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Contents / Sadržaj

„Ako nemate san,
kako će vam se taj san ostvariti?“

Ovim retoričkim pitanjem jednog aktera u mjuziklu „Južni vetar“ započeo sam svoj prvi urednički komentar davne 2002. godine.

„Stomatološki glasnik Srbije“ (SGS) časopis je Srpskog lekarskog društva osnovan 1953. godine i ove godine slavi 70 godina postojanja. Časopis objavljuje originalne naučne i stručne radove, prikaze iz prakse, pregledne radove, saopštenja, istoriografske radove, prikaze knjiga, komentare i pisma uredništvu, društvenu hroniku.

Časopis se nalazi na listi kategorisanih naučnih časopisa čiji su izdavači iz Republike Srbije u oblasti medicinskih nauka (M51). Do 1992. god. „Stomatološki glasnik“ je bio u bazi Medline, a nakon toga do 2000. god. nije redovno štampan zbog problema u finansiranju. Od 2003. redovno se štampa i izlazi četiri puta godišnje.

Od momenta kada je najstarija specijalistička sekcija Srpskog lekarskog društva pokrenula izdavanje stručnog časopisa „Stomatološki glasnik Srbije“ (1954) ovaj časopis je bio jedan od osnovnih izvora saznanja i dostignuća u stomatologiji u brojnim generacijama. Kao glasilo svih stomatologa Srbije SGS je imao, a i danas ima, presudnu ulogu u podsticanju stručne i naučne aktivnosti, ali i upoznavanju članstva sa najnovijim dostignućima u svim oblastima stomatologije. Preko društvene hronike stomatolozi su dobijali informacije o brojnim interesantnim događajima iz okvira stomatološke nauke i struke.

Vremenom se SGS usavršavao u pogledu kvaliteta radova i uređivanja časopisa, odnosno zadovoljenja osnovnih normativa vezanih za štampu, tako da je danas to vodeći nacionalni časopis iz oblasti stomatologije u Srbiji (M51). U skladu sa vremenom i trenutkom, ovaj časopis se stalno unapređuje koncepcijski i tehnički usavršava.

Od 2006. godine časopis dobija novu naslovnu stranu, novi dizajn i malo izmenjen koncept, koji je i danas osnova časopisa. Od januara 2006. godine SGS se nalazi u bazi DOI Serbia kao jedan od prvih naučno-stručnih časopisa sa ovih prostora.

U 2009. godini časopis je postao savremeniji i u pogledu forme i u pogledu grafičko- tehničkog dizajna. Radovi se u osnovnom obliku objavljuju na engleskom jeziku, zajedno sa prilozima, uz prevod celokupnog teksta na srpski jezik. Od 2009. godine SGS je sa sedištem u prostorijama Srpskog lekarskog društva, gde zajedno sa časopisom „Srpski arhiv za celokupno lekarstvo“ upotpunjuje izdavačku delatnost SLD-a, koja je jedan od najznačajnijih zadataka ovog udruženja.

Zahvaljujući elektronskoj verziji časopisa (od 2005. godine), SGS danas objavljuje radove autora iz čitavog sveta (Italije, Brazila, Indije, Nemačke, Španije, Bugarske, Hrvatske, Makedonije, Bosne i Hercegovine). Iako postoji hroničan problem sa malim brojem prispelih radova, uredništvo uspeva da svaku godinu uspešno realizuje i da svaka sveska (četiri puta godišnje) bude štampana na vreme i u predviđenoj dinamici. Zadovoljstvo je celog uređivačkog odbora što 50% objavljenih radova (originalni, pregledni, prikazi iz prakse) čine radovi iz inostranstva.

Promene u uređivačkom odboru su urađene u dva navrata (2010. i 2017. godine) sa članovima koji su se svojom naučnom i stručnom biografijom kvalifikovali za članstvo u ovom odboru. Promene iz 2017. su realizovane na osnovu ugovora o saradnji Srpskog lekarskog društva i Stomatološke komore Srbije, pa je uređivački odbor dodatno proširen sa predstavnicima Stomatološkog odseka medicinskih fakulteta u Kragujevcu, Kosovskoj Mitrovici, privatnog Stomatološkog fakulteta u Pančevu, kao i sa direktorom Stomatološke komore Srbije. Međunarodni uređivački odbor časopisa čine vrhunski svetski stručnjaci iz skoro svih oblasti stomatologije, a zamenik glavnog i odgovornog urednika je jedan od najplodnijih evropskih istraživača na području restaurativne stomatologije.

Nakon potpisivanja ugovora sa De Gruyter Openom (2015. godine) ostvareni su otvoreni pristup časopisa svetskim bibliotekama i automatsko detektovanje plagijata, čime su se stekli uslovi za povećanu citiranost časopisa. U 2015. je takođe potpisan ugovor o korišćenju naprednog paketa usluga u okviru SCI indeksa (Srpski citatni indeks) između CEON-a (Centar za evaluaciju u obrazovanju i nauci) i Srpskog lekarskog drištva sa osnovnim ciljem da se unaprede vidljivost, uticajnost i kvalitet samog časopisa.

Jedina želja glavnog i odgovornog urednika i celokupnog uređivačkog odbora je da časopis dopre do svakog stomatologa na terenu i ispuni osnovni zadatak širenja informacija o naučnim i stručnim dostignućima iz svih oblasti stomatologije. Glavni cilj je da se ostvare uslovi za indeksiranje u bazi MEDLINE (gde je bio indeksiran do 1992), ali i drugim prestižnim bazama naučnih i stručnih publikacija.

Poslednje dve decenije SGS „neguje" dva ozbiljna problema: materijalno-finansijski i nedovoljan priliv radova. Problemi u finansiranju časopisa su konstanta, ali ideje i entuzijazam odgovornih za „očuvanje" časopisa nisu poremetili redovnost štampanja, niti ugrozili njegov naučno-stručni nivo. Ovo je i osnovni razlog uvođenja obavezne naknade i pripreme članka (6000 din.) po predatom rukopisu. I pored brojnih problema, SGS je danas časopis sa visokim naučnim standardima i u skladu sa proklamovanim svetskim kriterijumima vezanim za časopise biomedicinskog profila.

Ozbiljan problem proteklih godina bio je nedovoljan priliv radova (25–40 godišnje), što je uz 10-20% odbijenih relativno mali broj na koji časopis može računati. Sa povećanjem broja kvalitetnih radova časopis bi u relativno kratkom roku mogao da ispuni uslove za indeksiranje i prelazak u neki viši rang i grupu časopisa sa impakt faktorom.

Zbog problema sa finansiranjem časopisa i teškoća vazanih za štampu, od ove (2023. god.) SGS se više neće štampati, već će imati samo elektronsku formu. Kao jedan od prvih naučno-stručnih časopisa sa ovih prostora sa elektronskom formom (od 2006), iskreno se nadam da će ova promena samo potvrditi ispravnost vizije vezane za sveprisutnu digitalizaciju svega, ali i značajno podići nivo i vidljivost časopisa.

Ovo je, dakle, prvi broj „Stomatološkog glasnika Srbije" koji će imati samo elektronsku formu, ali i poslednji broj koji uređuje aktuelni glavni i odgovorni urednik. U sledećem broju kao glavni i odgovorni urednik biće potpisan redovni profesor Stomatološkog fakulteta prof. dr Jugoslav Ilić. Njegova odgovornost, naučno-stručna kompetentnost i spremnost da se ozbiljno posveti očuvanju, odnosno unapređenju kvaliteta časopisa dobra su osnova za ovu inače vrlo odgovornu funkciju.

Bez obzira na brojne probleme tokom mandata, brojne obaveze i velike odgovornosti, posao glavnog i odgovornog urednika časopisa je pre svega izuzetna čast, a za mene lično privilegija i ostvarenje sna.

Mnogo je onih kojima bih se morao zahvaliti. Ali to bi oduzelo puno vremena i čitanje predugog teksta, pa ću od toga odustati. Svima koji su pomogli na očuvanju digniteta „Stomatološkog glasnika Srbije", ali i onima koji su se „ozbiljno" trudili da ga „ugase" neizmerno sam zahvalan. Prvima zato što su mi „olakšavali", a drugima zato što su me „naterali" da budem uporniji i istrajniji.

I ovaj urednički komentar ću završiti na uobičajen način i to citatom D. S. Džordana: „Mudrost je znati šta činiti, veština znati kako to da uradiš, a vrlina je to zaista i učiniti", jer on najbolje govori o viziji i entuzijazmu u nauci, praćenju svetskih trendova, suštinskoj otvorenosti prema svetu, ali i aktuelnim trendovima u izdavačkoj delatnosti.

