 3M EPSE, USA) i adheziv (Adper Single Bond, 3M ESPE) korišćeni su kao negativne kontrole. Otpuštanje fluorida je očitavano pomoću fluor-selektivne elektrode (Cole-Parmer, USA) i jon-metra (Oakton 700, Cole-Parmer, USA). Rezultati su statistički obrađeni ANOVA testom, regresionim i korelacionim analizama sa $\alpha = 0{,}05$.

Rezultati Ukupno otpuštanje fluorida je bilo najveće iz Alfagala (386 ± 61 ppm/g), značajno manje iz Equia Forte (188 ± 29 ppm/g), Fuji IX (143 ± 11 ppm/g), a najmanje iz Fuji II LC (104 ± 14 ppm/g) ($p < 0{,}05$). Svi GJC pokazali su najveće otpuštanje fluorida u toku prva 24 h po vezivanju. Posle tri dana otpuštanje fluorida je postajalo sporije dostižući plato za sva četiri materijala. Regresiona i Pirsonova korelaciona analiza pokazale su značajan obrnut odnos između otpuštanja fluorida i mase i gustine uzoraka ($p < 0{,}001$).

Zaključak Od GJC indikovanih za ispune, Equia Forte je otpustila najveću koncentraciju fluorida. Otpuštanje fluorida zavisi od materijala i njegove gustine, pri čemu GJC manje gustine otpuštaju više fluorida.

Ključne reči: fluoridi; glas-jonomer cement; hibridno staklo; smolom modifikovani glas-jonomer

UVOD

Jedan od glavnih uzroka neuspeha restauracije jeste sekundarni karijes [1, 2, 3], koji dovodi do daljeg gubitka zubnih tkiva, oslabljene strukture zuba, pa čak i gubitka zuba. Klinički je značajan svaki mehanizam koji umanjuje produkciju kiselina od strane bakterija, povećava otpornost na demineralizaciju ili potpomaže proces remineralizacije. Skorašnja studija je pokazala da najčešće korišćeni restaurativni materijali, kompoziti, ne poseduju puferski potencijal, te su stoga podložniji nastanku sekundarnog karijesa oko ruba ispuna [4].

Fluoridi otpušteni iz fluoridima bogatih materijala imaju sposobnost da stupe u hemijsku interakciju sa hidroksiapatitom iz gleđi i dentina u neposrednoj blizini ispuna, što za posledicu ima nastanak fluorapatita [5, 6], koji povećava otpornost na demineralizaciju i preventivno deluje na nastanak sekundarnog karijesa [7, 8]. Fluoridi koji su otpušteni iz materijala koji sadrže fluoride, kao što su glas-jonomer cementi (GJC), mogu poboljšati otpornost na demineralizaciju [9, 10], potpomoći proces remineralizacije [11, 12] ili čak direktno uticati na kariogene bakterije inhibiranjem njihovih metaboličkih enzima [13]. Registrovano je otpuštanje fluorida iz dubljih partija GJC ispuna (Fuji IX i unapređeni Fuji II LC) u dentinu veštački demineralizovanih zuba majmuna posle tri dana od restauracije [14]. Materijali koji otpuštaju fluoride pokazuju kariostatske osobine i mogu uticati na bakterijski metabolizam u simuliranim kariogenim uslovima *in vitro* [15].

GJC su poslednjih godina značajno unapređeni, pogotovo na polju mehaničkih karakteristika, što je dovelo do širenja indikacija za njihovo korišćenje. Ova unapređenja su dobijena uvođenjem svetlosno polimerizujućih smola (svetlosno polimerizujući GJC) ili dodavanjem ultrafinih, visoko reaktivnih staklenih punilaca raspršenih unutar konvencionalne glas-jonomerne strukture, kao i primenom poliakrilne kiseline veće molekulske mase u hibridnim GJC. To je vodilo i ka nedavnoj pojavi hibridnog GJC, Equia Forte, trenutno jedinog materijala u ovoj „klasi“ GJC.

Široko je prihvaćeno da tokom vezivanja GJC otpuštaju brojne jonske konstituente iz staklene faze, uključujući i fluoride. Fluoridi se prvobitno naglo otpuštaju brzim rastvaranjem spoljašnje površine postavljenog materijala u rastvor. Kontinuirano otpuštanje jona je posledica difuzije samih jona kroz cement. Uprkos skorašnjim unapređenjima, imperativ je da GJC zadrže obilno otpuštanje fluorida, kako bi ostvarili svoj antikariogeni potencijal.

Cilj ove studije je bio da se kvantifikuje i uporedi otpuštanje fluorida iz konvencionalnih, smolom modifikovanih GJC i novog hibridnog GJC u destilovanoj vodi nakon 1, 6, 24 h i nakon 3, 7, 14, 21 dana od momenta vezivanja materijala. Nulte hipoteze su glasile: (1) nema značajne razlike u otpuštanju fluorida iz različitih vrsta GJC i (2) ne postoji značajna veza između otpuštanja fluorida i mase/gustine samih uzoraka.

MATERIJAL I METOD

Detaljni opisi materijala koji su korišćeni u ovoj studiji dati su u Tabeli 1. Svi materijali korišćeni su po uputstvu proizvođača. Kapsule Fuji IX, Fuji II i Equia Forte su mešane u auto-mikseru 10 s. Alfagal je zamešan ručnim mešanjem jedne kašičice praha i dve kapi tečnosti plastičnom špatulom na papirnoj podlozi u trajanju od 30 s. Kompozit Z250 i Adper su korišćeni direktno iz tube ili bočice.

Standardni plastični kalupi visine 2 mm i širine 5 mm su postavljeni na celuloidnu traku i staklenu pločicu, potom su napunjeni GJC, kompozitom ili adhezivom. Tako napunjeni kalupi su zatim prekriveni drugom tračicom i pritisnuti drugom staklenom pločicom kako bi se eliminisao višak materijala i dobile glatke površine uzoraka. Pravljeno je po pet uzoraka u svakoj grupi, osim u adhezivnoj kontrolnoj grupi, gde je napravljen jedan uzorak kako bi se izbegao nepotreban gubitak materijala. Alfagal i Equia Forte su se vezivali šest min. odnosno dva min. i 30 s. Fuji II LC, Z250 i Adper su svetlosno polimerizovani kroz celuloidnu tračicu i staklenu pločicu debljine 1 mm po-

moću konvencionalne LED lampe (LEDition, Ivoclar Vivadent, Schaan, Lihtenštajn) iradijanse 800 mW/cm^2. Adper i Z250 su prosvetljavani po 40 s, a Fuji II LC po 20 s sa gornje i sa donje strane uzorka. Masa svakog uzorka je merena na analitičkoj vagi sa preciznošću od 0,1 mg (ACCULAB ALC-110.4, Sartorius group, Goettingen, Nemačka).

Svi uzorci su ostavljeni da stoje 24 h na temperaturi od 37°C, posle čega je svaki uzorak potopljen u 5 ml destilovane vode u sterilnoj staklenoj bočici i čuvan na temperaturi od 37°C. Koncentracije fluorida merene su nakon 1 h, 6 h, 24 h, 3, 7, 14 i 21 dana pomoću fluor-selektivne elektrode (Cole-Parmer, Bunker CT, Vernon Hills, Illinois) i jon-metra (OaktonpH/Ion 700 Bench Meter, Cole-Parmer, Bunker CT, Vernon Hills, Illinois). Pre svakog merenja elektroda je kalibrisana sa rastvorima poznatih koncentracija fluorida 0,1, 1 i 10 ppm F. Rastvor Ionic Strength Adjuster (0,5 ml) dodavan je u sve ispitivane rastvore pre merenja. Između svakog očitavanja elektroda je isprana destilovanom vodom.

Statistička analiza je urađena u statističkom programu Minitab 16 (Minitab Inc., State College, PA, USA). Podaci su analizirani jednofaktorskim ANOVA testom uz Tukey post-hoc test za multipla poređenja sa nivoom značajnosti $\alpha = 0,05$. Regresiona i Pirsonova korelaciona analiza su primenjene kako bi se utvrdila veza između otpuštanja fluorida i mase/gustine uzoraka.

REZULTATI

Izuzev kontrolne grupe (Z250), masa i gustina GJC uzoraka je značajno varirala, pri čemu je konvencionalni GJC Alfagal pokazao manju masu i gustinu nego ostali testirani GJC ($p < 0,001$) (Tabela 2).

Otpuštanje fluorida je variralo u koncentracijama između materijala i vremenskih intervala (Grafikon 1). Svi GJC pokazali su najveće otpuštanje fluorida u prva 24 h posle vezivanja. Relativno visoko otpuštanje fluorida se dešavalo i u sledećih 48 h (prva tri dana) sa izuzetkom Fuji II LC, kao što se vidi u Grafikonu 1. Dalje otpuštanje fluorida je beleženo do kraja eksperimenta (21 dan), pri čemu je Alfagal baza/lajner pokazao najveće otpuštanje fluorida u svim ispitivanim vremenskim intervalima (više nego duplo u odnosu na druge materijale) ($p < 0,05$). Otpuštanje fluorida iz Equia Forte je bilo više nego iz Fuji IX i Fuji II LC, pri čemu je Fuji II LC otpustio najmanju količinu fluorida od svih ispitivanih GJC ($p < 0,05$). Otpuštanje fluorida u negativnim kontrolnim grupama, kompozit Z250 i Adper adheziv, bilo je deset i više puta manje u poređenju sa kontrolama (11,46 ± 2,45 i 14,81 ppm po gramu kompozita i adheziva tokom 21 dana).

Regresiona i Pirsonova korelaciona analiza su pokazale značajnu obrnutu vezu između otpuštanja fluorida (F) i mase/gustine ($p < 0,001$) (Figure 2). Regresione jednačine mogu biti predstavljene kao jednačine 1 i 2.

$$F = 132,00 - 1020,43 \times masa \qquad \text{Jednačina 1.}$$
$$F = 132,00 - 40,072 \times gustina \qquad \text{Jednačina 2.}$$

sa R-sq = 86,52% and R-sq(adj.) = 85,77%

Pirsonov korelacioni koeficijent $r = -0,930$ i $p < 0,001$ ukazuje na jaku negativnu korelaciju između otpuštanja fluorida i mase/gustine. GJC manje gustine otpuštali su veću količinu fluorida.

DISKUSIJA

Obe nulte hipoteze su odbačene, jer su rezultati potvrdili značajne razlike u otpuštanju fluorida između GJC, kao i obrnutu vezu između otpuštanja fluorida i mase/gustine uzorka.

U ovoj studiji ispitano je više GJC različitog sastava, od „klasično" formulisanog GJC, Alfagala, ojačanog konvencionalnog Fuji IX, smolom modifikovanog i svetlosno polimerizujućeg Fuji II do GJC sa hibridnim staklenim puniocima, Equia. Ovaj izbor je napravljen kako bi se pokrio širok dijapazon različitih sastava GJC i kako bi se utvrdio opseg mogućeg otpuštanja fluorida tokom ispitivanog perioda od 21 dan.

U literaturi je primetno odsustvo standardizacije u smislu veličine i oblika uzorka, tipa i količine imerzionog medijuma, kao i u prezentaciji dobijenih podataka u trenutno dostupnoj literaturi. Upoređivanje dobijenih podataka je često teško zbog ovih razlika. Zbog toga je u ovoj studiji otpuštanje fluorida normirano na jedan gram materijala. Destilovana voda je korišćena kao imerzioni medijum, po ugledu na druga istraživanja, jer je pokazano da više olakšava otpuštanje fluorida u odnosu na veštačku pljuvačku [16].

Kratkoročno i dugoročno otpuštanje fluorida iz restaurativnih materijala je u vezi sa njihovim matriksom, mehanizmima vezivanja, količinom fluorida koje poseduju, a zavisi i od uslova okoline [15]. Rezultati našeg istraživanja pokazuju najveće otpuštanje fluorida iz konvencionalnog GJC Alfagala. Alfagal ima „najklasičniji" sastav od svih testiranih GJC. Počiva na vodenom rastvoru akrilnih i itakonskih kiselinskih kopolimera. Manje je gustine (manje viskoznosti) od ostalih ispitivanih GJC i usko je indikovan za korišćenje kao lajner/baza ispod direktnih i indirektnih ispuna. Alfagal nije indikovan za ispune, čak i u regijama koje ne trpe veliki pritisak žvakanja, za razliku od ostalih ispitivanih GJC. Dobro je poznato da su rani, konvencionalni GJC imali izrazito inferiorna mehanička svojstva u poređenju sa drugim materijalima za ispune i da su imali ozbiljnu osetljivost na disbalans vode, pogotovo u toku prva 24 h [17]. Dodatak smole konvencionalnoj formulaciji je rezultirao donekle unapređenim mehaničkim svojstvima GJC, ali je to i dalje bilo ispod nivoa kompozita [18]. Takođe, GJC koji su u svom sastavu imali više praha nego tečnosti imali su bolja mehanička svojstva [19]. Veća asorpcija vode i rastvorljivost, jednostavnija unutrašnja struktura u poređenju sa GJC, koji u svom sastavu imaju velike molekule poliakrilne i polikarboksilne kiseline i/ili smolu, može objasniti izraženije otpuštanje fluorida iz Alfagala nego iz ostalih ispitivanih GJC.

Equia Forte je pokazala veće otpuštanje fluorida u poređenju sa Fuji IX i Fuji II LC, ali ta razlika nije bila statistički značajna između Equia Forte i Fuji IX GJC, verovatno zbog relativno visokih vrednosti standardnih devijacija. Ipak rezultati ukazuju na tendenciju većeg otpuštanja fluorida iz Equia Forte nego iz Fuji IX. Ova dva GJC imaju sličan sastav, pri čemu se Equia smatra naslednikom Fuji IX. Veći depo fluorida, neznatno manja gustina ili druga modifikacija u sastavu, koju proizvođač drži u tajnosti, može biti razlog (ili razlozi) za donekle veće otpuštanje fluorida iz Equia Forte nego iz Fuji IX. U kliničkoj praksi je preporučeno da se površina Equia zaštiti premazom Equia Forte Coat. U pitanju je svetlosno polimerizujući lak na

bazi smole. Ovaj lak bi se verovatno ponašao kao semipermeabilna membrana, koja bi omogućila samo delimično otpuštanje fluorida u oralnu sredinu. Ipak, mala otpornost na trošenje smolastog laka koji u svom sastavu nema punioce bi značilo da bi lak mogao biti potrošen u funkciji žvakanja, što bi dovelo do direktne izloženosti Equia oralnoj sredini i nesmetanom otpuštanju fluorida iz nje. Kako je dugotrajnost premaza u kliničkim uslovima nepredvidiva i individualna, u ovoj studiji je korišćena Equa bez zaštitnog laka kako bi se omogućilo merenje mogućeg maksimalnog otpuštanja fluorida iz ispitivanog uzorka.

Ranija istraživanja su pokazala različite podatke vezane za otpuštanje fluorida iz smolom modifikovanih i konvencionalnih GJC. Nekoliko studija su pokazale da ne postoji značajna razlika u otpuštanju fluorida između konvencionalnih i smolom modifikovanih GJC [20, 21, 22]. U ovoj studiji uočeno je manje otpuštanje fluorida iz svetlosno polimerizujućeg Fuji II LC u odnosu na druge ispitivane GJC. Otpuštanje fluorida iz smolom modifikovanih GJC može biti otežano usled mreže polimera koja se prepliće sa poliakrilatnim lancima.

Posle najvećeg otpuštanja fluorida tokom prva 24 h od vezivanja, otpuštanje fluorida iz svakog materijala se smanjilo naglo tokom prve nedelje i nastavilo da se stalno smanjuje tokom naredne tri nedelje, što je u saglasnosti sa drugim studijama koje navode maksimalno otpuštanje fluorida tokom prvih 24–48 h [23–27]. U svim ispitivanim GJC tendencija ka otpuštanju fluorida je posmatrana prema povećanju nagiba u Grafikonu 1. Ovo ukazuje na to da nove formulacije sastava GJC takođe stvaraju rezervoar fluorida sa mogućim kontinuiranim otpuštanjem tokom dugog perioda, pogotovo u uslovima kiselog pH. Ranija istraživanja su pokazala stalno otpuštanje fluorida tokom dve godine iz konvencionalnih GJC [28].

Kontinuirano otpuštanje fluorida je uočeno i u retkoj *in vivo* studiji Kocha i sar. [29], koji su pokazali da količina fluorida u salivi neposredno posle postavljanja GJC ispuna raste sa 0,04 ppm na 0,8–1,2 ppm, ali lagano opada za oko 35% posle tri nedelje i dodatnih 30% posle šest nedelja.

Iako je klinička važnost fluorida kao antikariogenog činioca danas prihvaćena, dokazi koji potkrepljuju ovu tvrdnju dolaze iz *in vitro* i *in situ* studija. Randomizovana klinička ispitivanja nude nedovoljno dokaza o većem antikariogenom potencijalu GJC [30]. *Ex vivo* studije su pokazale potencijal fluoridnih jona da migriraju iz GJC ispuna u okolnu gleđ i dentin mlečnih molara [31, 32]. Takođe je pokazano da GJC inhibiraju nastanak sekundarnog karijesa *in vitro* u veštačkim biofilm modelima [33, 34]. U nedostatku konkluzivnih kliničkih dokaza, u smislu antikariogene efikasnosti materijala koji u svom sastavu sadrže fluor (na prvom mestu GJC), važno je da nove i unapređene formulacije ovih materijala zadrže visok nivo otpuštanja fluorida.

ZAKLJUČAK

Konvencionalni, staklohibridni i smolom modifikovani GJC su pokazali kontinuirano otpuštanje fluorida tokom 21 dana od vezivanja. Koncentracije otpuštenih fluorida variraju i zavise od sastava materijala i njegove gustine. Dodatak smole u formulaciju GJC smanjuje mogućnost otpuštanja fluorida. Viskozniji GJC mogli bi biti povezani sa slabijim otpuštanjem fluorida. Od tri GJC koji su indikovani kao materijali za ispune (Fuji II LC, Fuji IX, Equia Forte), najveće otpuštanje fluorida je pokazala Equia Forte.

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Microbiological status of root canal after unsuccessful endodontic treatment

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SUMMARY

Introduction The main objective of endodontic treatment is to eliminate infection from root canal and prevent re-infection by three-dimensional hermetic obturation of the canal system. Endodontic failure can occur due to inability of complete control and elimination of infection from the root canal.

The aim of this study is to investigate, by PCR technique, microbiological status of previously endodontically unsuccessfully treated teeth immediately after the removal of obturation material.

Material and Methods The analysis included 30 teeth indicated for endodontic retreatment. After removing previous root canal filling material, the bacteriological sample was taken by sterile instrument (# 15) and paper points. Standard PCR technique was used to analyze the incidence of *E.faecalis*, *P.micros*, *P.intermedia*, *P. endodontalis* and *A.actinomycetemcomitans*.

Results Positive bacteriological findings were registered in 80% of cases, while bacteria were not identified in 20% of all samples (all taken from the root canals without significant changes in periapical tissue). From 24 canals with identified bacteria, 17 had affected apical periodontium. The most dominant microbe in root canals with positive bacteriological finding was *E.faecalis* (83.3% of the canals) and *P.intermedia* (75%). In case of teeth with chronic periapical changes, the most common was *E. faecalis* (94%) and *P.intermedia* (82.3%).

Conclusion The presence of periapical lesions significantly affects microbiological status of endodontically treated teeth. The presence of bacteria was confirmed in most teeth with periapical lesions, while the most frequently identified bacteria were *E. faecalis*, *P.intermedia* and *P.micros*.

Keywords: PCR; *E. faecalis*; endodontic failure

INTRODUCTION

The main objective of endodontic treatment is to eliminate infection from the root canal and prevent reinfection by three-dimensional hermetic obturation of the canal system. However, sometimes even properly conducted endodontic treatment can fail. It has been confirmed that the outcome of endodontic treatment depends largely on the quality of endodontic procedures and possibility of eliminating infection from root canal system before obturation [1, 2]. Due to the complexity of canal systems their cleaning is difficult, therefore bacteria may remain in inaccessible parts of the canal, especially in the apical portion.

Persistant infection in the apical third is most often result of inadequate completion of endodontic treatment i.e. non-aseptic conditions with insufficiently extended and poorly designed access cavities, insufficient dimension of instrumentation, inadequate hermetic obturation or microleakage due to inadequate temporary or definitive restorations [3]. However, number of infections may persist as asymptomatic periapical radiolucency even if endodontic procedure is properly implemented. The reason is usually complex anatomy of the root canal system with regions that cannot be adequately treated and obturated by existing instruments, materials and techniques [4]. Some studies have shown that certain parts of the root canal space remain untouched during chemo-mechanical instrumentation regardless of the preparation technique or instruments used [5]. Untreated parts of the root canal can contain bacteria and necrotic tissues even when obturation seems to be radiographically correct [4]. It is believed that endodontic failure is caused by inability of complete control and elimination of infection in the root canal.

The aim of this study was, using PCR technique, to investigate microbiological status of endodontically treated teeth with persistent infection immediately after the removal of obturation material.

METHODS

Material for microbiological tests was obtained by taking samples from 30 patients who had root canal treatment done earlier but they needed retreatment. After obtaining dental history, and taking periapical radiographs, patients

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were clinically examined and failure of the initial endodontic treatment was diagnosed.

Microbiological study included 30 teeth (8 multi-rooted and 22 single-rooted) indicated for endodontic retreatment. Primary endodontic therapy was performed 12 months ago in 2 cases, 1-5 years in 8 cases, while for 20 teeth, primary endodontic treatment was done > 5 years ago. All 30 teeth had inadequate obturation and that was the failure criterion for which patients needed retreatment. Poor quality of obturation was assessed as short filling (in 22 teeth), "forgotten" canal (in 7 teeth), or separated instrument (in 1 tooth). Adequate restoration or prosthetic restorations (crowns) were observed in 13 teeth, 7 teeth were without coronal restoration for a longer period of time and 10 teeth did not have proper restoration. The presence of symptoms such as pain, swelling, presence of a fistula, sensitivity to percussion or pain when biting were observed in 10 patients, while the remaining 20 had no clinical signs or symptoms.

Status of apical periodontal tissue was evaluated by PAI index, where completely healthy periodontium (PAI 1) was radiographically registered in 6 cases; PAI 2 score (small changes in the bone structure that are not pathognomonic for apical periodontitis) was recorded in 7 cases; PAI 3 score (which includes changes in bone structure with decalcification characteristic for apical periodontitis) was registered in 13 teeth; PAI 4 (which represents periodontitis with clearly defined zone of radiolucency) was noted in 3 cases; the highest score of PAI 5 (advanced periodontitis with signs of exacerbation and expansion of the bone), was registered only in 1 case. Microbiological process involved first the removal of hard and soft deposits from teeth, restorations and decay and placing a rubber dam. Disinfection of the operative field was done with 30% hydrogen peroxide solution and 2.5% sodium hypochlorite solution, which is then inactivated with 5% sodium thiosulfate. Previous root canal filling material was removed without the use of any solvents, fillers, lubricants or irrigants. Gates Glidden drills were used to remove gutta-percha from the first two-thirds of the root canal, and Hedstrom files were used for the apical third of the root canal. During radiographic determination of working length, the quality of removal of obturation material was checked. Working length was determined with apex locator. In cases of forgotten canals, access cavity was extended, oriphicies located, and root canals were instrumented by modified double-cone technique - Gates Glidden drills, hand Hedstrom and K-Flex files to full working length. The size of apical preparation (ISO # 25 or # 30) depended on initial diameter and curvature of the root canal. Then after small amount of sterile saline was placed in the root canal and further instrumented in order to scrape the material from the canal walls. The sample was taken using sterile canal instrument – barbed broach type (# 15) or Hedstrom files (# 15) with the help of paper points that were used to dry the canal. The paper points were placed with sterile forceps and left in the canal for 60 seconds and then placed in sterile micro-tubes together with canal instruments whose handle was cut off with sterile forceps. The micro-tubes were stored at the temperature of -20 °C until microbiological analysis.

With the aim to isolate DNA, 100 ml of redistilled water was added in each micro-tube with paper points. Isolation of total bacterial DNA (Gram positive and Gram negative bacteria) was performed using commercial kit QIAamp DNA Mini Kit (Qiagen). After application of isolation protocol, bacterial DNA was dissolved in 100 ml of elution buffer.

The incidence of the following bacteria was analyzed by classical PCR: *Enterococcus faecalis, Peptostreptococcus micros, Porphyromonas endodontalis* and *Actinobacillus actinomycetemcomitans*. The sequences of used primers; the temperature profile and length of PCR products are shown in Table 1. In each PCR reaction, simultaneously with the test samples, positive and negative controls were used to avoid false positive and negative results. Reaction mixture of PCR with 25 ml volume was made up of the following components: 13 ml dH_2O shall, 2.5 ml PCR buffer, 1.5 ml of 25 mM $MgCl_2$, 1 ml of dNTP, per 1 ml of F and R primers, 0.2 ml of Taq polymerase and 5 ml bacterial DNA.

The effectiveness of PCR reaction was measured by electrophoresis on a vertical 8% polyacrylamide gel (PAA) in 1XTBE buffer, at constant voltage of 200 V for a period of 30 min. Visualization of PCR products was performed by staining with ethidium bromide.

RESULTS

PCR technique was used to analyze the presence of the following microorganisms: *E. faecalis*, *P. micros*, *P. inter-*

Table 1. Primer sequences, specific hybridization temperature and length of PCR products
Tabela 1. Sekvence prajmera, specifične temperature hibridizacije i dužine PCR produkata

Microorganism Mikroorganizam	Primer Prajmer	Temperature Profile Temperaturni profil	PCR Product PCR produkt
Enterococcus faecalis	F,TACTGACAAACCATTCATGATG R, AACTTCGTCACCAACGCGAAC	95°C 3min 95°C 45s, 55°C 1min, 72°C 1min, X35 cyclus 72°C 5min	112bp
Peptostreptococcus micros	F, AGAGTTTGATCCTGGCTCAG R,ATATCATGCGATTCTGTGGTCTC		207bp
Prevotela intermedia	F,CGTGGACCAAAGATTCATCGGTGGA R, CCGCTTTACTCCCCAACAAA		259bp
Porphyromonas endodontalis	F, GCTGCAGCTCAACTGTAGTC R, CCGCTTCATGTCACCATGTC		672bp
Actinobacilus actinomycetemcomitans	F, GCTAATACCGCGTAGAGTCGG R, ATTTCACACCTCACTTAAAGGT		500bp

Table 2. The presence of bacteria in the sample in relation to the status of apical periodontium
Tabela 2. Prisustvo bakterija u uzorku u odnosu na stanje apeksnog parodoncijuma

Status of Periodontium Stanje parodoncijuma	Negative bacteriological findings Negativan bakteriološki nalaz		Positive bacteriological findings Pozitivan bakteriološki nalaz		Total Ukupno
	N	%	N	%	
Healthy periodontium Zdrav parodoncijum PAI 1,2	6	20%	7	23.3%	13
Chronic apical periodontitis Hronični apikalni periodontitis PAI 3,4,5	0	0	17	56.6%	17
Σ	6	20%	24	80%	30

Table 3. The presence of bacterial species in the tested samples of root canals
Tabela 3. Zastupljenost bakterijskih vrsta u testiranim uzorcima kanala

Microorganism Vrsta bakterije	N	%
E. faecalis	20	66.6%
P. micros	14	46.6%
P. endodontalis	8	26.6%
P. intermedia	18	60%
A. actinomycetemcomitans	3	10%

Table 4. The presence of bacterial species in relation to the apical periodontal condition
Tabela 4. Zastupljenost bakterijskih vrsta u odnosu na stanje apeksnog parodoncijuma

Microorganism Vrsta bakterije	Teeth with healthy periodontium (PAI 1,2) 7 teeth Zubi sa zdravim parodoncijumom (PAI 1,2) 7 zuba		Teeth with periapical lesions (PAI3,4,5) 17 teeth Zubi sa periapikalnim lezijama (PAI3,4,5) 17 zuba		Total of 24 teeth Ukupno 24 zuba	
	N	%	N	%	N	%
E. faecalis	4	57%	16	94%	20	83.3%
P. micros	4	57%	10	58.8%	14	58.3%
P. endodontalis	4	57%	4	23.5%	8	33.3%
P. intermedia	4	57%	14	82.3%	18	75%
A. actinomycetemcomitans	0	0	3	17.6%	3	12.5%

Table 5. Distribution of the number of bacterial species in individual samples
Tabela 5. Distribucija broja bakterijskih vrsta u pojedinačnim uzorcima

Number of species per sample Broj vrsta u uzorku	N	%
0	6	20
1	4	13.3
2	6	20
3	10	33.3
4	3	10
5	1	3.3

media, *P. endodontalis* and *A. actinomycetemcomitans*. Positive bacteriological findings were registered in 80% of cases, while the bacteria were not identified in 20% of samples. All negative samples were taken from the root canal without significant changes in the apical periodontal tissue (PAI 1, 2) while 17 out of 24 canals with identified bacteria belonged to the teeth with damaged apical periodontium (PAI 3, 4, 5) (Table 2). All samples taken from the root canals with chronic periapical lesions were positive for bacteria (100%).

Table 6. The presence of bacterial species in tested samples in relation to the state of the apical periodontium (PAI index)
Tabela 6. Zastupljenost bakterijskih vrsta u testiranim uzorcima u odnosu na stanje apeksnog parodoncijuma (PAI index)

PAIN of species / Broj vrsta		1	2	3	4	5	Total Ukupno	
	N	6	7	13	3	1	N	%
	%	20	23.3	43.3	10	3.3		
0	N	5	1				6	20
	%	16.6	3.3					
1	N	1	2	1			4	13.3
	%	3.3	6.6	3.3				
2	N		1	4	1		6	20
	%		3.3	13.3	3.3			
3	N		3	7			10	33.3
	%		10	23.3				
4	N				2	1	3	10
	%				6.6	3.3		
5	N			1			1	3.3
	%			3.3				

Isolated bacteria mainly belonged to *E. faecalis* (66.6%) followed by *P.intermedia* (60%), *P. micros* (46.6%), *P. endodontalis* (26.6%) and *A. actinomycetemcomitans* (10%) (Table 3).

The most dominant microorganism in root canals with positive bacteriological findings was *E.faecalis* (83.3%), followed by *P. intermedia* (75%) and *P.micros* (58.3%) (Table 4). In the group of teeth with healthy apical pericapical tissue, all bacterial species (except *A. actinomycetemcomitans* which was not detected in any of the samples) were equally represented or were identified in 57.7% of canals. In the case of teeth with chronic periapical changes, the most common isolation was *E. faecalis* identified in 94% of the canals, then *P.intermedia* that was present in 82.3% of samples (Table 4).

In regards to the number of bacterial species contained in a single sample, monoinfection was registered in 13.3% of cases (*E.faecalis* was presented in half of the canals), while the most common bacteria identified in 33.3% of canals were 3 bacterial species per one canal *E. faecalis*, *P. intermedia* and *P.micros* (Table 5). It was observed that the samples taken from the tooth with healthy periodontal tissues around the root tip, showed mainly absence of bacteria or the presence of 1 or 2 bacterial species, while in the case of chronic periapical changes, in more than a half of samples, the presence of 3 or more bacterial species was identified (Table 6).

Table 7. Distribution of bacteria in correlation with the presence of certain symptoms
Tabela 7. Distribucija bakterija u odnosu na prisustvo određenih simptoma

Symptom Simptom	Pain Bol		Swelling Otok		Sinus tract Fistula		Percution sensitivity Perkutorna osetljivost		Pain when biting Bol na zagrižaj	
Microorganism Mikroorganizam	N	%	N	%	N	%	N	%	N	%
E. faecalis	3	100	1	50	1	50	8	100	3	100
P. micros	2	66.6	1	50	1	50	4	50	1	33.3
P. endodontalis	1	33.3					2	25		
P. intermedia	3	100	1	50	1	50	8	100	3	100
A. actinomycetemomitans					1	50	1	12.5	1	33.3
Number of patients with symptoms Broj pacijenata sa simptomima	3		2		2		8		3	

Correlation between the presence of certain symptoms after the initial endodontic treatment and findings of specific bacterial species in root canal are shown in Table 7. *E. faecalis* and *P. intermedia* were detected in all patients with pain, teeth sensitive to percussion and pain when biting, as well as the half of the samples taken from the root canal with sinus tract and swelling. *P.micros* was identified in 66.6% of patients with pain, 50% of patients with swelling, sinus tract and percussion sensitivity and 33.3% of patients with pain when biting. The presence of *P. endodontalis* was confirmed in 33.3% of the canals registered with spontaneous pain and 25% with sensitivity to percussion. *A. actinomycetemcomitans* was identified in 50% of the canals with sinus tract, 33.3% of patients with pain when biting and 12.5% of the samples taken from the root canal of patients with sensitivity to percussion (Table 7).