Prof. dr Slavoljub Živković

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Efficacy of removing calcium hydroxide deposits from endodontic instruments prior to sterilization using different cleaning methods

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SUMMARY

Introduction Endodontic instruments during root canal treatment come into contact with dentinal debris, irrigation solutions and medication agents, which reminants should be eliminated before sterilization. The aim of the study was to verify the effectiveness of different cleaning methods for removing calcium hydroxide paste residues from the surfaces of the working parts of hand instruments, as well as to propose an effective protocol for cleaning endodontic instruments before sterilization.

Material and methods Forty-two new hand endodontic instruments were used to remove calcium hydroxide paste from the filled canals of extracted teeth. After contamination with medication, they were divided into the four groups and subjected to individual decontamination methods - mechanical, chemical and ultrasonic, as well as a combined protocol. The instruments were then observed under a light microscope. The effectiveness of the methods was evaluated based on the amount of residual matter on the surface of the working parts of the instruments.

Results The combined protocol showed a statistically significant difference in the achieved level of cleanliness compared to mechanical ($\chi^2 = 12.00$ $p < 0.05$) and chemical methods ($\chi^2 = 12.00$ $p < 0.05$), but there was no statistically significant difference compared to ultrasonic cleaning in disinfectant solution ($\chi^2 = 2.4$ $p > 0.05$). By applying combined protocol, instruments with completely clean surfaces were found, as well as the lowest values of contamination at the level of the entire group of instruments (8.33%).

Conclusion The protocol that consisted of mechanical cleaning with a sponge soaked in chlorhexidine gluconate, chemical soaking in sodium hypochlorite, and ultrasonic cleaning in a disinfectant showed the best efficiency in removing calcium hydroxide residues.

Keywords: endodontic instruments; cleaning; calcium hydroxide

INTRODUCTION

As endodontic files and reamers are generally accepted as reusable instruments, the evaluation of residual debris on their working part after use has been a significant topic of numerous scientific studies for a long time [1]. Data from the literature do not provide consistent information on the procedure for removing residues from contaminated endodontic instruments [2]. The cleaning procedure mainly involves mechanical (different types of brushes, sponges, etc.) and chemical cleaning (soaking in sodium hypochlorite (NaOCl), detergents, enzymatic cleaners), application of ultrasound and final rinsing before placing the instruments in the sterilizer. Numerous authors who analyzed different decontamination methods proved that no single method completely removes residual debris [3]. Therefore, the choice of appropriate methods of preparation of endodontic instruments for sterilization, justifies the development of an efficient cleaning protocol, with the aim of obtaining clean surfaces without inorganic and biological residues.

In addition to dentine debris, endodontic instruments during root canal treatment come into contact with irrigation solutions and medicated agents, their remains should also be eliminated before sterilization. Bearing in mind different chemical composition and adherence of these agents to the surface of the instruments, it is important to check the effectiveness of decontamination methods in their removal [4].

Calcium hydroxide is the medicament of choice in multi-session treatment of endodontic infections. It is stable in the canal, harmless to the body and has a prolonged bactericidal effect. It induces the formation of hard tissues and is effective against exudation in inflammations [5]. However, its incomplete removal from the root canal can negatively affect the adhesion of the sealer for root canal obturation [6]. Although there are different methods of removal that involve the application of various irrigants, ultrasound waves or laser energy, the remains of calcium hydroxide paste in the root canal must also be removed mechanically using endodontic instruments [7–11]. The paste deposits are then retained in the instrument blades

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along with the dentine debris and may remain even after the sterilization process is completed [4].

The aim of the study was to assess the effectiveness of different cleaning methods for removing calcium hydroxide paste residues from the surfaces of the working parts of hand endodontic instruments, as well as to propose an effective protocol for cleaning endodontic instruments before sterilization.

MATERIAL AND METHODS

The research was carried out at the Department of Restorative Dentistry and Endodontics of the Clinic for Dental Medicine in Niš and at the Scientific Research Center for Biomedicine of the Faculty of Medicine in Niš. The materials used in this research were manual endodontic instruments made of stainless steel - reamers and files (NTI-Kahla GmbH, Germany). Forty-two new stainless steel endodontic instruments - reamers and files were taken from their original packaging and subjected to ultrasonic cleaning to remove manufacturing impurities. The study included single-rooted teeth, extracted due to severe marginal periodontitis. A standard endodontic access cavity was prepared with a diamond bur. Each root canal was treated with a set of 15-40 instruments using a standard manual technique. NaOCl in concentration 0.5% was used for canal irrigation. After drying, the prepared root canals were filled with calcium hydroxide paste (i-CAL, i-dental, Lithuania) using a lentulo spiral. Endodontic access cavities were temporarily filled and stored in hermetically sealed boxes at room temperature for 24 hours. After removal of the temporary filling, the calcium hydroxide paste was flushed from the canal using 0.5% NaOCl, and the wall debris was then removed mechanically with endodontic instruments to achieve contamination of the working surfaces with the paste. The instruments were kept in hermetic boxes on the endodontic stand until the cleaning procedure.

The instruments were divided into the four groups and subjected to different cleaning methods (Table 1):

Group I: mechanical cleaning: with a brush and sponge soaked in chlorhexidine gluconate.

Group II: chemical cleaning: soaking in 1% sodium hypochlorite (NaOCl) and enzymatic cleaner for 10 minutes.

Group III: ultrasonic cleaning in two different media, water and disinfectant for 10 minutes.

Group IV: combination of the most effective mechanical, chemical method and ultrasonic cleaning before placing in the sterilizer.

A 0.2% solution of chlorhexidine gluconate (Curaprox Perio plus forte, Curadent international AG, Switzerland) was used in the research. Enzymatic cleaner Instruton E (Antiseptica, GmbH), used as a general instrument cleaner, contains proteases, amylases, tissue solvents and a corrosion inhibitor. Orocid Multisept plus ("OCC" Switzerland) was used as a disinfectant in the ultrasonic bath. The presence of residual contamination was analyzed by light microscopic examination. The assessment of instrument contamination was performed according to the methodology of Linsuwanont et al. [14]. The assessment of the effectiveness of decontamination methods was evaluated only numerically, with grades from 0 to 4. It was not possible to use a descriptive scale because it was residual material that is not of organic origin and does not stain histologically.

Table 1. Groups of instruments on which residual deposits of calcium hydroxide were observed, according to the applied decontamination methods
Tabela 1. Podela instrumenata na kojima su posmatrani rezidualni ostaci kalcijum-hidroksida, prema primenjenim metodama dekontaminacije

Decontamination methods Metode dekontaminacije		n(B)	Σ(B)
I group Mechanical cleaning I grupa Mehaničko čišćenje	Manual brushing Čišćenje četkom	6	42
	Sponge soaked in chlorhexidine gluconate Sunđer natopljen hlorheksidin-glukonatom	6	
II group Chemical methods II grupa Hemijske metode	Soaking in 1% NaOCl Natapanje u 1% NaOCl	6	
	Soaking in enzymatic cleaner Natapanje u enzimski čistač	6	
III group Ultrasonic cleaning III grupa Ultrazvučno čišćenje	Ultrasonic cleaning in distilled water Ultrazvučno čišćenje u vodi	6	
	Ultrasonic cleaning in disinfectant solution Ultrazvučno čišćenje u dezinficijensu	6	
IV group Combination of efective methods IV grupa Kombinacija najefikasnijih metoda	Sponge, soaking in 1% NaOCl, ultrasonic cleaning in disinfectant solution Sunđer, natapanje u 1% NaOCl, ultrazvučno čišćenje u dezinficijensu	6	

n – number of samples decontaminated by a certain method
n – broj uzoraka dekontaminisanih određenom metodom

The amount of residual matter was determined using the following scale:

0 – clean surface without any residues

1 – only the presence of a residual film

2 – slight contamination (individual, rare particles of calcium hydroxide, scattered on the surface of the working part of the instrument)

3 – medium contamination (numerous particles with fields in the form of a continuous covering on the surface)

4 – heavy contamination (fields on the instruments where the grooves of the working surfaces are completely filled with debris).

Each instrument was evaluated in 12 positions (four sides of the instrument in the coronary, middle and apical thirds) covering the entire blade region of the instrument. The results of all positions were added up. The minimum value was 0 (no presence of intracanal medication residues), and the maximum was 48 (all surfaces were heavily contaminated). The mean value for each instrument (MC) was calculated and then converted to a percentage mean maximum contamination (%MC).