DISCUSSION

The analysis included 30 teeth that needed retreatment regardless of its causes - prosthetic indications, incidental finding or the patient reported having problems. In order to prevent contamination of canal samples, strictly aseptic conditions were conducted by the current protocol for disinfection of working fields [6–9]. This involved decontamination and disinfection of the operative field with 30% hydrogen peroxide solution and 2.5% sodium hypochlorite followed by inactivation of 5% sodium thiosulfate so that its remains would not affect the sample taken [7]. Also, complete removing of the root canals filings was done purely mechanically without use of any solvents, lubricants or irrigants. The micro-tubes with microbiological material were stored at -20° C, for no longer than a month.

PCR (Polymerase Chain Reaction) is a modern, fast (it takes a few hours), and a simple method for identification of microorganisms. It is extremely sensitive and highly specific. Theoretically, it is possible to demonstrate the presence of only single bacterial cell (living or dead) in the sample, although the number of 10 cells is considered to be the lowest limit for detection (e.g. 100 viable cells are necessary for one method of bacteria cultivation) [9]. Identification is based on *in vitro* amplification of target DNA fragment, which can be repeated up to a billion times. Because its detection works on the basis of genotypic structure, rather than phenotypic characteristics of microorganisms, identification is very reliable and precise. It is possible to identify those bacterial species that cannot be cultivated *in vitro* or cases where the number of cells in examined material is very small [9]. However, the PCR method of identification of microorganisms has its limitations. Firstly, the species that are not targeted or detected by other methods cannot be identified. Precisely determined conditions are required for reaction with specific primer pair that can detect only particular and specific bacterial species. It should be noted that PCR method can not determine if identified cell is alive or dead. Another limitation of the conventional PCR method is its inability to quantify the number of bacteria. This can be overcome by "real-time" PCR method where the number of bacterial cells can be determined [10].

Positive bacteriological findings were registered in 80% of cases, while no bacteria were identified in 20% of samples. All samples taken from root canals with chronic periapical lesions were positive for presence of bacteria (100%). Also, all negative samples were taken from root canal with healthy periapical tissue. This confirmed the hypothesis that without bacteria, there is no infection in periapical tissues, and consequently there is no failure of endodontic treatment. Similar results were obtained by Siqueira et al. [5], Gomes et al. [11,12], Roca et al. [13], Sedgley et al. [14] and Sakamoto et al. [15].

However, 23% of positive bacteriological samples were taken from root canals where periapical tissue had PAI score 2 (periapical radiolucency) with no pathognomonic signs of chronic inflammation. Kaufman et al. [16] and Olette et al. [17] reported the presence of bacteria in the root canal without any periapical changes detected radiographically. The explanation may lie in the fact that two-dimensional radiography is not sufficiently accurate to diagnose apical periodontitis with less destroyed bone tissue [18]. Also, it could be that small number and low virulent bacteria are present. Only if present in higher numbers and pathogenic bacteria persisting after primary endodontic treatment can cause or maintain periradicular inflammation [2]. However, there is a dilemma whether bacteria remained after primary endodontic treatment (persistent infection) or they are the result of re-infection (secondary infection). In the recent years, research has pointed out the importance of proper coronal restora-

tion in preventing re-infection of endodontic space with opinion that secondary infection is important cause of endodontic treatment failure [1, 19]. The most common microorganism in the canal system with positive bacteriological findings was *E. faecalis* followed by *P. intermedia* and *P. micros* accounting for 58.3%. *E. faecalis* was identified in 94% of root canals with chronic periapical lesions (PAI 3, 4, 5) which is similar to findings of Sedgley et al. who also used PCR identification method. They used 48 samples and showed that the incidence of *E. faecalis* finding was 90% [14]. Gomes et al. used PCR to analyze microbiological status of previously filled canals with periapical lesions and came to the conclusion that *E. faecalis* was present in 90% of bacteriologically positive root canals followed by *P. micros* 59%, *P. gingivalis* 41%, *P. endodontalis* 26% and *P. intermedia* 13% [12]. This high percentage of *E. faecalis* was probably the result of its numerous and diverse virulence factors and extraordinary ability to survive. This microorganism is small; it easily penetrates into dentinal tubules and has a good adherence to collagen [20]. It is resistant to calcium hydroxide [21] and has the ability to survive (as a single species) in dentinal tubules without the support of other bacteria [22]. Also, it has the ability to survive without nutrients and recover easily when they become available in the form of serum (the origin of alveolar bone and periodontal ligament) [23].

The results of this study confirmed the presence of *E. faecalis* in 57.7% of root canals without changes in apical periodontal tissue. However, *P. intermedia*, *P. endodontalis* and *P. micros* were also identified in 57.7% of root canals which leads to the conclusion that *E.faecalis* is not the only microorganism responsible for failure of endodontic treatment. Williams et al. showed that *E. faecalis* can survive all stages of endodontic treatment because RT-PCR detected its presence in samples taken immediately after instrumentation and irrigation as well as after medication [24].

Our research indicated that remaining microorganisms could be present in root canals due to inadequate coronal seal. In 70% of teeth with healthy periodontal apical tissue, coronal restoration did not show satisfactory quality that opened door for secondary infection of endodontic space. In addition, although present in root canal, the bacteria had no effect on periapical tissue. They probably remained blocked and trapped in dentinal tubules or root canal filling material blocked further progress to periapical tissues (microleakage).

Patients who took part in our study came from general dental practice and health centers where it was quite difficult to adhere to contemporary standards and good endodontic practice, therefore the incidence of residual bacteria after primary endodontic treatment was high. *Prevotella spp* and *Porphiromonas spp* (previously classified as *Bacteroides spp*) belong to the group of "black-pigmented bacteria" because in contact with agar, they form shiny, smooth colonies of gray or black. According to the new taxonomy, saharolytic Bacteroides spp species are classified as genus Prevotella, while asaharolytic species belong to genus *Porphiromonas*. Types of *P. intermedia*, *P. melaninogenica*, *P. denticola* and *P. dentalis* belong to gram-negative obligate anaerobes. Although they have limited ability of fermentation of amino acids and require the presence of hemin and menadione for growth, they can be observed in different parts of body (oral cavity, upper respiratory and urogenital system) [25]. Ruan et al. found that *P. intermedia* originating from oral cavity represents potential opportunistic microorganism associated with periodontal disease but also apical periodontitis due to its adhesiveness and competitiveness with surrounding microorganisms [25]. *P. endodontalis* is gram-negative microorganism associated with periodontitis, endodontic infections and gingivitis, and more frequently with symptomatic than asymptomatic infections in oral cavity [26].

One-third of patients who took part in the current study showed some clinical symptoms, but the most common symptom was sensitivity to percussion. The most common microorganisms present in the samples taken from such teeth were *E. faecalis* and *P. intermedia* (100%), followed by *P. micros* (50%). Gomes et al. also found statistically significant relationship between *P.micros* and tooth sensitivity to percussion [12] while Pinheiro et al. noticed association between *P.intermedia* and the presence of these symptoms [27]. *E.faecalis* and *P.intermedia* were detected in all patients with pain, tenderness to percussion and pain when biting, as well as one half of the samples taken from root canals of patients with swelling and fistula. *P. micros* was identified in 66.6% of patients with pain that is in accordance with the study of Pinheiro et al. who reported involvement of bacterial species *P.intermedia* and *P.micros* in teeth where pain was present [27].

CONCLUSION

The presence of periapical lesions significantly affects microbiological status of endodontically treated teeth. The presence of bacteria in root canals was confirmed in most cases of unsuccessful endodontic treatments, while the most frequently identified bacteria were *E. faecalis*, followed by *P. intermedia*, *P. micros* and *P. endodontalis*.

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Mikrobiološki status kanala korena endodontski neuspešno lečenih zuba

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KRATAK SADRŽAJ

Uvod Osnovni zadatak endodontskog tretmana je da eliminiše infekciju iz kanala korena i spreči reinfekciju trodimenzionalnom hermetičkom opturacijom kanalskog sistema. Usled nemogućnosti potpune kontrole i eliminacije infekcije iz kanala korena može doći do pojave endodontskog neuspeha.

Cilj ovog rada je bio da se neposredno posle uklanjanja materijala za opturaciju kod zuba sa neuspelim endodontskim lečenjem PCR tehnikom proveri mikrobiološki status endodontski lečenih zuba.

Metode Mikrobiološka studija je obuhvatila 30 zuba (osam višekorenih i 22 jednokorena) indikovanih za ponovljeni endodontski tretman. Posle dezinfekcije radnog polja i dezopturacije koja je urađena isključivo mehaničkim putem, uzorak je uzet sterilnim kanalnim instrumentom tipa pulpekstirpatora (#15) ili Hoedstrem-turpije (#15) i uz pomoć papirnih poena kojima je sušen kanal. Ependorfice su čuvane na temperaturi od -20˚C do mikrobiološke analize. Putem klasičnog PCR analizirana je zastupljenost bakterija: *Enterococcus faecalis, Peptostreptococcus micros, Prevotela intermedia, Porphyromonas endodontalis* i *Actinobacilus actinomycetemcomitans.*

Rezultati Pozitivan bakteriološki nalaz registrovan je u 80% slučajeva, dok bakterije nisu identifikovane u 20% uzoraka. Svi negativni uzorci su uzeti iz kanala korena zuba bez značajnih promena u apeksnom periodoncijumu, dok je 17 od 24 kanala sa identifikovanim bakterijama pripadalo zubima sa oštećenim apeksnim parodoncijumom. Najprisutniji mikroorganizam u kanalima sa pozitivnim bakteriološkim nalazom bio je *E. faecalis*, koji je identifikovan u 83,3% kanala, zatim *P. inermedia* sa 75% i *P. micros* sa zastupljenošću od 58,3%. Kod zuba sa hroničnim periapikalnim promenama najzastupljeniji je bio *E. faecalis*, koji je identifikovan u 94% kanala, zatim *P. intermedia*, koja je bila prisutna u 82,3% uzoraka. Mikroorganizmi *E. faecalis i P. intermedia* su registrovani kod svih pacijenata sa nekim od simptoma.

Zaključak Prisustvo periapikalnih lezija značajno utiče na mikrobiološki status kanala korena endodontski lečenih zuba. Prisustvo bakterija u kanalima korena potvrđeno je u većini endodontski neuspešno lečenih zuba, a najčešće identifikovane bakterije bile su *E. faecalis*, zatim *P. intermedia, P. micros i P. endodontalis.*

Ključne reči: PCR; *E. faecalis*; endodontski neuspeh

UVOD

Osnovni zadatak endodontskog tretmana je da eliminiše infekciju iz kanala korena i prevenira reinfekciju trodimenzionalnom hermetičkom opturacijom kanalskog sistema. Ponekad, međutim, i kod adekvatno sprovedenog endodontskog tretmana može doći do endodontskog neuspeha.

Potvrđeno je da ishod endodontskog tretmana umnogome zavisi od kvalitetno realizovane endodontske procedure i mogućnosti eliminacije infekcije iz kanalnog sistema korena pre opturacije [1, 2]. Zbog kompleksnosti kanalskog sistema njegovo čišćenje je prilično otežano pa bakterije mogu zaostati u nepristupačnim delovima kanala na svim njegovim nivoima, a naročito u apeksnom delu.

Intraradikularna infekcija koja perzistira u kompleksnoj apikalnoj trećini kanalnog sistema korena najčešće je posledica neadekvatno sprovedenog endodontskog tretmana, odnosno neaseptičnih uslova rada sa nedovoljno ekstendiranim i loše dizajniranim pristupnim kavitetom, kao i nedovoljne dimenzije instrumentacije, neadekvatne hermetične opturacije ili mikrocurenja usled neadekvatnog privremenog ili definitivnog ispuna [3].

Međutim, određeni broj lezija u periapeksu može da perzistira kao asimptomatsko radiografsko rasvetljenje čak i pored adekvatno realizovane endodontske procedure. Razlog je najčešće kompleksna anatomija kanalnog sistema sa regijama koje se ne mogu adekvatno ni obraditi niti opturisati postojećim instrumentima, materijalima i tehnikama [4]. Neke studije su pokazale da određeni delovi kanalnog prostora korena zuba ostaju netaknuti tokom hemomehaničke instrumentacije bez obzira na to koja se tehnika preparacije i kanalni instrumenti koriste [5]. Netaknute regije endodontskog prostora mogu sadržati bakterije i nekrotično tkivo čak i onda kada se čini da je opturacija kanala radiografski korektna [4]. Smatra se da do endodontskog neuspeha dolazi usled nemogućnosti potpune kontrole i eliminacije infekcije u kanalu korena.

Cilj ovog rada je bio da se neposredno posle uklanjanja materijala za opturaciju kod zuba sa neuspelim endodontskim lečenjem PCR tehnikom proveri mikrobiološki status endodontski lečenih zuba.

METODE

Materijal za mikrobiološka ispitivanja dobijen je uzimanjem uzoraka iz 30 endodontski lečenih kanala korena zuba kod kojih je postojala potreba za retretmanom. Kod svih pacijenata uzeti su anamnestički podaci, urađeni su klinički pregled i detaljna analiza retroalveolarne radiografije i na osnovu toga postavljana dijagnoza neuspeha inicijalnog endodontskog tretmana.

Mikrobiološka studija je obuhvatila 30 zuba (osam višekorenih i 22 jednokorena) indikovanih za ponovljeni endodontski tretman. Primarna endodontska terapija je u dva slučaja bila sprovedena pre 12 meseci, u osam slučajeva u periodu 1–5 godina, dok je kod 20 zuba primarno endodontsko lečenje bilo urađeno pre više od pet godina. Svih 30 zuba imalo je radiografski nalaz sa neadekvatnom opturacijom jer je to bio kriterijum neuspeha zbog kojeg su pacijenti i upućeni na ponovni endodontski tretman. Nezadovoljavajući nivo kvaliteta opturacije je ocenjen kao kratko punjenje (22 zuba), kao „zaboravljeni“ kanal (sedam zuba), odnosno kanal sa zalomljenim instrumentom (jedan

zub). Kod 13 zuba uočena je adekvatna restauracija ili protetska nadoknada, sedam zuba je bilo bez ispuna duži period, a 10 zuba nije imalo odgovarajući ispun. Prisustvo simptoma u vidu bola, otoka, prisustva fistule, osetljivosti na perkusiju ili bolovi na zagrižaj zabeleženi su kod 10 pacijenata, dok preostalih 20 nije imalo kliničke znake ni simptome.

Zdravlje apeksnog periodoncijuma ocenjivano je dodeljivanjem PAI indexa, pri čemu je potpuno zdrav parodoncijum (PAI 1) radiografski registrovan u šest slučajeva; PAI 2 skor (male promene u strukturi kosti koje nisu patognomonične za apikalni periodontitis) zabeležen je u sedam slučajeva; PAI 3 skor (koji podrazumeva promene u koštanoj strukturi sa dekalcifikacijom karakterističnom za apikalni periodontitis) registrovan je kod 13 zuba; PAI 4 (koji predstavlja periodontitis sa jasno definisanom zonom rasvetljenja) zabeležen je u tri slučaja, i najviši PAI 5 skor (uznapredovao periodontitis sa znacima egzacerbacije i ekspanzije kosti) dodeljen je samo jednom slučaju.

Mikrobiološki postupak je uključivao najpre uklanjanje čvrstih i mekih naslaga sa zuba, eventualno postojećih restauracija i karijesnih masa i postavljenje koferdama. Dezinfekcija operativnog polja urađena je 30% rastvorom vodonik-peroksida i 2,5% rastvorom natrijum-hipohlorita, koji je zatim inaktivisan 5% rastvorom natrijum-tiosulfata. Dezopturacija je urađena isključivo mehanički bez upotrebe bilo kakvih rastvarača, lubrikanata ili iriganasa. Gates-Glyden svrdlima je gutaperka uklonjena iz prve dve trećine kanala, a hoedstrem turpijama iz apikalne trećine kanala korena zuba. Tokom radiografskog postupka odontometrije kontrolisan je kvalitet uklanjanja materijala za opturaciju. Odontometrija je potvrđivana elektroodontometrom. U slučajevima zaboravljenih kanala ekstendiran je pristupni kavitet, korigovana trepanacija i locirani ulazi, a kanali su instrumentirani modifikovanom tehnikom dvostrukog konusa Gates-Glyden svrdlima i ručnim hoedstrem i k-flex turpijama do pune radne dužine. Veličina apeksne preparacije (ISO #25 ili #30) zavisila je od inicijalnog promera i povijenosti kanala korena zuba. Zatim je mala količina sterilnog fiziološkog rastvora ubrizgana u kanal tako da se isti ne prepuni. Kanal je potom dodatno instrumentiran kako bi se sastrugao materijal sa zidova kanala. Uzorak je uzet sterilnim kanalnim instrumentom tipa pulpekstirpatora (#15) ili hoedstrem turpije (#15) i uz pomoć papirnih poena kojima je sušen kanal. Papirni poeni su plasirani sterilnom pincetom, ostavljeni u kanalu tokom 60 sekundi, a zatim postavljeni u sterilne ependorfe zajedno sa kanalnim istrumentima čija je drška odsečena sterilnim kleštima. Ependorfice su čuvane na temperaturi od -20˚C do mikrobiološke analize.

Sa ciljem da se izoluje DNK, u svaku mikrotubu papirnim poenom dodato je 100 µl redestilovane H_2O. Za izolaciju totalne bakterijske DNK (gram-pozitivne i gram-negativne bakterije) korišćen je komercijalni kit Qiamp DNA Mini Kit (Qiagen). Posle primenjenog protokola za izolaciju bakterijska DNK je rastvorena u 100 µl pufera za eluciju.

Putem klasičnog PCR analizirana je zastupljenost bakterija: *Enterococcus faecalis, Peptostreptococcus micros, Prevotela intermedia, Porphyromonas endodontalis* i *Actinobacilus actinomycetemcomitans.* Sekvence korišćenih prajmera, temperaturni profil i dužine PCR produkata date su u Tabeli 1. U svakoj PCR reakciji istovremeno sa testiranim uzorcima korišćene su pozitivne i negativne kontrole, da bi se izbegli lažno pozitivni i negativni rezultati. Reakcion smešu PCR reakcije, volumena 25 µl, činile su sledeće komponente: 13 µl dH_2O, 2,5 µl PCR pufera, 1,5 µl 25 mM $MgCl_2$, 1 µl dNTP, po 1 µl F i R prajmera, 0,2 µl Taq polimeraze i 5 µl bakterijske DNK.

Uspešnost PCR reakcije je proveravana vertikalnom elektroforezom na 8% poliakrilamidnom gelu (PAA) u 1XTBE-puferu, pri konstantnom naponu struje od 200 V, u trajanju od 30 min. Vizuelizacija PCR produkata urađena je putem bojenja etidijum bromidom.

REZULTATI

PCR analizom je ispitivano prisustvo sledećih mikroorganizama: *Enterococcus faecalis, Peptostreptococcus micros, Prevotela intermedia, Porphyromonas endodontalis* i *Actinobacilus actinomicetemcomitans*.

Pozitivan bakteriološki nalaz registrovan je u 80% slučajeva, dok bakterije nisu identifikovane u 20% uzoraka. Svi negativni uzorci su uzeti iz kanala korena zuba bez značajnih promena u apeksnom periodoncijumu (PAI 1, 2) dok je 17 od 24 kanala sa identifikovanim bakterijama pripadalo zubima sa oštećenim apeksnim parodoncijumom (PAI 3, 4, 5) (Tabela 2). Svi uzorci uzeti iz kanala korena zuba sa hroničnim periapikalnim lezijama su bili pozitivni na prisustvo bakterija (100%).

Kada je u pitanju zastupljenost ispitivanih bakterijskih vrsta, najčešće identifikovana bakterija bila je *E. faecalis* (66,6%), potom *P. intermedia* (60%), *P. micros* (46,6%), *P. endodontalis* (26,6%) i *A. actinomycetemcomitans* (10%) (Tabela 3).

Najprisutniji mikroorganizam u kanalima sa pozitivnim bakteriološkim nalazom bio je *E. faecalis*, koji je identifikovan u 83,3% kanala, zatim *P. inermedia* sa 75% i *P. micros* sa zastupljenošću od 58,3% (Tabela 4). U grupi zuba sa zdravim apeksnim parodoncijumom sve bakterijske vrste (izuzev *A. actinomycetemcomitans*, koji nije detektovan ni u jednom uzorku) bile su jednako zastupljene, odnosno identifikovane su u 57,7% kanala. Kod zuba sa hroničnim periapikalnim promenama najzastupljeniji je bio *E. faecalis*, koji je identifikovan u 94% kanala, zatim *P. Intermedia*, koja je bila prisutna u 82,3% uzoraka (Tabela 4).

Kada je u pitanju broj bakterijskih vrsta prisutnih u pojedinačnom uzorku, samo u 13,3% slučajeva se radilo o monoinfekciji (u polovini takvih kanala u pitanju je bio *E. faecalis*), dok su najčešće (33,3% kanala) identifikovane tri bakterijske vrste u kanalu korena (*E. faecalis, P. intermedia i P. micros*) (Tabela 5).

Uočeno je da uzorci uzeti iz zuba sa zdravim parodontalnim tkivima oko vrha korena pokazuju uglavnom odsustvo bakterija ili prisustvo jedne ili dve bakterijske vrste, dok je u slučaju postojanja hroničnih periapikalnih promena u više od polovine uzoraka identifikovano prisustvo tri ili više bakterijskih vrsta (Tabela 6).

Korelacija između prisustva određenih simptoma posle primarnog endodontskog lečenja i nalaza određenih bakterijskih vrsta u kanalu korena prikazana je u Tabeli 7.

Mikroorganizmi *E. faecalis i P. intermedia* su registrovani kod svih pacijenata sa bolovima, kod zuba osetljivih na perkusiju i bolove pri zagrižaju, kao i kod polovine uzoraka uzetih iz kanala korena zuba sa otokom i fistulom.

Peptostreptococcus micros je identifikovan kod 66,6% pacijenata sa bolom, kod 50% pacijenata sa otokom, fistulom i perkutornom osetljivošću i kod 33,3% pacijenata sa prisutnim bolovima na zagrižaj. Prisustvo *P. endodontalis* je potvrđeno u 33,3% kanala sa registrovanim spontanim bolovima i 25% kanala sa perkutornom osetljivošću. Najređe identifikovani

mikroorganizam bio je *A. actinomyces*, koji je identifikovan u 50% kanala sa prisutnom fistulom, kod 33,3% pacijenata sa bolovima na zagrižaj i 12,5% uzoraka uzetih iz kanala korena zuba pacijenata sa osetljivošću pri perkusiji (Tabela 7).

DISKUSIJA

U analizu je uključeno 30 zuba kod kojih je postojala potreba za retretmanom bez obzira na to da li je u pitanju bila protetska indikacija, slučajan nalaz ili je pacijent imao određene tegobe.

Kako bi se sprečila kontaminacija uzoraka iz kanala, sprovođeni su striktno aseptični uslovi uzimanja i aktuelni protokol dezinfekcije radnog polja [6–9]. To je podrazumevalo dekontaminaciju radnog polja i dezinfekciju operativnog polja 30% rastvorom vodonik-peroksida i 2,5% rastvorom natrijum-hipohlorita i potom inaktivaciju 5% rastvorom natrijum-tiosulfata kako njegovi ostaci ne bi uticali na uzeti uzorak [7]. Takođe, kompletna dezopturacija je rađena isključivo mehanički bez upotrebe bilo kakvih rastvarača, lubrikanata ili iriganasa. Mikrotube sa mikrobiološkim materijalom su čuvane na temperaturi od -20 C, ne duže od mesec dana.

PCR (Polymerase Chain Reaction) savremena je, brza (potrebno je svega nekoliko sati) i jednostavna metoda za identifikaciju mikroorganizama. Izuzetno je osetljiva i visoko specifična. Teoretski je moguće dokazati prisustvo samo jedne bakterijske ćelije (žive ili mrtve) u uzorku, mada se broj od 10 ćelija smatra donjom granicom detekcije (primera radi, za metodu kultivisanja bakterija neophodno je 100 živih ćelija) [9]. Identifikacija se zasniva na *in vitro* amplifikaciji ciljnog fragmenta DNK, koju je moguće ponoviti i do milijardu puta. Zahvaljujući tome što se detekcija radi na osnovu genotipske strukture, a ne fenotipskih karakteristika mikroorganizama, identifikacija je vrlo pouzdana i precizna. Moguće je identifikovati i one bakerijske vrste koje se ne mogu kultivisati *in vitro* ili slučajeve kada je broj ćelija u ispitivanom materijalu jako mali [9].

Međutim, PCR metoda identifikacije mikroorganizama ima i svoja ograničenja. Na prvom mestu, vrste koje nisu otkrivene nekom drugom metodom i vrste koje se ciljano ne traže ne mogu se ni detektovati. Postavljaju se tačno determinisani uslovi izvođenja reakcije sa određenim parom prajmera koji mogu da detektuju samo tu, specifičnu, bakterijsku vrstu. Treba naglasiti da PCR metoda ne prepoznaje da li je identifikovana ćelija živa ili mrtva. Drugo ograničenje konvencionalne PCR metode odnosi se na nemogućnost apsolutne kvantifikacije prisutnih bakterija. Ovo se može prevazići „real-time“ PCR metodom, gde se može utvrditi i broj bakterijskih ćelija [10].

Pozitivan bakteriološki nalaz registrovan je u 80% slučajeva, dok bakterije nisu identifikovane u 20% uzoraka. Svi uzorci uzeti iz kanala korena zuba sa hroničnim periapikalnim lezijama su bili pozitivni na prisustvo bakterija (100%). Takođe, svi negativni uzorci su uzeti iz kanala korena zuba sa zdravim parodoncijumom. Na taj način je potvrđena hipoteza da bez bakterija nema infekcije u periapikalnim tkivima, a samim tim ni neuspeha endodontskog lečenja. Slične rezultate dobili su Siqueira i sar. [5], Gomes i sar. [11, 12], Rôças i sar. [13], Sedgley i sar. [14], Sakamoto i sar. [15].

Međutim, 23% bakteriološki pozitivnih uzoraka uzeto je iz kanala korena zuba čije je stanje apeksnog parodoncijuma ocenjeno vrednošću PAI indexa 2, što radiografski predstavlja blago proširen periodontalni prostor bez znakova patognomoničnih za hronična zapaljenja apeksnog parodoncijuma. Kaufman i sar. [16] i Zoletti i sar. [17] takođe su registrovali prisusutvo bakterija u kanalima korenova zuba bez radiografski uočljivih periapikalnih promena. Objašnjenje može ležati u činjenici da retroalveolarna radiografija nije dovoljno precizna u dijagnozi apeksnog parodontitisa sa manjim destrukcijama koštanih tkiva [18]. Drugi razlog za prisustvo bakterija u kanalu korena bi mogao biti mali broj i slaba virulencija bakterija. Da bi mikroorganizmi koji su opstali nakon primarnog endodontskog lečenja kanala korena izazvali ili održavali periradikularno zapaljenje, moraju biti dovoljno patogeni i u dovoljno velikom broju [2].

Kada se govori o bakterijama prisutnim u kanalima korena zuba sa periapikalnim promenama posle endodontskog lečenja, nameće se dilema da li su one zaostale posle primarnog endodontskog tretmana (perzistirajuća infekcija) ili su posledica reinfekcije (sekundarna infekcija). Poslednjih godina su istraživači ukazivali na značaj koronarne restauracije u prevenciji reinfekcije endodontskog prostora i izneli stav da je sekundarna infekcija važan uzročnik neuspeha endodontskog lečenja [1, 19].

Najzastupljeniji mikroorganizam u kanalima sa pozitivnim bakteriološkim nalazom bio je *E. faecalis*, zatim *P. intermedia* i *P. micros* sa zastupljenošću od 58,3%. *E. faecalis* je identifikovan u 94% kanala korena zuba sa hroničnim periapikalnim lezijama (PAI 3, 4, 5), što je slično nalazima Sledgley i sar., koji su takođe PCR metodom identifikacije na 48 uzoraka dobili 90% učestalost ovog gram-pozitivnog mikrorganizma [14]. Gomes i sar. su PCR-om analizirali mikrobiološki status prethodno punjenih kanala sa periapikalnim lezijama i došli do zaključka da je *E. faecalis* prisutan u 90% bakteriološki pozitivnih kanala, potom slede *P. micros* – 59%, *P. gingivalis* – 41%, *P. endodontalis* – 26% i *P. intermedia* – 13% [12].

Ovako visok procenat prisustva *E. faecalisa* verovatno je posledica njegovih brojnih i raznovrsnih faktora virulencije i izuzetne sposobnosti preživljavanja. Ovaj mikroorganizam je dovoljno mali i lako prodire u dentinske tubule, a ima i dobru sposobnost adherencije za kolagen [20]; rezistencije na kalcijum-hidroksid [21]; sposobnost da preživi (kao jedina vrsta) u dentinskim kanalićima kanala bez podrške drugih bakterija [22]; sposobnost da preživi bez ishrane i oporavi se čim nutritijenti u vidu seruma (porekla alveolarne kosti i periodontalnog ligamenta) postanu dostupni [23].

Rezultati ove studije potvrđuju prisustvo *E. faecalisa* u 57,7% kanala korena zuba bez promena u apeksnom parodoncijumu. Međutim, *P. intermedia*, *P. endodontalis i P. micros* su takođe identifikovane u 57,7% kanala, što navodi na zaključak da *E. faecalis* nije jedini mikroorganizam odgovoran za neuspeh endodontskog tretmana. Williams i sar. su pokazali da *E. faecalis* može da preživi sve etape endodontskog tretmana jer su RT-PCR-om detektovali njegovo prisustvo u uzorcima uzetim neposredno posle instrumentacije i irigacije, kao i posle interseansne medikacije [24].

Ovo istraživanje ukazuje na to da su preostali mikroorganizmi verovatno dospeli u kanal zahvaljujući neadekvatnom kruničnom zaptivanju. Naime, kod 70% zuba sa zdravim apeksnim parodoncijumom krunične restauracije nisu bile zadovoljavajućeg kvaliteta, što predstavlja ulazna vrata za sekundarnu infekciju endodontskog prostora. Međutim, iako prisutne u kanalu korena, ove bakterije nisu imale uticaja na periradikularna tkiva. Prvi razlog bi mogao biti da su ostale blokirane i zarobljene u dentinskim kanalićima materijalom za definitivnu opturaciju kanala, a

drugi da je na njihovom putu od usne duplje (mikrocurenjem) kanalno punjenje blokiralo dalji prolaz ka periapeksnim tkivima.

Pacijenti koji su uzeli učešća u ovoj studiji su dolazili iz ordinacija opšte stomatološke prakse i domova zdravlja, gde je mogućnost poštovanja savremenih standarda i dobre endodontske prakse uglavnom neadekvatna, tako da je učestalost bakterija zaostalih posle primarnog endodontskog lečenja mogla biti visoka (kako za *E. faecalis* tako i za druge mikroorganizme).

Prevotella spp i Porphiromonas spp (ranije klasifikovani kao *Bacteroides spp*) pripadaju grupi „crnopigmentisanih bakterija" jer na agaru formiraju sjajne, glatke kolonije sive ili crne boje. Po novoj taksonimiji, saharolitične vrste *Bacteroides spp* su svrstane u rod *Prevotella*, a asaharolitične u rod *Porphiromonas*. Vrste *P. intermedia, P. melaninigenica*, *P. denticola* i *P. dentalis* pripadaju gram-negativnim obligatnim anaerobima. Iako imaju ograničenu sposobnost fermentacije aminokiselina i zahtevaju prisustvo hemina i menadoina za rast, mogu se uočiti u različitim delovima organizma (usna duplja, gornji respiratorni i urogenitalni trakt) [25]. Ruan i sar. su ustanovili da *Prevotella intermedia* poreklom iz usne duplje predstavlja potencijalno oportunistički mikroorganizam koji se povezuje sa periodontalnim oboljenjima ali i sa periapikalnim periodontitisom jer poseduje adhezivnost i kompetitivnost sa okolnim mikroorganizmima [25].

Porphyromonas endodontalis je gram-negativan mikroorganizam koji se dovodi u vezu sa periodontitisom, endodontskom infekcijom i gingivitisom, i to češće sa simptomatskim nego asimptomatskim infekcijama u usnoj duplji [26].

Trećina pacijenata koji su uzeli učešća u studiji su prijavali neki od kliničkih simptoma, a najčešće registrovani simptom bila je osetljivost na perkusiju. Najčešće prisutni mikroorganizmi u uzorcima uzetih iz takvih kanala su bili *E. faecalis* i *P. intermedia* (100%), a zatim *P. micros* (50%). Gomes i sar. su takođe našli statistički značajnu povezanost *P. microsa* i osetljivosti zuba na perkusiju [12], a Pinheiro i sar. su sa ovim simptomom povezali prisustvo *P. intermedia* [27].