Descriptive and analytical statistical methods were used for statistical data processing. The results were tabulated. Depending on the type of data, statistical parameters were

Table 2. Efficacy of removing calcium hydroxide from instrument surfaces by different methods
Tabela 2. Rezultati efikasnosti metoda uklanjanja kalcijum-hidroksida sa površine instrumenata različitim metodama

Cleaning method Metoda čišćenja	Cleaning results Rezultat čišćenja				
	0	1	2	3	Total Ukupno
Manual brushing Ručno četkanje	0 (0,0%)	0 (0,0%)	3 (7.1%)	3 (7.1%)	6 (14.3%)
Cleaning in sponge Čišćenje u sunđeru	0 (0,0%)	0 (0,00%)	5 (11.9%)	1 (2.4%)	6 (14.3%)
Soaking in NaOCl Natapanje u NaOCl	0 (0,0%)	0 (0,00%)	6 (14.3%)	0 (0,00%)	6 (14.3%)
Soaking in enzymatic cleaner Natapanje u enzimski čistač	0 (0,0%)	0 (0,0%)	2 (4.8%)	4 (9.5%)	6 (14.3%)
Ultrasonic cleaning – water Ultrazvučno čišćenje – voda	0 (0,0%)	4 (9.5%)	2 (4.8%)	0 (0,0%)	6 (14.29%)
Ultrasonic cleaning – disinfectant Ultrazvučno čišćenje – dezinficijens	0 (0,0%)	6 (14.3%)	0 (0.0%)	0 (0,0%)	6 (14.3%)
Combined method Kombinovana metoda	2 (4,8%)	4 (9.5%)	0 (0,0%)	0 (0,0%)	6 (14.3%)
Total Ukupno	2 (4,8%)	14 (33.3%)	18 (42.9%)	8 (19.0%)	42 (100,0%)

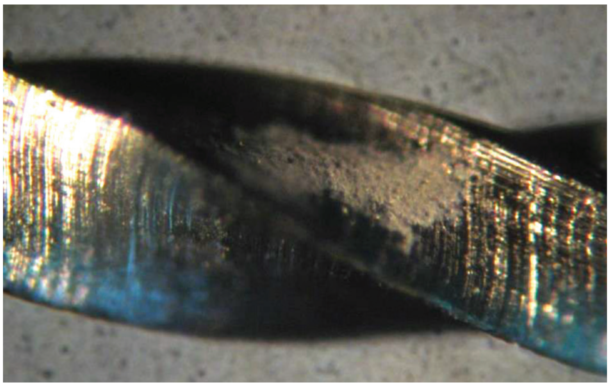

Figure 1. Residual deposit of calcium hydroxide on the working part of a size 30 stainless steel reamer, after the applied hand brushing method (score 3)
Slika 1. Ostaci naslaga kalcijum-hidroksida na radnom delu proširivača od nerđajućeg čelika veličine 30, posle primenjene metode ručnog četkanja (ocena 3)

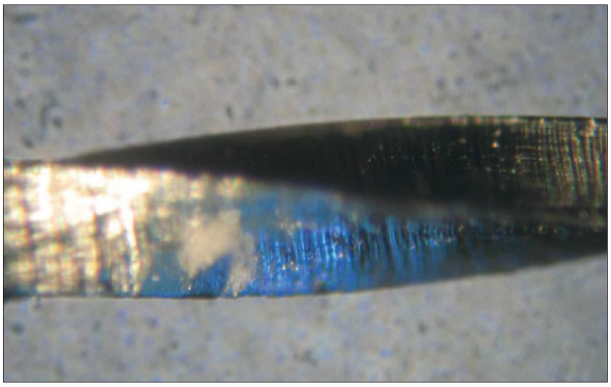

Figure 2. Surface of a size 30 reamer with residual calcium hydroxide particles after treatment with a sponge soaked in chlorhexidine gluconate (score 2)
Slika 2. Površina proširivača veličine 30 sa rezidualnim partikulama kalcijum-hidroksida posle tretmana sunđerom natopljenim hlorheksidin-glukonatom (ocena 2)

tested with appropriate tests. Analysis of variance (ANOVA) with post-hoc test was used for data related to a continuous feature, and the sample was homogeneous. Non-parametric chi squared and Fisher exact tests were used for data related to discontinuous feature.

Statistical processing was done using computer software packages Microsoft Excel and SPSS 20.0.

RESULTS

From the total number of instruments on which the presence of residual calcium hydroxide contamination was examined (42 instruments), 8 instruments were scored 3, 18 were scored 2, and 14 were scored 1 and 2 were scored 0. Subgroup 1, manually cleaned with a brush, showed a high degree of residual contamination. Of the six instruments, 3 were scored 2 and 3 separately (Table 2; Figure 1).

A subgroup of instruments subjected to mechanical cleaning with a sponge soaked in chlorhexidine gluconate showed lower cleanliness scores compared to manual brushing. Only 1 instrument was scored 3, and the other 5 were scored 2 (Table 2; Figure 2).

The effectiveness of the chemical soaking of instruments in sodium hypochlorite showed effectiveness with a score of 2 at the level of the entire subgroup. All 6 instruments had residual contamination in the form of scattered white particles (Table 2; Figure 3).

The chemical method of soaking instruments in an enzymatic cleaner showed the highest degree of residual contamination. Four instruments were scored 3, and 2 were scored 2 (Table 2; Figure 4).

In the subgroup of instruments cleaned in an ultrasonic bath, where distilled water was used as the liquid medium, 2 instruments were scored 2, and 4 were scored 1 (Table 2; Figure 5).

Ultrasonic cleaning in disinfectant solution showed that all 6 instruments were scored as 1 (Table 2; Figure 6).

Figure 3. Residual white deposit of calcium hydroxide on a size 35 file, after soaking in sodium hypochlorite (score 2)
Slika 3. Zaostala bela masa kalcijum-hidroksida na turpiji veličine 35, posle natapanja u natrijum-hipohlorit (ocena 2)

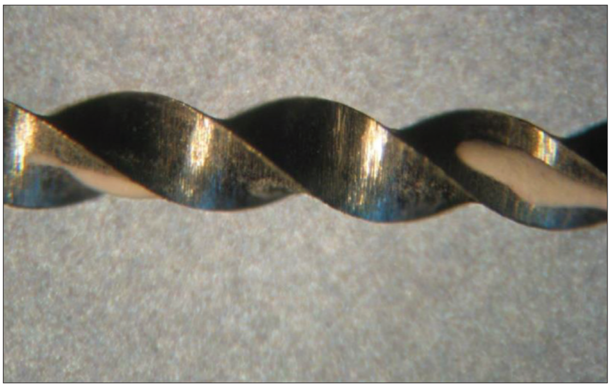

Figure 4. Residual deposits of calcium hydroxide on a size 35 file, after treatment with an enzymatic cleaner (grade 3)
Slika 4. Zaostale naslage kalcijum-hidroksida na površini turpije veličine 35, nakon tretmana u enzimskom čistaču (ocena 3)

Figure 6. Residual film of calcium hydroxide on a size 35 file treated in an ultrasonic bath with disinfectant solution
Slika 6. Preostali film od kalcijum-hidroksida na turpiji veličine 35 tretirane u ultrazvučnom kupatilu sa dezinficijensom

Figure 5. A size 25 file with residual deposit of calcium hydroxide over the entire surface (score 2)
Slika 5. Turpija veličine 25 sa zaostalim naslagama kalcijum-hidroksida na čitavoj površini (ocena 2)

Figure 7. Clean instrument surface without calcium hydroxide deposits subjected to the combined decontamination method
Slika 7. Čista površina instrumenta bez naslaga kalcijum-hidroksida podvrgnuta kombinovanoj metodi dekontaminacije

By decontamination of instruments using the combined method, 4 instruments were scored 1 and 2 were scored 0 (Table 2; Figure 7).

Table 2 shows the cleaning results in relation to the agent used. In an attempt to select the most adequate method, first pairs of means with a similar effect were tested in order to select the more effective one, and then the combined method was tested in relation to the more effective method of the previous pairs. It was calculated that there is no statistically significant difference in the effect of cleaning with a brush or sponge (Fisher exact test $p > 0.05$), ultrasonic cleaning with water versus ultrasonic cleaning with disinfectant ($\chi^2 = 0.60$ Fisher exact $p < 0.05$), but there was a statistically significant difference between chemical cleaning in NaOCl and enzymatic cleaner ($\chi^2 = 3.38$ Fisher exact $p > 0.05$).

Combined method showed a statistically significant difference in the achieved level of cleanliness compared to mechanical and chemical methods (combined method : sponge - $\chi^2 = 12.00$ $p < 0.05$, combined method : NaOCl - $\chi^2 = 12.00$ $p < 0.05$), but there was no statistically significant difference compared to ultrasonic cleaning in disinfection (combined method : ultrasound in disinfectant - $\chi^2 = 2.4$ $p > 0.05$).