E. faecalis i P. intermedia su registrovani kod svih pacijenata sa bolovima, osetljivošću na perkusiju i bolovima na zagrižaj, kao i kod polovine uzoraka uzetih iz kanala korena zuba pacijenata sa otokom i fistulom. *P. micros* je identifikovan kod 66,6% pacijenata sa bolom, a Pinheiro i sar. ovo takođe povezuju sa prisusutvom bakterijskih vrsta *P. intermedia i P. micros* [27].

ZAKLJUČAK

Prisustvo periapikalnih lezija značajno utiče na mikrobiološki status kanala korena endodontski lečenih zuba. Prisustvo bakterija u kanalima korena potvrđeno je u većini endodontski neuspešno lečenih zuba, a najčešće identifikovane bakterije bile su *E. faecalis*, zatim *P. intermedia, P. micros i P. endodontalis.*

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Serbian Dental Institutions Top Cited Research (1996–2018)

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SUMMARY
Introduction Citation analysis, as one of the best-known bibliometric approaches, is widely used in the evaluation of research output and assessment of research performance and impact. The purpose of this study was to identify the top cited articles published by researchers affiliated to Serbian dental institutions and to analyze their characteristics in order to describe cooperation at individual and institutional, as well as at national and international level.
Material and Methods Articles that cited the most 2018 publication in the Web of Science were assessed. The analyzed aspects covered distribution of annual production, annual citations, journals, categories, countries, institutions, authors, research focuses and trends by author keywords and KeyWords Plus.
Results The top cited papers were published in 60 journals from 1997 to 2016, with the mean number of 45.08 citations per article. These papers were co-authored by 449 authors, responsible for 726 authorships, affiliated to 149 institutions from 41 countries. Among the overall number of authors, 238 were from Serbia, while 211 were affiliated with international institutions. The most productive institutions were the University of Belgrade and Military Medical Academy from Serbia, whilst the international contributing institutions were primarily from the USA, England, and Italy.
Conclusion The present study presented useful insight into the most influential Serbian dental institutions research, revealing the most productive actors and multidisciplinary nature of the research contribution.
Keywords: bibliometrics; citation analysis; Serbia; Dentistry, Oral Surgery and Medicine

INTRODUCTION

Bibliometrics has been applied in various forms for studying the structure and process of scholarly communication [1]. This powerful set of methods and measures has been used as a practical tool that can provide the knowledge of scholarly communicative behavior through the overall production of scientific literature on a macro or a micro level of analysis. Bibliometric indicators offer quantitative perspective of scientific activities and interactions, and can be used in combination with other indicators. Measuring ideas, as the main products of science, is difficult and each indicator has its advantages and limitations that must be taken into consideration. The first bibliometric indicator - count of published papers - reflects scientific output. Paper counts provide an initial and simplified measure of the quantity of work that a researcher, a department, an institution, a national or international scientific team, a country produced. Citations, as one of the best-known bibliometric indicators, may be considered as a measure of the cited articles' impact and reflection of their utility, recognized by the scientific community. The number of co-authored papers is an indicator of cooperative research achieved at individual or institutional, as well as national or international level. With the development of various networked information technologies, highly evolved quantitative and qualitative bibliometric methods emerged.

The number of times an individual paper is cited still presents the primary indicator, often linked to the outstanding research attributes. Along with the increasing attention directed to research evaluation, the number of bibliometric studies, dealing with the highly cited papers in various scientific disciplines, has been growing [2–5]. Highly cited papers concept has been described in different fields and named as the most cited papers, classic publications, top cited articles etc. Research articles, classified as classic publications or top cited, were investigated in a variety of disciplines, including oral cancer [6, 7, 8], maxillofacial surgery [9, 10], implant dentistry [3, 11, 12], endodontics [13], orthodontics [14, 15], or periodontology [16, 17]. Most of the conducted bibliometric studies analyzed the most cited articles retrieved from the Web of Science database by Clarivate Analytics, which has been widely recognized as the most comprehensive source of the highest impact journals worldwide. Serbian dental academic research and clinical institutions are characterized with long and prosperous tradition of scientific production. Identification of the articles that have influenced the most, as measured by citation count, may provide the starting point for the description of the Serbian dental scientific communication and its world-class research.

The purpose of this study was to identify, using the established method in evaluative bibliometrics, the top cited articles published by authors affiliated to Serbian dental institutions or departments, and analyze their characteristics in order to describe the cooperation at individual and institutional level, as well as national and international level. This analysis covered annual production from 1996 to 2018

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through the Web of Science database, including citations, journals, the Web of Science categories, countries, institutions, authorship, author keywords, and KeyWords Plus.

MATERIAL AND METHODS

The top cited articles authored by researchers affiliated to Serbian dental departments or institutions were retrieved from Clarivate Analytics' Web of Science and used for further analysis. The search was performed with the Web of Science Core Collection Citation Indexes that included Science Citation Index Expanded (SCI-EXPANDED), Social Sciences Citation Index (SSCI), Arts & Humanities Citation Index (A&HCI), Conference Proceedings Citation Index-Science (CPCI-S), Conference Proceedings Citation Index-Social Science & Humanities (CPCI-SSH) and Emerging Sources Citation Index (ESCI). Web of Science does not cover the majority of dental conferences, besides those whose proceedings papers were published in special issues of some referenced journals. The following search strategy produced a sample of documents used for further analysis:

ADDRESS: ((srb* OR serb*) SAME (dent* OR stom* OR maxill* OR oral OR prosthod* OR orthodon* OR endod* OR periodon*))

Indexes = SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI

Timespan = 1996-2018

All articles that resulted with this search procedure were included without restrictions regarding the article type. In the first step total of 1,451 articles published by authors affiliated to Serbian dental institutions or departments were obtained. Articles were sorted by option "times cited" in the descending order from the highest to the lowest cited article (all articles were ranked by the number of citations listed in the Web of Science). Thereafter, the 100 top cited articles published between 1996 and 2018 were identified. Since the articles ranked 101 and 102 received the same citation count as the last two articles, they were also included in the study. Out of 1 451 articles, 462 have never been cited in the Web of Science database, while 989 articles had at least one citation. For the top 102 articles RIS format file was generated and imported to BibExcel [18] in order to perform bibliometric analysis. BibExcel is a software designed to assist in analyzing bibliographic data, or any data of a textual nature formatted in a similar manner. It enables work with large datasets and it is compatible with various software applications aimed for network analysis. For the analysis of bibliometric data and their visualization in networks VOSviewer was used [19].

The following variables were recorded for each article and included in the analysis: author information (first author, co-author), article title, article type, cited references, country and institution of origin, keywords, KeyWords Plus, number of citations (Web of Science Core Collection), journal and its published year, the Web of Science category and Research Area. Each document was considered with respect to all authors' institutions and countries, and not only by the first or corresponding author. Records were manually refined and normalized in order to unify terms and remove typographical, transcription and/or indexing errors. Data regarding the authors, institutions, countries and journal titles were normalized. There were several different entries for a single author or institution to be checked and unified, both on macro (i.e. universities and research centers) and micro (i.e. individual departments and research units) levels. Articles originating from Serbia and Montenegro, since they were published after 1996, were reclassified as being from Serbia. Regarding the journals, only one had different entries that had to be unified due to the change of title.

RESULTS

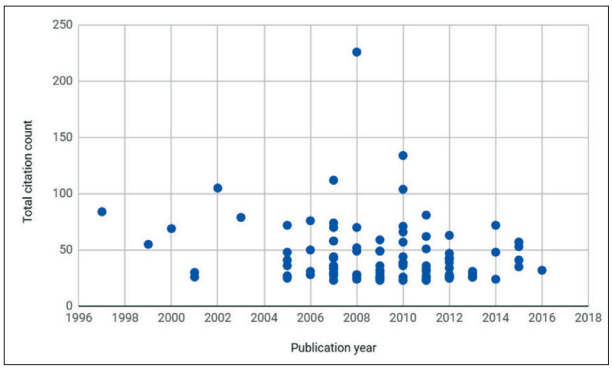
Complete details of the 102 top cited articles affiliated to Serbian dental institutions or departments were listed in the Appendix (available online) in descending order based on the number of citations obtained. The equally cited articles were sorted chronologically in accordance with the publication date (e.g. papers with the Article ID 11 and 12 in the Appendix).

The set of studied articles received in total 4,598 citations during the period 1996-2018. The average number of citations per article found in this study was 45.08. The most common document type were journal papers, including original research (n=92) and review articles (n=8), as well as two proceedings papers, published between 1997 and 2016 (Table 1).

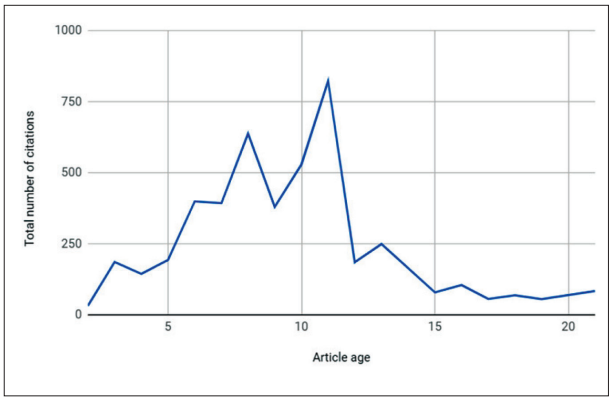
Table 1. Number and types of top cited articles
Tabela 1. Broj i tip najcitiranijih radova

Year Godina	Article Članak	Proceedings Paper Radovi sa kongresa	Review Revijski radovi	Total Ukupno
1997	1			1
1999	1			1
2000	1			1
2001	2			2
2002	1			1
2003	1			1
2005	6			6
2006	4			4
2007	17		1	18
2008	6	1	2	9
2009	10		1	11
2010	10		1	11
2011	10			10
2012	10		1	11
2013	6		1	7
2014	2	1		3
2015	3		1	4
2016	1			1
Total Ukupno	92	2	8	102

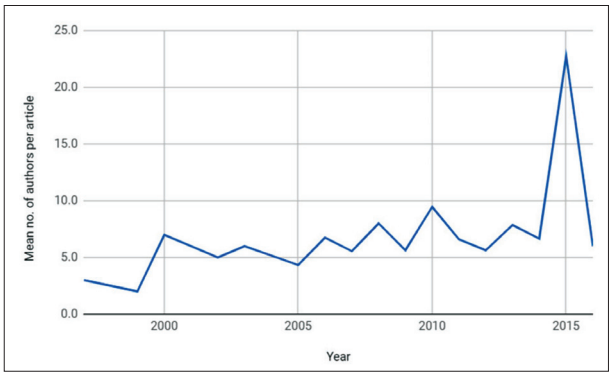
The distribution of these top cited documents with respect to the publication year and total citation count is shown in Graph 1. The citation count of selected articles ranged between 23 (ArticleID 99-102) and 226 (top cited article). The top five articles were cited more than 100



Graph 1. Distribution of the 102 top cited documents per year of publication and total citation count.
Grafikon 1. Raspodela najbolje citirana 102 članka po godini publikovanja i ukupnom broju citata



Graph 2. The total number of citations with respect to age of articles
Grafikon 2. Ukupan broj citata prema godini starosti članaka



Graph 3. Mean number of authors per paper with respect to year
Grafikon 3. Srednja vrednost ukupnog broj autora po članku prema godini publikovanja

times, while the most referenced article (c=226) was a review paper published in 2008. Out of total number of selected papers, nearly 52% appeared in period 2006-2010, while 35.3% and 10.8% were published in 2011-2016 and 2000-2005, respectively. Only a small number of papers (n=2) date from 1996-1999. The year 2007 was the year with the greatest number of published top cited articles (n=18), followed by 2009, 2010 and 2012 (n=11). In addition, the five top cited articles were published in 2002, 2007, 2008 and 2010.

The top 102 articles' total number of citations with respect to their age is shown in Graph 2. The greatest total number of citations (c=822) had 18 articles published 11 years ago. On the other hand, the articles 8 years old received the total of 638 citations and it was the greatest number of citations per year (μ=79.8). The most cited original research article was: *Jensen SB, Pedersen AML, Vissink A et al. A systematic review of salivary gland hypofunction and xerostomia induced by cancer therapies: management strategies and economic impact. Supportive Care in Cancer. 2010;18(8):1061-79* (Article ID 1); while the most cited review paper was: *Radovic I, Monticelli F, Goracci C, Vulicevic ZR, Ferrari M. Self-adhesive resin cements: A literature review. Journal of Adhesive Dentistry. 2008;10(4):251-8* (Article ID 2).

Journals

One hundred and two (102) most influential articles affiliated to Serbian dental institutions or departments were published in 60 journals. Journals with the highest contribution of articles were *Journal of Periodontology*, *Dental Materials* and *Clinical Oral Implants Research* (n=15) followed by the *Journal of Clinical Periodontology*, *Journal of Endodontics* and *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology* (n=12). Out of the total number of journals, 39 (65%) contained only one article, while the remaining 25% of the journal titles had two or three articles. Table 2 displays the most influential journal titles with respect to received number of citations, as well as their Quartile rankings based on Impact Factor data from the 2017 edition of Journal Citation Reports. Even though the most of the top cited articles were published in high ranked journals, article that stands out in terms of received citation number was published in *American Journal of Dentistry*, which is ranked at the lowest position of the subject category IF distribution. Source publications of the top cited articles are distributed in 40 the Web of Science subject categories. Slightly more than a half of journals belong to Dentistry, Oral Surgery & Medicine, while the other subject areas have assigned 12% (e.g. Materials Science, Biomaterials; Engineering, Biomedical; Surgery) or less journal titles.

Authors, Institutions and Countries

The frequency of occurrence of all authors was recorded in detail. There were 449 authors, responsible for 726 authorships, affiliated to 149 institutions from 41 countries. The number of authors of the top cited articles ranged from 2-32, while the mean number of authors per paper was 7. Since the three large studies (Article ID 23, 43, 54), authored with 22.8 authors per paper, were conducted in 2015, the average number of authors per article increased from 2.5 in 1990s to 7.5 in 2000s (Graph 3). Beside these articles published in 2015, there were three other studies with more than 20 co-authors (Article ID 2, 5, 32). A great deal of articles (40.2%) was co-authored by more than 6 researchers, whereas 22 articles had 6 authors. Out of the 102 top cited papers, 5, 8, 10 and 16 papers were written by two, three, four and five authors, respectively.

There were 72 researchers who contributed to the top cited articles as first authors. Table 3 outlines the most

Table 2. Characteristics of the top 15 journals
Tabela 2. Karakteristike najboljih 15 časopisa

Journal Časopis	Total citations Ukupan broj citata	Total articles Ukupan broj članaka	Citations per article Broj citata po članku	Quartile in Category Kvartil	WoS category WoS kategorija
JOURNAL OF PERIODONTOLOGY	261	5	52.2	Q1	Dentistry, Oral Surgery & Medicine
JOURNAL OF ADHESIVE DENTISTRY	260	2	130.0	Q2	Dentistry, Oral Surgery & Medicine
SUPPORTIVE CARE IN CANCER	238	2	119.0	Q1	Health Care Sciences & Services
					Oncology
JOURNAL OF CLINICAL PERIODONTOLOGY	203	4	50.8	Q1	Dentistry, Oral Surgery & Medicine
JOURNAL OF ENDODONTICS	198	4	49.5	Q1	Dentistry, Oral Surgery & Medicine
DENTAL MATERIALS	173	5	34.6	Q1	Dentistry, Oral Surgery & Medicine
					Materials Science, Biomaterials
INTERNATIONAL JOURNAL OF ORAL AND MAXILLOFACIAL SURGERY	172	3	57.3	Q2	Dentistry, Oral Surgery & Medicine
					Surgery
ORAL ONCOLOGY	162	3	54	Q1	Dentistry, Oral Surgery & Medicine
					Oncology
CLINICAL ORAL IMPLANTS RESEARCH	150	5	30	Q1	Dentistry, Oral Surgery & Medicine
					Engineering, Biomedical
EUROPEAN JOURNAL OF ORAL SCIENCES	139	3	46.3	Q2	Dentistry, Oral Surgery & Medicine
CLINICAL ORAL INVESTIGATIONS	123	2	61.5	Q1	Dentistry, Oral Surgery & Medicine
JOURNAL OF DENTISTRY	119	3	39.7	Q1	Dentistry, Oral Surgery & Medicine
AMERICAN JOURNAL OF DENTISTRY	112	1	112.0	Q4	Dentistry, Oral Surgery & Medicine
ORAL SURGERY ORAL MEDICINE ORAL PATHOLOGY ORAL RADIOLOGY	106	4	26.5	Q2	Dentistry, Oral Surgery & Medicine
JOURNAL OF MATERIALS SCIENCE-MATERIALS IN MEDICINE	106	3	35.3	Q2	Engineering, Biomedical
					Materials Science, Biomaterials

Table 3. Top 15 contributing authors with respect to the number of published papers
Tabela 3. Najboljih 15 autora prema broju publikovanih članaka

Authors Autori	Affiliations Afilijacija	Number of articles Broj članaka	Total number of citations Ukupan broj citata	First author Prvi autor
Ferrari, Marco	Univ Siena, Policlin Le Scotte, Italy	13	830	1
Radović, Ivana	Univ Belgrade, Sch Dent, Serbia	12	782	4
Leković, Vojislav	Univ Belgrade, Sch Dent, Serbia	12	658	4
Kenney, E. Barrie	Univ Calif Los Angeles, Sch Dent, USA	9	530	0
Goracci, Cecilia	Univ Siena, Policlin Le Scotte, Italy	8	581	1
Camargo, Paulo M.	Univ Calif Los Angeles, Sch Dent, USA	8	456	4
Weinlaender, Michael	Univ Calif Los Angeles, Sch Dent, USA	8	436	1
Milašin, Jelena	Univ Belgrade, Sch Dent, Serbia	7	289	0
Čolić, Miodrag	Mil Med Acad, Inst Med Res, Serbia	7	279	4
Ignjatović, Nenad	Serbian Acad Arts & Sci, Inst Tech Sci, Serbia	7	194	6
Uskoković, Dragan	Serbian Acad Arts & Sci, Inst Tech Sci, Serbia	7	194	0
Vasilić, N	Univ Calif Los Angeles, Sch Dent, USA	6	381	0
Kozomara, Ružica J.	Mil Med Acad, Clin Maxillofacial & Oral Surg, Serbia	6	265	1
Marković, Aleksa	Univ Belgrade, Sch Dent, Serbia	6	198	4
Ajduković, Zorica	Univ Nis, Fac Med Clin Stomatol, Serbia	6	166	1

productive authors regarding the total number of published papers. As indicated, Ferrari tops the list wić with a total of 13 publications, followed by Radović, Leković, Kenney, Goracci, Camargo and Weinlaender, each of which has more than 7 publications. It should be noted that a number of papers were co-authored by these authors. For instance, Ferrari, Radović and Goracci have co-authored 12 papers in the past 11 years. Furthermore, another group of authors i.e. Leković, Kenney, Camargo and Weinlaender have co-authored 7 papers.

Among the overall number of authors, 238 were from Serbia, while 211 were affiliated with foreign institutions. The top 20 Serbian authors who contributed the most as first authors are listed in Table 4. Ignjatović was the author with the highest number of first author papers, followed by Radović, Leković, Čolić and Marković.

According to the information on author addresses contained in the research papers, 69 articles were contributed by international collaboration, while the remaining 33 were independent Serbian articles. There were 108 authors from Serbian dental institutions or departments, whereas 130 were from other Serbian institutions. Countries contributing to the top cited articles affiliated to Serbian dental institutions or departments are depicted

Table 4. Top 20 contributing authors affiliated to Serbian institutions, with respect to the number of papers where they are first author, total number of published papers and cumulative number of citations for all their articles
Tabela 4. Najboljih 20 autora iz institucija iz Srbije prema broju članaka gde su prvi autori, ukupan broj objavljenih članaka i ukupan broj citata za sve njihove članke

Authors Autori	Affiliations Afilijacija	First Author Prvi autor	Total number of citations Ukupan broj citata	Number of articles Broj članaka
Ignjatović, Nenad	Serbian Acad Arts & Sci, Inst Tech Sci	6	194	7
Radović, Ivana	Univ Belgrade, Sch Dent	4	782	12
Leković, Vojislav	Univ Belgrade, Sch Dent	4	658	12
Čolić, Miodrag	Mil Med Acad, Inst Med Res	4	279	7
Marković, Aleksa	Univ Belgrade, Sch Dent	4	198	6
Šupić, Gordana	Mil Med Acad, Inst Med Res	3	239	5
Brković, Božidar M. B.	Univ Belgrade, Sch Dent	2	103	3
Djurić, Marija R.	Univ Belgrade, Fac Med	2	93	3
Mandinić, Zoran	Univ Belgrade, Sch Dent	2	83	2
Pejčić, Ana	Univ Nis, Fac Med Clin Stomatol	2	65	2
Kozomara, Ružica J.	Mil Med Acad, Clin Maxillofacial & Oral Surg	1	265	6
Ajduković, Zorica	Univ Nis, Fac Med Clin Stomatol	1	166	6
Gazivoda, Dragan	Mil Med Acad, Clin Maxillofacial & Oral Surg	1	147	4
Miletić, Vesna	Univ Belgrade, Sch Dent	1	109	3
Zeljić, Katarina	Mil Med Acad, Inst Med Res	1	109	3
Dimitrijević, Milovan V.	Clin Ctr Serbia, Clin Otorhinolaryngol & Maxillofacial Surg	1	98	2
Jevremović, Danimir	Univ Business Acad Novi Sad, Fac Stomatol Pancevo	1	79	2
Obradović, Radmila R.	Univ Nis, Fac Med Clin Stomatol	1	68	2
Daković, Dragana	Mil Med Acad	1	60	2
Jurišić, Milan	Univ Belgrade, Sch Dent	1	52	2

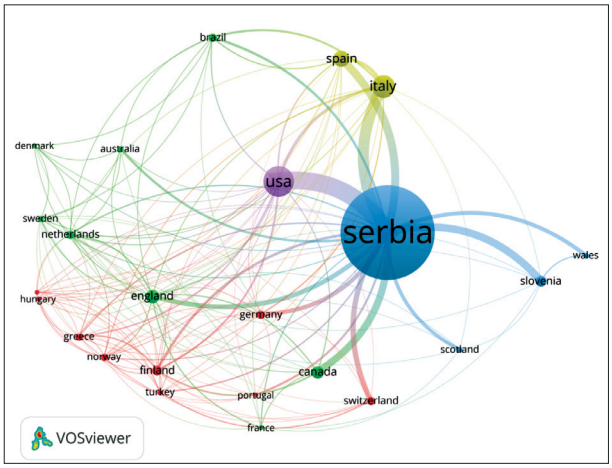


Figure 1. Contributing countries
Slika 1. Države iz kojih dolaze članci

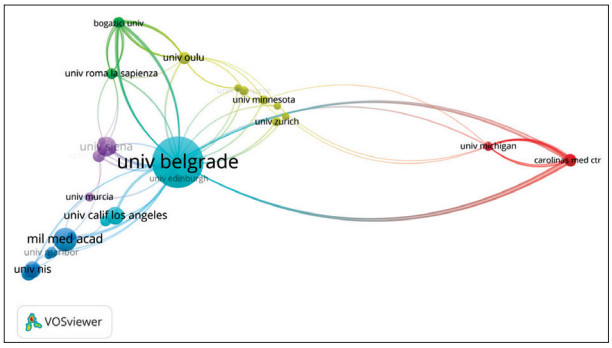


Figure 2. Most productive universities
Slika 2. Najproduktivniji univerziteti

Table 5. Most productive top cited institutions
Tabela 5. Najproduktivnije institucije, sa najvećim brojem citata

Institution Institucija	Number of articles Broj članaka
University of Belgrade, Serbia	80
Military Medical Academy, Serbia	18
University of Siena, Italy	12
University of Niš, Serbia	11
University of California Los Angeles, USA	10
Serbian Academy of Arts & Sciences, Serbia	7
University Business Academy Novi Sad, Serbia	6
University of Granada, Spain	5
University of Novi Sad, Serbia	5
University of Oulu, Finland	5

in Figure 1, with respect to their number of co-authored articles. Among 41 countries only 13 had first author articles (Austria, Canada, Denmark, England, Germany, Italy, Netherlands, Scotland, Serbia, Spain, Sweden, Switzerland, and USA). Beside Serbia, the top countries were represented with more than 6 national institutions. The greatest number of institutions (n=23) originated from the USA, followed by England (n=10), Italy (n=9), Germany (n=8), and Canada and Spain (n=6). The top two most productive institutions were the University of Belgrade and Military Medical Academy from Serbia (Table 5). They were followed by University of Siena, University of Niš, University of California Los Angeles etc. Out of the total number of institutions, there were 102 universities and the most productive are illustrated in Figure 2.

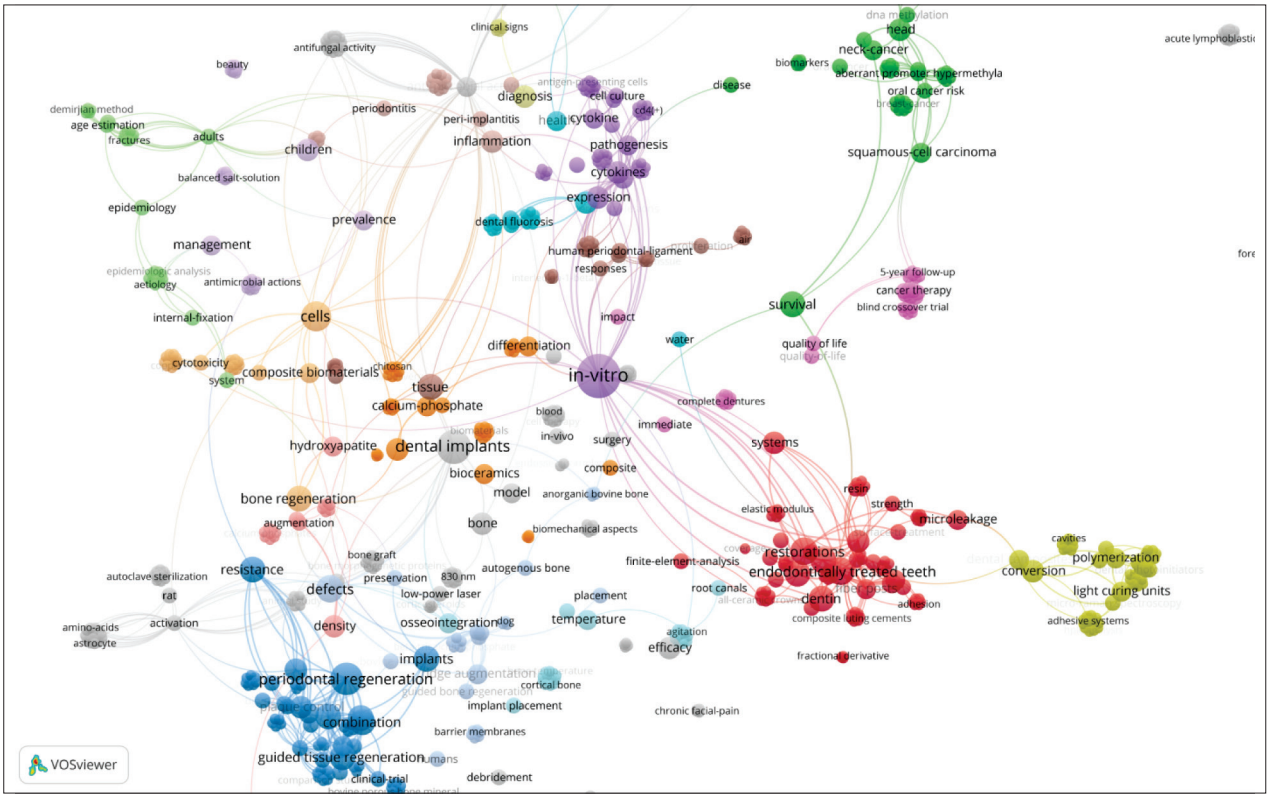


Figure 3. Clusters based on co-occurrence of keywords
Slika 3. Klasteri zasnovani na zajedničkom pojavljivanju ključnih reči

KEYWORDS

Of the 102 top cited articles, a total of 176 keywords were identified. The most frequently used author keywords (n=77) and KeyWords Plus (n=99), with respect to the number of times they appear in the analyzed articles are presented in Figure 3. The most productive keywords with interconnected links are shown, while the different clusters are illustrated through coloring.

DISCUSSION

This bibliometric study aimed to identify the top cited articles affiliated to Serbian dental institutions or departments and their main characteristics, in order to reveal the achieved Serbian scientific progress in the field of dentistry, as well as trends in Serbian dental research. Even though the studying of the citation count is widespread [2, 3, 6, 8–11, 15, 20–27], this value could not be treated as an absolute scientific quality indicator, but it can provide an objective and quantitative measure that an article has made on the scientific community in its respective field [28, 29]. The top 102 articles affiliated to Serbian dental institutions or departments were cited between 23 and 261 times. The top five articles were cited at least 100 times, and according to the previous studies in the field, they could be characterized as citation classics [16]. However, it should be noted that the obtained number of citations is far below the citation rates in larger medical fields [30, 31].

In our study, almost all investigated articles were published in journals that occupy top or middle-high positions in their respective subject category. Citations are largely driven by publication records, but they are also substantially increased by larger research teams and co-author networks [32]. Collaboration is a significant factor in scholarly productivity, but varies by discipline [1]. Collaborative research is typical of most scientific disciplines, as well of medicine. The mean number of authors per paper found in this study was 7, which is slightly higher than the mean number of authors per article revealed in other studies in the field of dentistry [11]. Since the six large studies, authored with more than 22 authors per paper, were conducted, the mean number of authors per article increased from 2.5 in 1990s to 7.5 in 2000s. The identified co-author networks were the crucial factors in determining the distribution of institutions and countries. The majority of articles originated from the collaboration with the researchers from the USA, as well as European institutions, i.e. Italy, Spain and Finland.

The author keywords provide reasonably detailed picture of the article's themes, while KeyWords Plus, generated independently of the author keywords, describe the article's content with greater depth and variety [33]. In order to overcome the limitations of investigating relatively small sample of author keywords, for the analysis and representation of the article's themes KeyWords Plus were also used.

From a methodological point of view, there are some limitations associated with the study. For the identification of top cited articles affiliated to Serbian dental insti-

tutions and departments only the Web of Science database was used, while other databases were not covered. Therefore, some important articles published in journals that are not included in the Web of Science might be missed. In addition, the self-citations were not excluded from the total number of citations encountered by an article, which may inflate the citation rate. Besides, for the analysis of authorships, the whole counting method was used instead of fractional. Finally, a content analysis of the influential articles was not performed, including the most frequent scope and design of study, as well as level of evidence.

Despite these obvious limitations, the data presented in this study still provide significant insight into the achievements and evolving trends in Serbian dental research over the past decades. To the best of authors' knowledge, this is the first report of the top cited articles affiliated to Serbian dental institutions or departments.

CONCLUSION

The present work explores the characteristics of the 102 top cited articles affiliated to Serbian dental institutions or departments during 1996-2018 within the Web of Science database. This bibliometric analysis reveals the escalating course in the mean citation number of these top articles, highlighting the trends in the Serbian dental research literature. Since the citation counts are continuously changing, results presented in this study provide a snapshot of the most influential articles affiliated to Serbian dental institutions and departments of the current time.

The set of top cited papers were published in 60 journals from 1997 to 2016, with the mean number of 45.08 citations per article. These papers were co-authored by 449 authors, responsible for 726 authorships, affiliated to 149 institutions from 41 countries. Among the overall number of authors, 238 were from Serbia, while 211 were affiliated with foreign institutions. The top Serbian authors who contributed the most with respect to the number of published articles were Radović Ivana and Leković Vojislav, followed by Milašin Jelena, Čolić Miodrag and Ignjatović Nenad. The most productive institutions were the University of Belgrade and Military Medical Academy from Serbia, whilest the foreign contributing institutions were primariliy from the USA, England, Italy, Germany, Canada and Spain. The revealed multi-institutional and international collaborations encourage higher quality research. Beside Dentistry, Oral Surgery and Medicine, the top cited articles were published in journals that belong to various Web of Science subject categories, revealing in that way the multidisciplinary nature of the investigated top cited articles research.

In order to provide more comprehensive insight into this literature, it would be very useful to conduct finer content analysis and classification of the investigated top cited articles affiliated to Serbian dental institutions and departments. Future studies covering these issues are desirable in order to introduce to new researchers approaches and methods applied in the top cited papers and help them to spot current impactful and emergent topics.