The effectiveness of the decontamination methods was also analyzed through the average values of the mean percentage of maximum contamination (MC). Instruments of group 1, decontaminated by manual brushing, showed a MC value of 24.00 for instruments with score 3, and 20.67 for instruments with score 2. The mean percentage of MC value for score 3 was 50.00%, and for score 2 – 43.06%. The mean value of the MC for the entire subgroup is 22.33, i.e. 46.53%.

Group 2, decontaminated with a sponge soaked in chlorhexidine gluconate, had a MC value of 22.00 for score 3 instruments and 20.00 for score 2 instruments. Within the subgroup, the mean MC value was 20.33 and 42.36%, respectively. All instruments of group 3, soaked in NaOCl, had a score of 2, so the mean MC for this group was 21.33, i.e. 44.44%.

Group 4 of instruments soaked in enzymatic cleaner showed MC values of 22.25 for instruments with grade 3, and 23.50 for those with grade 2. The mean percentage of MC for grade 3 was 46.35%, and for grade 2 – 48.96%. At the level of the entire subgroup, the average rating of MC was 22.67, i.e. 47.22%.

Subgroup 5 of instruments, cleaned in an ultrasonic cleaner with distilled water, had a MC value of 12.50 for score 2 and 11.75 for score 1. The mean percentage of MC for score 2 was 26.04%, and for score 1 24.48%. Within the entire subgroup, the average value of the MC was 12.00, or 25.00%. Decontamination of the instruments

Table 3. Average values of %MC after cleaning with different methods
Tabela 3. Prosečne vrednosti %MC posle čišćenja različitim metodama

Cleaning method Metoda čišćenja	Cleaning result Rezultat čišćenja				
	0	1	2	3	Total Ukupno
Manual brushing Ručno četkanje			43.06 ± 2.41	50.00 ± 2.08	46.53 ± 4.30
Cleaning in sponge Čišćenje u sunđeru			41.67 ± 4.42	45.83 ± 0.00	42.36 ± 4.30
Soaking in NaOCl Natapanje u NaOCl			44.44 ± 3.14		44.44 ± 3.14
Soaking in enzymatic cleaner Natapanje u enzimski čistač			48.96 ± 1.47	46.35 ± 3.56	47.22 ± 3.14
Ultrasonic cleaning – water Ultrazvučno čišćenje – voda		24.48 ± 1.04	26.04 ± 1.47		25.00 ± 1.32
Ultrasonic cleaning – disinfectant Ultrazvučno čišćenje – dezinficijens		25.00 ± 0.00			25.00 ± 0.00
Combined method Kombinovana metoda	0.00	8.33 ± 1.32			22.92 ± 1.32
Total Ukupno	0.00	19.26 ± 1.31	41.90 ± 6.81	47.66 ± 3.23	36.21 ± 10.90

of subgroup 6 in the ultrasonic bath with disinfectant resulted in a score of 1 at the level of the entire subgroup, and thus the mean value of the MC was calculated, which was 12.00, i.e. 25.00% for the entire subgroup. Using the combined method, the lowest values of MC were obtained, which for this group of instruments was 4, i.e. 8.33% (Table 3).

The average values of % MC after cleaning with different methods were tested by analysis of variance, and the value F = 87,431 was calculated, which was statistically significant ($p < 0.05$). The post hoc test found that all average values for cleaning scores differ from each other.

DISCUSSION

The presence of debris on the surface of endodontic files and reamers can be detected by various methods. The application of scanning electron microscopy enables a detailed, three-dimensional observation of the surface of the instrument with all irregularities and defects that arise during the production process, including the possible presence of impurities, however, it is difficult to determine the nature and origin of residual matter [12]. In order to observe the colored or uncolored residual material or the highlighting of a thin film on the surface of the grooves of endodontic instruments, the blades of the instruments were observed under a light microscope [3]. Since there is currently no recognized method for testing cleanliness, recommendations regarding cleaning and sterilization are based on currently available scientifically derived and clinically relevant data.

Although there are views supporting the concept of single-use endodontic instruments, the recommendations remain controversial in the published literature. Arguments supporting multiple use mostly concern economic reasons but are supported by studies that guarantee the safety of their reuse [13]. Due to the necessity of eliminating all links in the chain of contamination, cleaning, disinfection and sterilization of endodontic instruments are imperative in the prevention of cross-infections in dental practice. It has been observed that different types of particles (production debris, biological residues or medicament residues) on the surface of new or used endodontic instruments can cause problems during canal preparation, so their elimination is necessary.

Calcium hydroxide is the intracanal medication of choice in endodontics due to its superior antimicrobial properties, ability to inhibit osteoclast activity and induce tissue reparative response. However, its residues in the canal system have a negative effect on the sealer's hermetic obturation, increase the apex permeability and have a negative effect on zinc oxide-eugenol-based filling agents. All methods for its removal from the canal can be divided into 3 categories: instrumentation with irrigation; manual irrigation using a syringe; irrigation performed using different equipment – passive ultrasonic irrigation, Endo-Vac, RinseEndo [15]. The removal of this medication from the root canal is influenced by the type of irrigant and the method of its activation in the canal. Chelating solutions such as ethylenediaminetetraacetic acid (EDTA), citric and maleic acids have shown a better effect of dissolving calcium hydroxide in the canal [16]. However, irrigants such as NaOCl, distilled water and saline, which do not chelate calcium hydroxide, can effectively remove it only when used with different activation systems [17]. Due to the fact that in clinical conditions, during the mechanical removal of calcium hydroxide paste on the surface of the instruments, both medication and dentin particles are simultaneously deposited, the effectiveness of NaOCl was examined in the study because it effectively dissolves dentin and has a disinfectant effect.

The aim of this study was to examine alternative combinations of mechanical, chemical and ultrasonic cleaning methods to perform a simple protocol effective in removing calcium hydroxide paste deposits from endodontic instrument surfaces. The study evaluated the effectiveness of different, in practice most often applied individual methods, for obtaining instruments that did not contain residual remains of calcium hydroxide paste at the microscopic level. In order to perform the final protocol, the results of pairs of methods with a similar effect were first tested. The final protocol was equally effective and applicable for different types of endodontic instruments.

After comparing the cleaning results of two mechanical methods - manual cleaning with a brush and cleaning with a sponge soaked in chlorhexidine gluconate, it was proven that cleaning with a sponge was significantly more effective. The high values of the maximum contamination for the instruments cleaned with a brush can be explained by the inability of the wide fibers to pass through all the grooves. The disadvantage is also that the effectiveness depends to a large extent on the commitment of the staff. Adequate execution of this technique takes a lot of time, which is impractical for application in everyday practice

[18]. Linsuwanont et al. [14] showed that mechanical cleaning removes a significant amount of debris, but is unable to completely clean the instruments. When viewed from the aspect of the risk of injury during work, there is a possibility of puncture, so it is recommended that brushing be performed on an endodontic stand. The use of a sponge soaked in chlorhexidine gluconate provided a satisfactory level of initial cleaning. Lower values of the maximum contamination were achieved due to the fact that all sides of the instrument during operation were affected by the spongy mass. Using a sponge is safer, because the operator does not come into direct contact with sharp parts of the instruments [19]. This method is also important in moist storage of instruments after clinical use, which is the basis for effective cleaning [14, 18].

Analyzing the results of chemical soaking of instruments in sodium hypochlorite and enzymatic cleaner, a significant difference in the efficiency of residual debris removal was observed. Sodium hypochlorite has been used as an endodontic irrigant for over eighty years. It is a widely used disinfectant and oxidizing agent and has the ability to dissolve organic tissue, which largely depends on the ratio of hypochlorite to organic matter [20]. If used as a disinfectant when cleaning nickel titanium endodontic instruments, it can lead to micro-point corrosion. Enzymes and tissue solvents are effective in removing proteins, lipids and carbohydrates from their surfaces [21]. Although both agents exhibit a strong dissolving effect of proteins and organic matter, sodium hypochlorite showed significantly higher efficiency in removing calcium hydroxide. In performing the final decontamination protocol, preference was given to sodium hypochlorite.