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Najcitiraniji radovi istraživača srpskih stomatoloških institucija (1996–2018)

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KRATAK SADRŽAJ

Uvod Citatna analiza, kao jedan od najpoznatijih bibliometrijskih pristupa, u velikoj meri se koristi prilikom procene naučnih rezultata, postignutog istraživačkog učinka i uticaja. Cilj ovoga rada bio je identifikacija najcitiranijih članaka koje su objavili istraživači povezani sa srpskim stomatološkim institutcijama, kao i analiza njihovih odlika, radi opisa ostvarene saradnje, kako na individualnom i institucionalnom, tako i na nacionalnom i internacionalnom nivou.

Materijal i metode Članci sa najvećim brojem citata dobijenih u okviru baze *Web of Science* od datuma publikovanja do 2018. godine bili su predmet procene. Aspekti koji su analizirani obuhvatali su podatke o broju objavljenih članaka na godišnjem nivou, broju dobijenih citata u toku godine, časopisima, predmetnim kategorijama, zemljama, institucijama, autorima, ali i centralnim temama i trendovima u istraživanjima, posmatranim na osnovu ključnih reči koje je autor dodelio članku, ali i dodatnih ključnih reči, generisanih u okviru korišćene baze *Web of Science* (*KeyWords Plus*).

Rezultati Posmatrana grupa najcitiranijih radova objavljena je u okviru 60 različitih časopisa, u periodu od 1997. do 2016. godine, uz 45,08 prosečan broj citata po radu. U izradi ovih članaka učestvovalo je ukupno 449 autora, odgovornih za 726 autorstava i povezanih sa 149 institucija iz 41 zemlje. Od ukupnog broja autora, 238 je iz Srbije, dok je 211 autora vezano za strane institucije. Najproduktivnije ustanove iz Srbije bile su Univerzitet u Beogradu i Vojnomedicinska akademija, dok su strane institucije koje su učestvovale u izradi radova prevashodno dolazile iz SAD, Engleske i Italije.

Zaključak Ova studija pruža koristan uvid u to koja su istraživanja srpskih stomatoloških ustanova izvršila najveći uticaj, otkrivajući i najproduktivnije aktere i multidisciplinarnu prirodu naučnog doprinosa.

Ključne reči: bibliometrija; citatna analiza; Srbija; stomatologija, oralna hirurgija i medicina

UVOD

Bibliometrija je primenjivana u različitim oblicima za izučavanje strukture i procesa naučnih komunikacija [1]. Ovaj moćan skup metoda i mera korišćen je kao praktičan alat koji nam omogućava upoznavanje sa ponašanjem svojstvenim naučnim komunikacijama kroz ukupnu produkciju naučne literature na makronivoima, ali i mikronivoima analize. Bibliometrijski pokazatelji nude mogućnost kvantitativnog sagledavanja naučnih aktivnosti i interakcija, a mogu da se koriste i u kombinaciji sa nekim drugim indikatorima. Merenje ideja, kao glavnih produkta nauke, nije jednostavno, a i svaki pokazatelj ima svoje prednosti i mane koje je potrebno uzeti u obzir. Prvi bibliometrijski pokazatelj – broj publikovanih radova – odražava rezultate naučnog rada. Broj publikovanih radova pruža inicijalnu i pojednostavljenu meru količine rada koju je istraživač, odeljenje, institutcija, nacionalni ili međunarodni naučni tim, zemlja proizvela. Citiranost, kao jedan od najpoznatijih bibliometrijskih indikatora, može se posmatrati kao mera uticaja citiranog članka i odraz njegove korisnosti koju naučno društvo prepoznaje. Broj koautorskih radova pokazatelj je naučno-istraživačke saradnje ostvarene na individualnom ili institucionalnom, kao i nacionalnom ili međunarodnom nivou. Sa razvojem različitih mrežnih informacionih tehnologija pojavile su se i visoko razvijene kvantitativne i kvalitativne bibliometrijske metode.

Broj citata koji je dobio neki rad još uvek predstavlja osnovni pokazatelj, često povezan sa izvanrednim naučno-istraživačkim odlikama. Uporedo sa porastom interesovanja za vrednovanje naučno-istraživačkih rezultata raste i broj bibliometrijskih studija koje se bave visoko citiranim radovima različitih naučnih disciplina [2–5]. Pojam visoko citiranih članaka opisivan je u radovima različitih naučnih oblasti i imenovan na više načina, poput najcitiraniji radovi, klasici, vrhunski citirani radovi itd. Naučno-istraživački radovi, okarakterisani kao klasici ili vrhunski citirani radovi, bili su predmet istraživanja više disciplina, uključujući karcinom usne duplje [6, 7, 8], maksilofacijalnu hirurgiju [9, 10], stomatološku implantologiju [3, 11, 12], endodonciju [13], ortodonciju [14, 15], ili parodontologiju [16]. U većini sprovedenih bibliometrijskih studija analizirani su najcitiraniji članci preuzeti iz baze *Web of Science Clarivate Analytics*, koja je široko prepoznata kao najsveobuhvatniji izvor najuticajnijih časopisa širom sveta.

Srpske stomatološke akademske, istraživačke i kliničke ustanove imaju dugu i bogatu tradiciju objavljivanja naučno-istraživačkih rezultata. Identifikacija članaka koji su izvršili najveći uticaj, mereno brojem dobijenih citata, može da bude polazna tačka za opis srpske stomatološke naučne komunikacije i njenih istraživanja svetskog ranga. Svrha ove studije je bila da se, koristeći ustanovljeni metod evaluativne bibliometrije, identifikuju najčešće citirani članci koje su objavili autori povezani sa srpskim stomatološkim institucijama i odeljenjima, kao i da se analiziraju njihove karakteristike radi opisa saradnje ostvarene na individualnom i institucionalnom, ali i na nacionalnom i internacionalnom nivou. Ovom analizom obuhvaćeni su naučni radovi objavljeni u periodu od 1996. do 2008. godine, prikupljeni putem baze *Web of Science*, uključujući broj dobijenih citata, časopise u kojima su objavljeni, pripadajuće *Web of Science* kategorije, zemlje, ustanove, podatke o autorstvima, ključne reči koje su autori dodelili i generički dodeljene ključne reči u okviru *Web of Science KeyWords Plus*.

MATERIJAL I METODE RADA

Korpus najcitiranijih radova istraživača povezanih sa srpskim stomatološkim ustanovama i odeljenjima, dobijen iz baze *Web of Science Clarivate Analytics*, upotrebljen je za dalju analizu. Pretraživanje je sprovedeno uz pomoć *Web of Science Core Collection*

citatnih indeksa, koji se sastoje od sledećeg: *Science Citation Index Expanded (SCI-EXPANDED)*, *Social Sciences Citation Index (SSCI)*, *Arts & Humanities Citation Index (A&HCI)*, *Conference Proceedings Citation Index-Science (CPCI-S), Conference Proceedings Citation Index-Social Science & Humanities (CPCI-SSH)* i *Emerging Sources Citation Index (ESCI)*. Citatna baza *Web of Science* ne pokriva većinu održanih stomatoloških simpozijuma, osim onih čiji su izlagani radovi objavljeni u specijalnim brojevima određenog časopisa, referisanog u bazi *Web of Science*. Sledeći princip pretrage bio je osnova za dalju analizu:

ADDRESS: ((srb* OR serb*) SAME (dent* OR stom* OR maxill* OR oral OR prosthod* OR orthodon* OR endod* OR periodon*))

Indexes = SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI

Timespan = 1996-2018

Svi radovi dobijeni ovom pretragom uključeni su u analizu bez ograničenja vezanih za sam tip rada. Na samom početku dobijen je 1.451 rad koji su objavili autori povezani sa srpskim stomatološkim ustanovama i odeljenjima. Radovi su sortirani uz pomoć opcije *times cited* u opadajućem nizu, od najviše ka najmanje citiranim radovima (svi radovi su rangirani po broju citata dobijenih u bazi *Web of Science*).

Nakon toga izdvojeno je 100 najcitiranijih radova objavljenih u periodu od 1996. do 2018. godine. Imajući u vidu da su radovi rangirani kao 101. i 102. imali isti broj citata kao i poslednja dva izdvojena rada, i oni su uključeni u ovu analizu. Od 1.451 rada, 462 rada nikada nisu bila citirana u bazi *Web of Science*, dok je 989 radova bilo citirano bar jednom. Za 102 najcitiranija rada generisan je dokument u RIS formatu, koji je uvezen u *BibExcel* [18] radi sprovođenja bibliometrijske analize. *BibExcel* je program napravljen tako da pomaže pri analizi bibliometrijskih podataka, ili bilo kakvih tekstualnih podataka formatiranih na sličan način. Ovaj program omogućava rad sa velikim kolekcijama podataka i kompatibilan je sa mnogim drugim programima koji se koriste za analizu umreženih podataka. Za analizu bibliometrijskih podataka i njihovu vizualizaciju korišćen je *VOSviewer* [19].

Za svaki članak su zabeležene i uključene u analizu sledeće promenljive: podaci o autorima (prvi autor, koautor), naziv članka, tip rada, citirana literatura, zemlja porekla i naziv institucije, ključne reči, *KeyWords Plus*, broj dobijenih citata (*Web of Science Core Collection*), naziv časopisa i godina publikovanja, *Web of Science* kategorija i oblast istraživanja.

Svaki rad je analiziran uzimajući u obzir institucije i zemlje svih autora rada, a ne samo prvog autora ili autora određenog za prepisku. Zapisi su pregledani i usaglašavani radi ujednačavanja termina i uklanjanja tipografskih grešaka, kao i grešaka nastalih prilikom transkripcije ili indeksiranja. Podaci koji su usaglašeni su podaci o autoru, instituciji, zemlji porekla i nazivu časopisa. Postojalo je nekoliko različitih varijanti imena jednog autora ili institucije koje je trebalo proveriti i ujednačiti, kako na makronivou, tj. nivou univerziteta i istraživačkih centara, tako i na mikronivou, tj. nivou pojedinačnih odseka i istraživačkih organizacionih jedinica. Članci kojima je za zemlju porekla bila navedena Srbija i Crna Gora, budući da su objavljeni nakon 1996. godine, iznova su klasifikovani kao radovi iz Srbije. Kada je reč o časopisima, samo je jedan, zbog promene naslova časopisa, imao navedene različite varijante, koje je bilo potrebno ujednačiti.

REZULTATI

U Dodatku rada (dostupno onlajn), u opadajućem redosledu zasnovanom na broju dobijenih citata, prikazani su detaljni podaci o 102 najčešće citirana rada povezana sa srpskim stomatološkim institucijama i odsecima. Podjednako citirani članci sortirani su hronološki u skladu sa njihovim datumom objavljivanja (npr. članci sa oznakama Article ID 11 i 12 u Dodatku rada).

Skup posmatranih članaka citiran je ukupno 4.598 puta 1996–2018. godine. U ovoj studiji utvrđena srednja vrednost dobijenih citata po članku iznosi 45,08. Najuobičajeniji tipovi radova bili su radovi objavljeni u časopisima između 1997. i 2016, uključujući originalne naučne (n = 92) i pregledne radove (n = 8), kao i dva rada izlagana na kongresima (Tabela 1).

Distribucija najcitiranijih radova u odnosu na godinu publikovanja i ukupan broj dobijenih citata prikazani su na Grafikonu 1. Broj dobijenih citata posmatranih članaka nalazi se u rangu između 23 (Article ID 99-102) i 226 (najcitiraniji rad). Pet najcitiranijih članaka citirano je više od 100 puta, pri čemu je onaj najčešće navođen (c = 226) pregledni rad objavljen 2008. godine. Od ukupnog broja posmatranih radova, gotovo 52% se pojavilo u periodu 2006–2010, dok je 35,3% i 10,8% publikovano u periodu 2011–2016, odnosno 2000–2005. Veoma mali broj radova (n = 2) datira iz perioda 1996–1999. Godine 2007. objavljeno je najviše (n = 18) najcitiranijih radova, a slede je 2009, 2010 i 2012. godina (n = 11). Osim toga, pet najcitiranijih članaka objavljeno je 2002, 2007, 2008 i 2010. godine.

Ukupan broj citata koje su dobila ova 102 najcitiranija rada u odnosu na njihovu starost prikazan je na Grafikonu 2. Najveći ukupan broj citata (c = 822) dobilo je 18 radova publikovanih pre 11 godina. S druge strane, radovi stari osam godina dobili su ukupno 638 citata, što predstavlja najveći broj dobijenih citata po godini (μ = 79,8). Najcitiraniji originalni naučni rad je: *Jensen SB, Pedersen AML, Vissink A, Andersen E, Brown CG, Davies AN, et al. A systematic review of salivary gland hypofunction and xerostomia induced by cancer therapies: management strategies and economic impact. Supportive Care in Cancer. 2010; 18(8):1061–79.* (Article ID 1); dok je najcitiraniji pregledni rad: *Radovic I, Monticelli F, Goracci C, Vulicevic ZR, Ferrari M. Self-adhesive resin cements: A literature review. Journal of Adhesive Dentistry. 2008; 10(4):251–8.* (Article ID 2).

Časopisi

Najuticajnija 102 rada stomatoloških institucija ili odseka objavljena su u 60 naučnih časopisa. Časopisi čiji je doprinos najveći u smislu broja objavljenih radova su *Journal of Periodontology*, *Dental Materials* i *Clinical Oral Implants Research* (n = 15), zatim *Journal of Clinical Periodontology*, *Journal of Endodontics* i *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology* (n = 12). Od ukupnog broja časopisa, u 39 (65%) naslova objavljen je samo po jedan rad, dok je u oko 25% časopisa objavljeno po dva ili tri rada. U Tabeli 2 prikazani su najuticajniji časopisi prema broju dobijenih citata, kao i njihov rang na kvartalnim listama, zasnovan na podacima o impakt faktoru iz *Journal Citation Reports* liste iz 2017. godine. Iako je većina najcitiranijih radova objavljena u visoko rangiranim časopisima, članak koji se ističe po broju dobijenih citata objavljen je u časopisu *American Journal of Dentistry*, koji se na osnovu distribucije impakt

faktora predmetne kategorije kojoj pripada nalazi u najnižem kvartalnom rangu. Časopisi u kojima su objavljeni ovi najcitiraniji radovi razvrstani u 40 predmetnih kategorija *Web of Science*. Nešto više od polovine ovih časopisa pripada kategoriji stomatologija, oralna hirurgija i medicina (eng. *Dentistry, Oral Surgery & Medicine*), dok ostalim kategorijama (npr. Materials Science, Biomaterials; Engineering, Biomedical; Surgery) pripada 12% ili manje naslova.

Autori, institucije i zemlje

Učestalost pojavljivanja svih autora detaljno je zabeležena. Zabeleženo je ukupno 446 autora, odgovornih za 726 autorstava, povezanih sa 149 institucija iz 41 zemlje. Broj autora u najcitiranijim radovima kreće se od dva do 32, dok je utvrđeni prosečan broj autora po radu sedam. S obzirom na to da su 2015. godine sprovedene tri velike studije (Article ID 23, 43, 54), koje u proseku imaju 22,8 autora po radu, prosečan broj autora po radu povećao se sa 2,5 u 1990-im na 7,5 u 2000-im (Grafikon 3). Osim ovih objavljenih 2015. godine, postoje još tri studije sa po više od 20 koautora (Article ID 2, 5, 32). Veliki deo radova (40,2%) ima više od po šest autora, dok 22 članka imaju po šest autora. Od 102 najčešće citirana rada, njih pet, osam, 10 i 16 imaju po dva, odnosno tri, četiri i pet autora.

Utvrđeno je da su 72 istraživača doprineli izradi ovih najcitiranijih radova kao prvi autori. U Tabeli 3 prikazani su najproduktivniji autori prema broju objavljenih radova. Kao što je navedeno, Ferrari je prvi na listi sa 13 objavljenih radova, zatim Radović, Leković, Kenney, Goracci, Camargo i Weinlaender, svako sa više od po sedam objavljenih članaka. Treba napomenuti da su ovi autori u velikom broju radova koautori. Na primer, Ferrari, Radović i Goracci su koautori u 12 radova u proteklih 11 godina. Osim toga, druga grupa autora, tj. Leković, Kenney, Camargo i Weinlaender koautori su u sedam radova.

Od ukupnog broja autora, 238 autora je iz Srbije, dok je njih 211 povezano sa stranim institucijama. U Tabeli 4 prikazano je 20 autora iz Srbije, čiji je doprinos, pre svega kao prvih autora, najveći. Ignjatović je istraživač sa najvećim brojem članaka u kojima je on prvi autor, zatim Radović, Leković, Čolić i Marković.

Na osnovu adresa autora navedenih u radovima, 69 članaka je plod međunarodne saradnje, dok su preostala 33 rada rezultat institucija iz Srbije. Ukupno 108 autora je iz srpskih stomatoloških institucija ili odseka, dok je ostalih 130 iz drugih srpskih institucija. Učešće drugih zemalja u najcitiranijim radovima autora iz srpskih stomatoloških institucija ili odseka prikazano je na Slici 1, prema broju koautorstava. Od ukupno 41 zemlje, prvi autori radova potiču iz samo 13 (Austrija, Kanada, Danska, Engleska, Nemačka, Italija, Holandija, Škotska, Srbija, Španija, Švedska, Švajcarska i SAD). Osim Srbije, i druge zemlje čiji je doprinos visok, zastupljene su sa po više od šest nacionalnih institucija. Najveći broj institucija (n = 23) potiče iz SAD, zatim Engleske (n = 10), Italije (n = 9), Nemačke (n = 8), kao i Kanade i Španije (n = 6). Dve najproduktivnije institucije su Univerzitet u Beogradu i Vojnomedicinska akademija (Tabela 5). Ove dve najproduktivnije institucije slede Univerzitet u Sijeni, Univerzitet u Nišu, Univerzitet Kalifornije (Los Anđeles) i drugi. Od ukupnog broja institucija, 102 najproduktivnije prikazane su na Slici 2.

Ključne reči

U 102 najčešće citirana rada utvrđeno je ukupno 176 upotrebljenih ključnih reči. Na Slici 3 prikazane su najčešće korišćene ključne reči autora (n = 77) i *KeyWords Plus* (n = 99), prema tome koliko puta su korišćene u analiziranim radovima. Prikazane su najčešće upotrebljavane ključne reči, sa vezama koje postoje među njima, dok su različiti klasteri obeleženi različitim bojama.

DISKUSIJA

Cilj ove bibliometrijske studije je identifikovanje najčešće citiranih radova povezanih sa srpskim stomatološkim institucijama i odeljenjima i njihovih osnovnih karakteristika, radi prikazivanja srpskog naučnog napretka postignutog na polju stomatologije, ali i pravaca stomatoloških istraživanja. Iako je proučavanje citiranosti široko rasprostranjeno [3–8, 10, 12–20], ova vrednost ne bi mogla da se posmatra kao apsolutni pokazatelj naučnog rada, ali može da pruži objektivan i merljiv uvid u to koliko je neki članak ostvario uticaj na naučnu zajednicu odgovarajuće oblasti [21, 22]. Najcitiranija 102 rada, povezana sa srpskim stomatološkim institucijama ili odeljenjima, citirana su između 23 i 261 puta. Pet najčešće citiranih radova navođeni su više od 100 puta i, shodno dosadašnjim sprovedenim studijama u ovoj oblasti, oni se mogu posmatrati kao citatni klasici [23]. Ipak, treba napomenuti da je broj dobijenih citata značajno manji u odnosu na citatne stope klasika većih medicinskih oblasti [24, 25].

Gotovo svi radovi obuhvaćeni ovom studijom objavljeni su u časopisima koji zauzimaju najviše ili visoke pozicije u svojim predmetnim kategorijama. Citiranost radova nekog pojedinca u velikoj meri zavisi i od broja njegovih publikovanih radova, ali značajno je uvećava i rad u okviru velikih istraživačkih timova i koautorskih grupa [26]. Saradnja je značajan faktor u naučnoj produktivnosti, ali u zavisnosti od discipline [27]. Zajednički rad je uobičajen za većinu naučnih disciplina, pa tako i za medicinu. Prosečan broj autora po radu utvrđen u ovoj studiji je sedam, što je malo više od prosečnog broja autora po radu prema ostalim studijama iz oblasti stomatologije [20]. Budući da je u ispitivanje uključeno i šest velikih studija, sa po više od 22 autora po radu, prosečan broj autora po radu porastao je sa 2,5 u 1990-im na 7,5 u 2000-im. Identifikovane koautorske mreže ključne su za utvrđivanje distribucije institucija i zemalja. Većina članaka rezultat je saradnje sa istraživačima iz institucija SAD, kao i Evrope, tj. Italije, Španije i Finske.

Ključne reči koje su autori izabrali pružaju prihvatljivu sliku o tome šta je tema članka, dok *KeyWords Plus*, generisane nezavisno od ključnih reči autora, opisuju njegov sadržaj sa većom dubinom i raznolikošću [28]. Radi prevazilaženja ograničenja koja mogu biti rezultat upotrebe relativno malog uzorka ključnih reči autora, za analizu i prikaz tema članaka korišćene su i ključne reči *KeyWords Plus*.

Sa metodološke tačke gledišta, postoje određena ograničenja sprovedene studije. Za utvrđivanje najčešće citiranih radova povezanih sa srpskim stomatološkim institucijama i odeljenjima korišćena je samo baza *Web of Science*, dok ostale baze nisu bile obuhvaćene. Stoga su možda propušteni neki važni članci, objavljeni u časopisima koji nisu uključeni u *Web of Science*.

Osim toga, autocitati nisu bili isključeni iz ukupnog broja dobijenih citata članaka, što u određenoj meri može da poveća stopu citiranosti. Pored toga, prilikom analize autorstava svim autorima nekog rada priznat je kompletan doprinos, bez obzira na ukupan broj koautora u radu. Na samom kraju, nije sprovedena sadržinska analiza ovih uticajnih članaka, uključujući najčešće područje ispitivanja i dizajn studije, kao i nivo dokaza.

Uprkos ovim očiglednim ograničenjima, podaci predstavljeni u ovom radu ipak pružaju značajan uvid u dostignuća i nove pravce srpskih stomatoloških istraživanja tokom prošlih decenija. Prema saznanjima autora, ovo je prvi izveštaj o najčešće citiranim radovima povezanim sa srpskim stomatološkim institucijama ili odeljenjima.

ZAKLJUČAK

Ovaj rad se bavi analizom karakteristika 102 najčešće citirana članka, povezana sa srpskim stomatološkim institucijama ili odeljenjima 1996–2018. godine u okviru baze *Web of Science*. Sprovedena bibliometrijska analiza prikazuje porast prosečnog broja citiranosti ovih najcitiranijih članaka i ističe pravce srpskih stomatoloških naučno-istraživačkih radova. S obzirom na to da se broj citata neprekidno menja, rezultati predstavljeni u ovom radu daju prikaz ovog trenutka najuticajnijih članaka povezanih sa srpskim stomatološkim institucijama i odeljenjima.

Najčešće citirani radovi objavljeni su u 60 časopisa u periodu od 1997. do 2016. godine, sa prosečnim brojem dobijenih citata po radu od 45,08. Ove radove je napisalo 449 autora, odgovornih za 726 autorstava, povezanih sa 149 institucija iz 41 zemlje. Od ukupnog broja autora, 238 je bilo iz Srbije, dok je 211 bilo povezano sa stranim institucijama. Srpski autori koji su najviše doprineli u pogledu broja publikovanih radova su Radović Ivana i Leković Vojislav, zatim Milašin Jelena, Čolić Miodrag i Ignjatović Nenad. Najproduktivnije institucije bile su Univerzitet u Beogradu i Vojnomedicinska akademija iz Srbije, dok su kod stranih institucija najveći doprinos dale prevashodno one koje potiču iz SAD, Engleske, Italije, Nemačke, Kanade i Španije. Sarađivanje više institucija, kao i međunarodna saradnja, pospešuju sprovođenje visoko kvalitetnih istraživanja. Osim kategorija stomatologija, oralna hirurgija i medicina, najviše citirani članci su objavljivani u časopisima koji pripadaju i drugim raznovrsnim predmetnim kategorijama Web of Science, na taj način otkrivajući multidisciplinarnu prirodu istraživanja ispitivanih najcitiranijih članaka.

Radi sveobuhvatnijeg uvida u ovu literaturu bilo bi veoma korisno sprovesti precizniju analizu sadržaja i klasifikaciju istraživanih najcitiranijih radova povezanih sa srpskim stomatološkim institucijama i odeljenjima. Poželjna su buduća istraživanja koja će se baviti ovim pitanjima kako bi se novim istraživačima omogućilo upoznavanje sa pristupima i metodama koiršćenim u istraživanjima najcitiranijih radova, ali i pomoglo da uoče aktuelne uticajne teme.

Da li ste pažljivo čitali radove?

1. SEM analizom je proveravana efikasnost čišćenja:
   a) celog kanala
   b) samo krunične trećine
   c) samo apikalne trećine

2. Mikrobiološki status kanala posle endodontskog neuspeha je analiziran:
   a) standardnom mikrobiološkom procedurom
   b) PCR tehnikom
   c) i jednom i drugom tehnikom

3. Nedostatak prostora u ortodontskoj terapiji se najčešće rešava:
   a) ekstrakcijom zuba
   b) restaurativnom terapijom
   c) endodontskom terapijom

4. Otpuštanje fluorida je testirano kod:
   a) 5 GJC
   b) 4 GJC
   c) 3 GJC

5. Preparacija kanala kod sistema sa recipročnim pokretima je urađena turpijom:
   a) UNICONE
   b) reciproc
   c) reciproc blue

6. Mikrobiološka studija je obuhvatila:
   a) 20 zuba
   b) 25 zuba
   c) 30 zuba

7. Zatvaranje prostora u zubnom nizu posle ekstrakcije se može rešiti primenom kliznog mehanizma sa elastičnim lancima?
   a) Da
   b) Ne
   c) Samo u izuzetnim slučajevima

8. Diskovi GJC za proveru otpuštanja fluorida su bili dimenzija:
   a) 5 × 2 mm
   b) 4 × 3 mm
   c) 6 × 2 mm

9. Čist kanal uzorka sa recipročnim pokretima je registrovan u:
   a) 60% slučajeva
   b) 76% slučajeva
   c) 80% slučajeva

10. Dezopturacija kanala kod endodontski neuspelih lečenja je urađena:
    a) isključivo mehaničkim putem
    b) isključivo ultrazvukom
    c) i mehanički i ultrazvukom

11. Kao uzorak za zatvaranje prostora u zubnom nizu posle ekstrakcije poslužilo je:
    a) 50 postekstrakcionih prostora
    b) 46 postekstrakcionih prostora
    c) 36 postekstrakcionih prostora

12. Otpuštanje fluorida iz GJC je testirano posle:
    a) 14 dana
    b) 21 dan
    c) 28 dana

13. Turpija sa punom rotacijom XPS je bila:
    a) efikasnija u čišćenju apikalne trećine
    b) manje efikasna u čišćenju apikalne trećine
    c) neefikasna u čišćenju apikalne trećine

14. Kao uzorak za zatvaranje prostora u zubnom nizu korišćeni su slučajevi sa ekstrahovanim:
    a) sekutićima
    b) premolarima
    c) molarima

15. Svaki uzorak GJC je posle svakog mernog intervala potapan u:
    a) 3 ml destilovane vode
    b) 4 ml destilovane vode
    c) 5 ml destilovane vode

16. U SEM analizi je korišćeno:
    a) 20 jednokorenih zuba
    b) 30 jednokorenih zuba
    c) 40 jednokorenih zuba

17. Uzorci sa mikrobiološkim materjalom za analizu su čuvani na:
    a) -200°C
    b) -400°C
    c) -500°C

18. Postekstrakcioni prostori su praćeni:
    a) tokom četiri sedmice
    b) tokom četiri meseca
    c) tokom četiri godine

19. Najveće otpuštanje fluorida je registrovano iz:
    a) Alfagala
    b) Equia forte
    c) Fuji IX

20. Efikasnost čišćenja apikalne trećine kanala je proveravana kod:
    a) gornjih sekutića
    b) gornjih premolara
    c) donjih premolara

21. Kod endodontskih neuspeha bakterije nisu nađene u kanalu:
    a) u 10% slučajeva
    b) u 20% slučajeva
    c) u 30% slučajeva

22. Širine postekstrakcionih prostora su merene:
    a) šestarom
    b) lenjirom
    c) digitalnim nonijusom

23. Najmanje otpuštanje fluorida je uočeno kod:
    a) Alfagala
    b) Fuji IX
    c) Fuji II LC

24. Instrumentacija kanala za SEM analizu je realizovana:
    a) setom od pet instrumenata
    b) setom od tri instrumenta
    c) samo jednim instrumentom

25. P. intermedia je uočena u:
    a) 85% kanala
    b) 75% kanala
    c) 82,2% kanala

26. Veće smanjenje postekstrakcionog prostora je uočeno posle primene:
    a) NiTi zatvorene opruge
    b) mehanizma sa elastičnim lancima
    c) nije bilo razlike između testiranih mehanizama

27. Svi GJC su pokazali najveće otpuštanje fluorida:
    a) posle dva sata
    b) posle 24 sata
    c) posle sedam dana

28. Instrument za preparaciju kanala u sistemu pune rotacije je bio:
    a) XP-endo finisher
    b) XP-endo finisher R
    c) XP-endo shaper

29. Najčešće identifikovane bakterije u kanalu sa endodontskim neuspehom su bile:
    a) E. faecalis i P. intermedia
    b) P. intermedia i P. micros
    c) P. micros i P. endondontalis

30. Prosečna vrednost smanjenja postekstrahovanog prostora posle primene NiTi zatvorene spirale je iznosila:
    a) 3,94 mm
    b) 3,10 mm
    c) 0,84 mm

31. Otpušanje fluorida kod svih GJC je bilo sporije:
    a) posle 48 sati
    b) posle tri dana
    c) posle sedam dana

32. Kao sredstvo za irigaciju kod uzorka za SEM analizu korišćen je:
    a) 1% NaOCl
    b) 2% NaOCl
    c) 5% NaOCl

33. Klasičnom PCR tehnikom je analizirana zastupljenost:
    a) svih bakterija u kanalu
    b) 10 vrsta bakterija
    c) pet vrsta bakterija

34. Prosečna vrednost smanjenja postekstrahovanog prostora posle primene metode elastičnog lanca je iznosila:
    a) 3,94 mm
    b) 3,10 mm
    c) 0,84 mm

35. Čist kanal kod uzoraka prebrisanih sistemom pune rotacije je zabeležen u:
    a) 60% slučajeva
    b) 76% slučajeva
    c) 80% slučajeva

36. U kanalu endodontski neuspešnih lečenja je analizirana zastupljenost bakterije Enterococus faecalis?
    a) Da
    b) Ne
    c) Samo u pojedinim kanalima

37. Najveću količinu fluorida je otpustio GJC:
    a) Equia forte
    b) Fuji IX
    c) Alfagal

38. Najzastupljenija bakterija u kanalima sa endodontskim neuspehom je bila:
    a) E. faecalis
    b) P. Intermedia
    c) P. Endodontalis

39. Najmanja vrednost za NiTi zatvorene opruge je iznosila:
    a) 0,84 mm
    b) 1,29 mm
    c) 2,19 mm

40. Za irigaciju kod uzoraka za SEM analizu je korišćen:
    a) 15% EDTA
    b) 17% EDTA
    c) 20% EDTA

41. Pozitivan bakteriološki nalaz kod analiziranih zuba sa neuspehom je registrovan:
    a) u 80% slučajeva
    b) u 60% slučajeva
    c) u 50% slučajeva

42. Najveće pomeranje zuba posle primene metode sa elastičnim lancima je iznosilo:
    a) 5,70 mm
    b) 4,80 mm
    c) 3,10 mm

43. NiTi sistemi sa samo jednom turpijom:
    a) potpuno uklanjaju razmazni sloj
    b) ne uklanjaju potpuno razmazni sloj
    c) u kanalu ostavljaju dosta razmaznog sloja

44. E. faecalis je identifikovan u:
    a) 58,3% kanala
    b) 75% kanala
    c) 83,3% kanala

45. Najveće pomeranje zuba posle primene NiTi zatvorene opruge je iznosilo:
    a) 5,70 mm
    b) 4,80 mm
    c) 3,10 mm

46. Turpija sa recipročnim pokretima UNICONE je bila:
    a) efikasnija u čišćenju apikalne trećine
    b) manje efikasna u čišćenju apikalne trećine
    c) neefikasna u čišćenju apikalne trećine

47. P. Micros je bio zastupljen u:
    a) 60% kanala
    b) 58,3% kanala
    c) 52,4% kanala

48. Sa oštećenim apeksnim parodoncijumom je bilo:
    a) 10 kanala
    b) 15 kanala
    c) 17 kanala

49. Kod zuba sa PA promenama E. faecalis je bio zastupljen u:
    a) 82% kanala
    b) 85% kanala
    c) 94% kanala

50. Kod pacijenata sa kliničkim simptomima bili su registrovani:
    a) E. faecalis
    b) P. micros
    c) A. actinomicetomcomitoris

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