By comparing the results of cleaning with two ultrasonic methods - in water and disinfectant, no statistically significant difference in efficiency was observed. The results showed that both ultrasonic methods removed a significant amount of residual debris, but no instrument with a completely clean surface was obtained. These results are in accordance with the results of Aasim et al. [4] who stated in their study that calcium hydroxide deposits were resistant to ultrasonic cleaning. Cleaning in disinfection showed better results, so it was included in the final protocol as a more efficient method. This research showed that the use of ultrasound is an important step in instrument cleaning, which is consistent with the studies of other authors [22, 23]. It improves worker safety compared to manual brushing. If combined with disinfectant solutions, ultrasonic cleaners can also have an antimicrobial effect, thereby reducing residual contamination and ensuring safer handling of instruments and more efficient sterilization [24]. In this study, satisfactory cleaning results were achieved with ultrasonic cleaning for 15 minutes. The same time is recommended by other authors [4]. It is extremely important to rinse the instruments after treatment in order to remove the residual contaminated solution [4, 25]. The results showed that the use of ultrasound, without prior preparation of the instruments, did not give completely clean surfaces of the instruments. Similar results were obtained by Linsuwanont et al. [14]. Their study showed that the combined use of 1% NaOCl and an ultrasonic bath for 5 minutes could not completely remove organic material from instruments without first removing a large amount of debris by brushing. The results of this study showed that all procedures in the protocol are necessary and emphasize the importance of mechanical removal of impurities before chemical and ultrasonic cleaning.

For this reason, a protocol was developed that represented a combination of the most effective methods from the previous three groups - mechanical, chemical and ultrasonic cleaning. It consisted of mechanical cleaning with a sponge soaked in chlorhexidine, soaking in sodium hypochlorite and ultrasonic cleaning in a disinfectant solution. By comparing the results of the protocol in relation to the more efficient method of the previous pairs, it was shown that there is a significant difference in cleaning efficiency. Only the application of the protocol resulted in instruments with completely clean surfaces. The protocol presented in this research relies more on chemical means and equipment than on the human factor in order to obtain satisfactory results of cleaning endodontic instruments. Initial cleaning with a sponge soaked in chlorhexidine gluconate is simple and can be done quickly. Chemical soaking and ultrasonic cleaning are two very important stages and must be carried out consecutively. This protocol is very simple and can be easily adopted and applied routine dental practice.

CONCLUSIONS

Individual mechanical, chemical or ultrasonic methods of decontamination of endodontic instruments are not effective enough to remove calcium hydroxide residues from the working parts of endodontic instruments. Methods that rely on manual cleaning of instruments are insufficiently efficient and do not always produce the same results, as they mostly depend on the motivation of the person performing them. The method of mechanical cleaning of endodontic instruments using a sponge soaked in chlorhexidine showed significantly higher efficiency than mechanical cleaning with a brush in a moist environment. The method of chemical soaking of endodontic instruments in sodium hypochlorite was more effective than soaking in an enzymatic cleaner. The method of ultrasonic cleaning of endodontic instruments in disinfection was significantly more effective compared to ultrasonic cleaning in water. The protocol that consisted of mechanical cleaning with a sponge soaked in chlorhexidine, chemical soaking in sodium hypochlorite, and ultrasonic cleaning in a disinfectant showed the best efficiency in removing calcium hydroxide residues.

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Procena efikasnosti različitih metoda uklanjanja naslaga kalcijum-hidroksida sa endodontskih instrumenata pre sterilizacije

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KRATAK SADRŽAJ

Uvod Endodontski instrumenti tokom mehaničko-medikamentozne obrade kanala dolaze u kontakt sa dentinskim debrisom, rastvorima za irigaciju i sredstvima za medikaciju, čiji ostaci treba da budu eliminisani pre sterilizacije.

Cilj istraživanja je bio da se proveri efikasnost različitih metoda čišćenja za uklanjanje ostataka paste kalcijum-hidroksida sa površina radnih delova ručnih instrumenata, kao i da se predloži efikasan protokol čišćenja endodontskih instrumenata pre sterilizacije.

Materijal i metode Četrdeset dva nova ručna endodontska instrumenta korišćena su za uklanjanje paste kalcijum-hidroksida iz napunjenih kanala ekstrahovanih zuba. Posle kontaminacije medikamentom podeljeni su u četiri grupe i podvrgnuti pojedinačnim metodama dekontaminacije – mehaničkim, hemijskim i ultrazvučnim, kao i kombinovanom protokolu. Instrumenti su zatim posmatrani na svetlosnom mikroskopu. Procena efikasnosti metoda je procenjivana na osnovu količine rezidualne materije na površini radnih delova instrumenata.

Rezultati Kombinovani protokol je pokazao statistički značajnu razliku u postignutom stepenu čistoće u odnosu na mehaničke ($\chi^2 = 12{,}00$, $p < 0{,}05$) i hemijske metode ($\chi^2 = 12{,}00$, $p < 0{,}05$), ali nije postojala statistički značajna razlika u odnosu na ultrazvučno čišćenje u dezinficijensu ($\chi^2 = 2{,}4$, $p > 0{,}05$). Primenom kombinovanog protokola dobijeni su instrumenti sa potpuno čistim površinama, kao i najniže vrednosti maksimuma kontaminacije na nivou cele grupe instrumenata (8,33%).

Zaključak Protokol koji se sastojao od mehaničkog čišćenja sunđerom natopljenim hlorheksidin-glukonatom, hemijskog natapanja natrijum-hipohloritom i ultrazvučnog čišćenja u dezinfekcionom sredstvu bio je najefikasniji u uklanjanju ostataka kalcijum-hidroksida.

Ključne reči: endodontski instrumenti; čišćenje; kalcijum-hidroksid

UVOD

Kako su endodontske turpije i proširivači generalno prihvaćeni kao instrumenti za višekratnu upotrebu, procena rezidualnih ostataka na njihovom radnom delu nakon upotrebe je već duže vreme značajna tema brojnih naučnih studija [1]. Podaci iz literature ne daju usaglašene informacije o postupku uklanjanja ostataka sa kontaminiranih endodontskih instrumenata [2]. Postupak čišćenja uglavnom podrazumeva mehaničko (različite vrste četkica, sunđera i sl.) i hemijsko čišćenje (natapanje u NaOCl, deterdžente, enzimske čistače), primenu ultrazvuka i završno ispiranje pre postavljanja instrumenata u sterilizator. Brojni autori koji su se bavili analizom različitih metoda dekontaminacije su dokazali da nijedna pojedinačna metoda potpuno ne uklanja rezidulani debris [3]. Stoga, izbor odgovarajućih metoda pripreme endodontskih instrumenata za sterilizaciju opravdava razvijanje efikasnog protokola čišćenja, sa ciljem dobijanja čistih površina bez neorganskih i bioloških ostataka.

Osim sa dentinskim debrisom, endodontski instrumenti tokom mehaničko-medikamentozne obrade kanala dolaze u kontakt i sa rastvorima za irigaciju i sredstvima za medikaciju, čiji ostaci takođe treba da budu eliminisani pre sterilizacije. Imajući u vidu različiti hemijski sastav i adherentnost ovih sredstava za površinu instrumenata, od značaja je izvršiti proveru efikasnosti dekontaminacionih metoda kod njihovog uklanjanja [4].

Kalcijum-hidroksid predstavlja medikament izbora u višeseansnom lečenju endodontskih infekcija. Stabilan je u kanalu, neškodljiv za organizam i ima vremenski ograničeno baktericidno dejstvo. Indukuje formiranje tvrdih tkiva i efikasno deluje na eksudaciju kod inflamacija [5]. Međutim, njegovo nekompletno uklanjanje iz kanala korena može negativno uticati na adherentnost silera za definitivnu opturaciju [6]. Iako postoje različite metode uklanjanja koje podrazumevaju primenu različitih iriganasa, ultrazvučnih talasa ili energije lasera, ostaci paste kalcijum-hidroksida u kanalu korena moraju se ukloniti i mehanički korišćenjem endodontskih instrumenata [7–11]. Naslage paste se tada zadržavaju u sečivima instrumenata zajedno sa dentinskim opiljcima i mogu zaostati i posle završenog procesa sterilizacije [4].

Cilj istraživanja je bio da se proveri efikasnost različitih metoda čišćenja za uklanjanje ostataka paste kalcijum-hidroksida sa površina radnih delova ručnih instrumenata, kao i da se predloži efikasan protokol čišćenja endodontskih instrumenata pre sterilizacije.

MATERIJAL I METODE

Istraživanje je obavljeno na Odeljenju za bolesti zuba i endodonciju Klinike za dentalnu medicinu u Nišu i u Naučnoistraživačkom centru za biomedicinu Medicinskog fakulteta u Nišu.

Kao materijal u ovom istraživanju korišćeni su ručni endodontski instrumenti od nerđajućeg čelika – proširivači i turpije (NTI-Kahla GmbH, Germany). Četrdeset dva nova endodontska instrumenta – proširivači i turpije od nerđajućeg čelika uzeti su iz originalnih pakovanja i podvrgnuti ultrazvučnom čišćenju da bi se oslobodili proizvodnih nečistoća.

U studiju su bili uključeni jednokoreni zubi, ekstrahovani zbog uznapredovale parodontopatije. Standardni endodontski pristupni kavitet je preparisan dijamantskim borerom u visokoturažnoj mašini. Svaki kanal korena je obrađen setom instrumenata 15-40 standardnom ručnom tehnikom. Za irigaciju kanala korišćen je 0,5% NaOCl. Nakon sušenja, ispreparisani kanali korena su lentulo spiralom napunjeni pastom na bazi kalcijum-hidroksida (i-CAL, i-dental, Lithuania). Endodontski pristupni kaviteti su zatvoreni privremenim ispunom i čuvani u hermetički zatvorenim kutijama na sobnoj temperaturi 24 sata. Posle uklanjanja privremenog ispuna, pasta kalciijum-hidroksida je ispirana iz kanala korišćenjem 0,5% NaOCl, a ostaci sa zidova su nakon toga uklanjani mehanički endodontskim turpijama da bi se postigla kontaminacija površina radnih delova sa pastom. Instrumenti su na endodontskom stalku do postupka čišćenja čuvani u hermetičkim kutijama.

Instrumenati su podeljeni u četiri grupe i podvrgnuti različitim metodama čišćenja (Tabela 1):

I grupa – mehaničko čišćenje: četkom i sunđerom natopljenim u hlorheksidin-glukonat.

II grupa – hemijsko čišćenje: natapanje u 1% natrijum-hipohlorit (NaOCl) i enzimski čistač u trajanju od 10 minuta.

III grupa – čišćenje ultrazvukom u dva različita medijuma, vodi i dezinficijensu, u trajanju od 10 minuta.

IV grupa – kombinacija najefikasnije mehaničke, hemijske metode i ultrazvučnog čišćenja pre postavljanja u sterilizator.

U istraživanju je korišćen 0,2% rastvor hlorheksidin-glukonata (Curaprox Perio plus forte, Curadent international AG, Switzerland). Enzimski čistač Instruton E (Antiseptica, GmbH), koji se koristi kao opšti čistač instrumenata, sadrži proteaze, amilaze, tkivne rastvarače i inhibitor korozije. Kao dezinficijens u ultrazvučnom kupatilu korišćen je Orocid Multisept plus („OCC“ Switzerland).

Prisustvo rezidualne kontaminacije je analizirano svetlosnomikroskopskim ispitivanjem. Procena kontaminacije instrumenata je vršena prema metodologiji koju su koristili Linsuwanont i saradnici [14].

Procena efikasnosti dekontaminacionih metoda je ocenjena samo numerički, ocenama od 0 do 4. Deskriptivnu skalu nije bilo moguće koristiti jer je bio u pitanju rezidualni materijal koji nije organskog porekla i histološki se ne boji.

Količina rezidualne materije je utvrđivana pomoću sledeće skale:

0 – čista površina bez ikakvih ostataka

1 – samo prisustvo rezidualnog filma

2 – neznatna kontaminacija (pojedinačne, retke partikule kalcijum-hidroksida, rasute po površini radnog dela instrumenta)

3 – srednja kontaminacija

4 – izrazita kontaminacija (polja na instrumentima gde su žlebovi radnih površina potpuno ispunjeni debrisom)

Svaki instrument je ocenjen u 12 položaja (četiri strane instrumenta i koronarna, srednja i apeksna trećina), što je obuhvatilo čitavu sečivnu regiju instrumenta. Rezultati svih položaja su sabrani. Minimalna vrednost je bila 0 (nema prisustva ostataka intrakanalnog medikamenta), a maksimalna je bila 48 (sve površine su bile jako kontaminirane). Izračunata je srednja vrednost za svaki instrument (MK), a zatim je preračunata u procentualnu srednju vrednost maksimuma kontaminacije (%MK).

Za statističku obradu podataka korišćen je deskriptivni i analitički statistički metod. Rezultati su prikazani tabelarno. U zavisnosti od vrste podataka, statistički parametri su testirani odgovarajućim testovima. Za podatke koji se odnose na kontinuirano obeležje, a uzorak je homogen, korišćena je analiza varijanse (ANOVA) sa post-hok testom. Za podatke koji se odnose na diskontinuirano obeležje korišćeni su neparametrijski testovi χ^2 test i Fišerov test.

Statistička obrada je urađena korišćenjem računarskih programskih paketa Microsoft Excel i SPSS 20.0.

REZULTATI

Od ukupnog broja instrumenata na kojima je ispitivano prisustvo rezidualne kontaminacije kalcijum-hidroksida (42 instrumenta), osam instrumenata je ocenjeno ocenom 3, 18 ocenom 2, a 14 ocenom 1 i dva ocenom 0.

Podgrupa 1, ručno čišćena četkom, pokazala je visok stepen rezidualne kontaminacije. Od šest instrumenata, po tri su ocenjena ocenom 2 i ocenom 3 (Tabela 2; Slika 1).

Podgrupa instrumenata podvrgnuta mehaničkom čišćenju sunđerom natopljenim hlorheksidin-glukonatom pokazala je niže ocene čistoće u poređenju sa ručnim četkanjem. Samo jedan instrument je ocenjen ocenom 3, a ostalih pet je ocenjeno ocenom 2 (Tabela 2; Slika 2).

Efikasnost hemijskog natapanja instrumenata u natrijum-hipohlorit pokazala je efikasnost ocenom 2 na nivou cele podgrupe. Svih šest instrumenata je imalo rezidualnu kontaminaciju u vidu razbacanih partikula bele boje (Tabela 2; Slika 3).

Hemijska metoda natapanja instrumenata u enzimski čistač je pokazala najviši stepen rezidualne kontaminacije. Četiri instrumenta su ocenjena ocenom 3, a dva ocenom 2 (Tabela 2; Slika 4).

U podgrupi instrumenata očišćenih u ultrazvučnom kupatilu, gde je kao tečni medijum korišćena voda, dva instrumenta su ocenjena ocenom 2, a četiri ocenom 1 (Tabela 2; Slika 5).

Ultrazvučno čišćenje u dezinficijensu je pokazalo da je svih šest instrumenata ocenjeno ocenom 1 (Tabela 2; Slika 6).

Dekontaminacijom instrumenata kombinovanom metodom četiri instrumenta su ocenjena ocenom 1 i dva ocenom 0 (Tabela 2; Slika 7).

U Tabeli 2 su prikazani rezultati čišćenja u odnosu na upotrebljeno sredstvo. U pokušaju odabira najadekvatnije metode, testirani su prvo parovi sredstava sličnog efekta radi odabiranja efikasnijeg, a potom je testirana kombinovana metoda u odnosu na efikasniji metod od prethodnih parova. Izračunato je da ne postoji statistički značajna razlika efekta čišćenja četkom ili sunđerom (Fišerov test $p > 0,05$), ultrazvučnog čišćenja vodom u odnosu na ultrazvučno čišćenje dezinficijensom ($\chi^2 = 0,60$ Fišerov test $p < 0,05$), ali postoji statistički značajna razlika između hemijskog čišćenja u NaOCl i enzimskom čistaču ($\chi^2 = 3,38$ Fišerov test $p > 0,05$).

Kombinovana metoda pokazala je statistički značajnu razliku u postignutom stepenu čistoće u odnosu na mehaničke i hemijske metode (kombinovana metoda : sunđer – $\chi^2 = 12,00$ $p < 0,05$, kombinovana metoda : NaOCl – $\chi^2 = 12,00$, $p < 0,05$), ali nije postojala statistički značajna razlika u odnosu na ultrazvučno čišćenje u dezinficijensu (kombinovana metoda : ultrazvučno čišćenje u dezinficijensu – $\chi^2 = 2,4$, $p > 0,05$).

Efikasnost metoda dekontaminacije je analizirana i kroz prosečne vrednosti srednjeg procenta maksimuma kontaminacije (MK).

Instrumenti grupe 1, dekontaminisani ručnim četkanjem, pokazali su vrednost MK od 24,00 za instrumente sa ocenom 3, i 20,67 za instrumente sa ocenom 2. Srednja vrednost procenta za ocenu 3 je iznosila 50,00%, a za ocenu 2 – 43,06%. Srednja vrednost MK za celu podgrupu iznosila je 22,33, odnosno 46,53%.

Grupa 2, dekontaminisana sunđerom natopljenim hlorheksidin-glukonatom, imala je vrednost MK od 22,00 za instrumente sa ocenom 3 i 20,00 za instrumente sa ocenom 2. U okviru podgrupe srednja vrednost MK je iznosila 20,33, odnosno 42,36%.

Svi instrumenti grupe 3, natapani u NaOCl, imali su ocenu 2, tako da je prosečan MK za ovu grupu iznosio 21,33, odnosno 44,44%.

Natapanje instrumenata grupe 4 u enzimski čistač je pokazalo vrednosti MK od 22,25 za instrumente sa ocenom 3, i 23,50 za one sa ocenom 2. Srednji procenat MK za ocenu 3 je iznosio 46,35%, a za ocenu 2 – 48,96%. Na nivou cele podgrupe srednja ocena MK je iznosila 22,67, odnosno 47,22%.

Instrumenti podgrupe 5, potapani u ultrazvučno kupatilo sa vodom, imali su vrednost MK 12,50 za ocenu 2 i 11,75 za ocenu 1. Srednja vrednost procenata MK za ocenu 2 je iznosila 26,04%, a za ocenu 1 – 24,48%. U okviru cele podgrupe prosečna vrednost MK je iznosila 12,00, odnosno 25,00%.

Dekontaminacija instrumenata podgrupe 6 u ultrazvučnom kupatilu sa dezinficijensom je kao rezultat imala ocenu 1 na nivou cele podgrupe, a samim tim je izračunata srednja vrednost MK, koja je iznosila 12,00, odnosno 25,00% za celu podgrupu.

Primenom kombinovane metode dobijene su najniže vrednosti MK, koji je kod ove grupe instrumenata iznosio 4, odnosno 8,33% (Tabela 3).

Prosečne vrednosti % MK posle čišćenja različitim sredstvima testirane su analizom varijanse, i izračunata je vrednost $F = 87{,}431$, koja je statistički značajna ($p < 0{,}05$). Post hok testom je ustanovljeno da se sve prosečne vrednosti po ocenama čistoće međusobno razlikuju.

DISKUSIJA

Postojanje debrisa na površini endodontskih turpija i proširivača može se detektovati različitim metodama. Primenom skening elektronske mikroskopije omogućeno je detaljno, trodimenzionalno sagledavanje površine instrumenta sa svim iregularnostima i defektima koji nastaju u toku procesa proizvodnje, uključujući i eventualno prisustvo nečistoća, međutim, teško je utvrditi prirodu i poreklo rezidualne materije [12]. Radi uočavanja obojenog ili neobojenog rezidualnog materijala ili isticanja tankog filma na površini žlebova endodontskih instrumenata, sečiva instrumenata se posmatraju pod svetlosnim mikroskopom [3]. S obzirom na to da trenutno ne postoji ni jedan priznati metod za ispitivanje čistoće, preporuke koje se tiču čišćenja i sterilizacije baziraju se na trenutno dostupnim naučno dobijenim i klinički relevantnim podacima.

Iako postoje stavovi koji podržavaju koncept jednokratne upotrebe endodontskih instrumenata, u objavljenoj literaturi preporuke ostaju kontroverzne. Argumenti koji podržavaju višestruku upotrebu najviše se tiču ekonomskih razloga, ali su podržani studijama koje garantuju bezbednost njihove ponovne upotrebe [13]. Zbog neophodnosti eliminacije svih veza u lancu kontaminacije, čišćenje, dezinfekcija i sterilizacija endodontskih instrumenata predstavljaju imperativ u prevenciji unakrsnih infekcija u stomatološkoj praksi. Uočeno je da različiti tipovi čestica (proizvodni debris, biološki ostaci ili ostaci medikamenata) na površini novih ili već korišćenih endodontskih instrumenata mogu da prouzrokuju probleme tokom pripreme kanala, pa je neophodna njihova eliminacija.

Kalcijum-hidroksid je intrakanalni medikament izbora u endodonciji zbog superiornih antimikrobnih osobina, sposobnosti da inhibira osteoklastnu aktivnost i izazivanja reparativnog odgovora tkiva. Međutim, njegovi ostaci u kanalnom sistemu loše utiču na hermetičku opturaciju silera, povećavaju apeksnu propustljivost i loše utiču na sredstva za punjenje na bazi cink-oksid eugenola. Sve metode za njegovo uklanjanje iz kanala mogu da se podele u tri kategorije: instrumentacija sa irigacijom; manuelno izvedena irigacija pomoću šprica; irigacija izvedena pomoću različite opreme – pasivna ultrazvučna irigacija, Endo-Vac, RinsEndo [15]. Na uklanjanje ovog medikamenta iz kanala korena utiče vrsta iriganса i način njegove aktivacije u kanalu. Helatni rastvori kao što su etilendiamintetrasirćetna kiselina (EDTA), limunska i maleinska kiselina pokazale su bolji efekat rastvaranja kalcijum-hidroksida u kanalu [16]. Međutim, irigansi kao što su NaOCl, destilovana voda i fiziološki rastvor, koji nemaju helatno dejstvo na kalcijum-hidroksid, mogu ga efikasno ukloniti samo kada se koriste uz različite aktivacione sisteme [17]. Zbog činjenice da se u kliničkim uslovima prilikom mehaničkog uklanjanja paste kalcijum-hidroksida na površini instrumenata istovremeno talože i medikament i opiljci dentina, u studiji je ispitivana efikasnost NaOCl jer efikasno rastvara dentin i ima dezinficijentno dejstvo.

Cilj ove studije je bio da se ispitaju alternativne kombinacije metoda mehaničkog, hemijskog i ultrazvučnog čišćenja radi izvođenja jednostavnog protokola efikasnog u uklanjanju naslaga paste kalcijum-hidrokida sa površina endodontskih instrumenata. U radu su procenjivane efikasnosti različitih, u praksi najčešće primenjivanih pojedinačnih metoda, za dobijanje instrumenata koji nisu sadržali rezidualne ostatke paste kalcijum-hidroksida na mikroskopskom nivou. U cilju izvođenja konačnog protokola najpre su testirani rezultati parova metoda sličnog efekta. Konačni protokol je bio jednako efikasan i primenjiv za različite tipove endodontskih instrumenata.

Posle poređenja rezultata čišćenja dve mehaničke metode – ručnog čišćenja četkom i čišćenja sunđerom natopljenim hlorheksidinom, dokazano je značajno efikasnije čišćenje sunđerom. Visoke vrednosti koeficijenta maksimuma kontaminacije kod instrumenata čišćenih četkom mogu se objasniti nemogućnošću širokih vlakana da prođu kroz sve žlebove. Nedostatak je i u tome što efikasnost u velikoj meri zavisi od posvećenosti osoblja. Adekvatno izvođenje ove tehnike oduzima dosta vremena, što je nepraktično za primenu u svakodnevnoj praksi [18]. Linsuwanont i saradnici [14] pokazali su da mehaničko čišćenje uklanja značajnu količinu debrisa, ali nije u mogućnosti da potpuno očisti instrumente. Kada se posmatra sa aspekta rizika od povređivanja u toku rada, postoji mogućnost uboda, pa se preporučuje da se četkanje izvodi na endodontskom stalku. Upotreba sunđera natopljenog u hlorheksidin-glukonat obezbedila je zadovoljavajući stepen inicijalnog čišćenja Niže vrednosti koeficijenta maksimuma kontaminacije postignute su zahvaljujući tome što su sve strane instrumenta u toku rada zahvaćene sunđerastom masom. Korišćenje sunđera je bezbednije, jer operator ne dolazi u direktan kontakt sa oštrim delovima instrumenata [19]. Ova metoda ima značaja i u vlažnom čuvanju instrumenata posle kliničke upotrebe, koja je osnova za efikasno čišćenje [14, 18].

Analizom rezultata hemijskog natapanja instrumenata u natrijum-hipohlorit i enzimski čistač uočena je značajna razlika u efikasnosti uklanjanja rezidualnog debrisa. Natrijum-hipohlorit se koristi kao endodontsko sredstvo za ispiranje više od 80 godina. On je široko primenjivano dezinfekciono sredstvo i oksidacioni agens i poseduje sposobnost rastvaranja organskog tkiva, koja u velikoj meri zavisi od odnosa hipohlorita i organske materije [20]. Ukoliko se koristi kao dezinfekciono sredstvo pri čišćenju endodontskih instrumenata od nikl-titanijuma, može da dovede do mikrotačkaste korozije. Enzimi i tkivni rastvarači su efikasni u uklanjanju proteina, lipida i ugljenih hidrata sa njihovih površina [21]. Iako oba sredstva ispoljavaju jak rastvarački efekat proteina i organske materije, natrijum-hipohlorit je pokazao značajno veću efikasnost u uklanjanju kalcijum-hidroksida. U izvođenju krajnjeg protokola dekontaminacije data je prednost natrijum-hipohloritu.

Poređenjem rezultata čišćenja kod dve ultrazvučne metode – u vodi i dezinficijensu, nije uočena statistički značajna razlika u efikasnosti. Rezultati su pokazali da su obe ultrazvučne metode uklonile znatnu količinu rezidualnih ostataka, ali nije dobijen nijedan instrument sa potpuno čistom površinom. Ovi rezultati su u skladu sa rezultatima Aasima i saradnika [4], koji su u svojoj studiji izneli da su depoziti kalcijum-hidroksida bili rezistentni na ultrazvučno čišćenje. Čišćenje u dezinficijensu je pokazalo bolje rezultate, pa je kao efikasnija metoda uključena u izvođenje konačnog protokola. Ovo istraživanje je pokazalo da je korišćenje ultrazvuka važan korak u čišćenju instrumenata, što se podudara sa studijama drugih autora [22, 23]. Ono unapređuje bezbednost radnika u poređenju sa ručnim četkanjem. Ukoliko se kombinuju sa dezinficijentnim rastvorima, ultrazvučni čistači mogu imati i antimikrobno dejstvo, time se smanjuje rezidualna konataminacija i obezbeđuje bezbednije rukovanje sa instrumentima i efikasnija sterilizacija [24]. U ovoj studiji zadovoljavajući rezultati čišćenja postignuti su ultrazvučnim čišćenjem u trajanju od 15 minuta. Isto vreme je preporučeno od strane drugih autora [4]. Od izuzetne važnosti je ispiranje instrumenata posle tretmana radi otklanjanja rezidualnog kontaminiranog rastvora [4, 25]. Rezultati su pokazali da upotreba samog ultrazvuka, bez prethodne pripreme instrumenata, nije dala potpuno čiste površine instrumenata. Slične rezultate su dobili Linsuwanont i saradnici [14]. Njihova studija je pokazala da kombinovana upotreba 1% NaOCl i ultrazvučnog kupatila u toku pet minuta ne može potpuno ukloniti organski materijal sa instrumenata bez prethodnog uklanjanja velike količine debrisa četkanjem. Rezultati ove studije pokazuju da su svi postupci u protokolu neophodni i naglašavaju važnost mehaničkog uklanjanja nečistoća pre hemijskog i ultrazvučnog čišćenja.

Iz tog razloga je razvijen protokol koji je predstavljao kombinaciju najefikasnijih metoda iz prethodne tri grupe – mehaničkog, hemijskog i ultrazvučnog čišćenja. Sastojao se iz mehaničkog čišćenja sunđerom natopljenim hlor-heksidinom, natapanja u natrijum-hipohlorit i ultrazvučnog čišćenja u rastvoru deterdženta. Poređenjem rezultata protokola u odnosu na efikasniji metod od prethodnih parova, pokazano je da postoji značajna razlika u efikasnosti čišćenja. Jedino je primena protokola kao rezultat imala instrumente potpuno čistih površina. Protokol predstavljen u ovom istraživanju se više oslanja na hemijska sredstva i opremu nego na ljudski faktor u cilju dobijanja zadovoljavajućih rezultata čišćenja endodontskih instrumenata. Početno čišćenje sunđerom natopljnim u hlorheksidin-glukonatu je jednostavno i može se brzo obaviti. Hemijsko natapanje i ultrazvučno čišćenje su dve vrlo važne faze i moraju se izvršiti uzastopno. Ovakav protokol je vrlo jednostavan i lako se može usvojiti i primenjivati kako u privatnoj praksi tako i u institucionalnom okruženju.

ZAKLJUČAK

Pojedinačne mehaničke, hemijske ili ultrazvučne metode dekontaminacije endodontskih instrumenata nisu dovoljno efikasne za uklanjanje ostataka kalcijum-hidroksida sa radnih delova endodontskih instrumenata. Metode koje se oslanjaju na ručno čišćenje instrumenata su nedovoljno efikasne i ne daju uvek iste rezultate, jer uglavnom zavise od motivacije osobe koja ih izvodi. Metoda mehaničkog čišćenja endodontskih instrumenata pomoću sunđera natopljenim hlorheksidinom je pokazala značajno veću efikasnost od mehaničkog čišćenja četkom u vlažnoj sredini. Metoda hemijskog natapanja endodontskih instrumenata u natrijum-hipohlorit je bila efikasnija od natapanja u enzimski čistač. Metoda ultrazvučnog čišćenja endodontskih instrumenata u dezinficijensu je bila značajno efikasnija u odnosu na ultrazvučno čišćenje u vodi. Najbolju efikasnost uklanjanja ostataka kalcijum-hidroksida je pokazao protokol koji se sastojao iz mehaničkog čišćenja u sunđeru natopljenim hlorheksidinom, hemijskog natapanja u natrijum-hipohlorit i ultrazvučnog čišćenja u dezinficijensu.

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Vascular endothelial growth factor as a response of denture bearing tissues on mechanical stress in diabetes mellitus

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SUMMARY

Introduction Vascular endothelial growth factor (VEGF) is signal molecule enrolled in diabetes mellitus type 2 (DM type 2) oral complications, but there are no studies showing the relation between VEGF and pressure caused by denture wearing in diabetic conditions.
The aim of this study is to compare tissue VEGF levels in patients and animals with/without DM in conditions of chronic and acute pressure.
Methods Research was conducted on DM type 2 and healthy partial denture wearers for more than 5 years (78), candidates for teeth extractions and experimental animals of the Wistar rats (40). For chronic conditions, VEGF was measured in 2 mucosal samples covered and not covered by denture in all denture wearers. Demonstrating acute conditions, after 3 days of wearing experimental plate VEGF was measured in 2 gingival samples of palatal mucosa of DM (20) and control rats (20). The concentrations of VEGF (pg/ml) in human and animal tissues were measured by commercially available ELISA kit.
Results Tissue VEGF levels in control and diabetic partial denture wearers not covered by denture were without statistical difference. In comparison to noncovered tissue, VEGF decreased in samples covered by denture, being significantly lower in DM type 2, comparing to healthiest. VEGF levels in palatal mucosa without palatal base did not significantly differ in control and DM rats. VEGF levels under palatal base increased being significantly lower in DM rats comparing to controls.
Conclusion Both, chronic and acute mechanical stress caused by wearing palatal denture (plate) decreased the VEGF levels in diabetic conditions comparing the health's suggesting the altered homeostasis.
Keywords: compression; diabetes mellitus type 2; denture; mucosa; VEGF

INTRODUCTION

Diabetes mellitus type 2 (DM type 2) is associated with increased incidence of several oral conditions: periodontal disease, impaired healing of oral wounds and mucosa ulcerations-especially under the base of complete denture [1, 2]. Prosthetic rehabilitation of reduced masticatory function in DM patients is of great relevance having in mind the importance of good and well-balanced diet as a part of diabetes therapy. However, the presence of denture and its compression might compromise oral homeostasis [3]. Namely, subjected to denture wearing, oral mucosa has to endure mechanical loads of various levels and durations. In addition to the persistent load required for static support and retention, oral mucosa must be resistant to various levels and durations of acute and chronic load during functional and para functional behaviors [4]. Studies have shown morphological and histological changes in oral mucosa induced by chronic stress caused by wearing dentures in human subjects and rodents [5, 6, 7]. Examinations of mechanical compression on mucosa caused by denture base showed ischemia *in vivo* and disruption of multiple functions of endothelial basal cells [3, 8, 9]. Besides mentioned, mechanical forces and compression are also known to induce the secretion of soluble mediators, including cytokines and growth factors [10, 11, 12]. One of them, vascular endothelial growth factor (VEGF) is an angiogenetic signaling molecule that elicits cellular responses to hypoxia [13]. Tsuruoka et al. reported increased VEGF levels under experimental palatal plate in the connective tissues and osteoblasts in the periosteum of maxilla in healthy rats [12].

Studies have shown altered levels of VEGF in saliva, gingival tissues and crevicular fluids of diabetic subjects [14, 15], pointing this factor as one of the crucial mediators of oral complications in DM type 2. However, there is luck of data about changes in VEGF level as cellular

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response on mechanical loads cased by denture wearing in underlying tissues of diabetics.

The aim of this study was 1) to determine tissue VEGF levels produced during chronic pressure caused by wearing partial acrylic denture in patients with and without DM type 2, and 2) to determine tissue VEGF levels produced during acute pressure caused by experimental palatal plate in experimental animals with and without DM.

MATERIAL AND METHODS

Study population

Seventy-eight participants aged 45 to 64 years were included into the study. The study consisted of participants with diagnosed DM type 2 (42) recruited from Clinical department for endocrinology, diabetes and metabolic diseases, University Medical Center, Belgrade and participants without diabetes (36) from the Department of Prosthodontics, Faculty of Dental Medicine, University of Belgrade. Participants enrolled in the study were wearing maxillary partial removable acrylic dentures and mandibular complete dentures at least for 5 years and had teeth present with signs of severe periodontitis in the anterior and premolar maxillary regions [16, 17, 18]. The criteria for diabetic participants included the following: history of disease for approximately 5 years with a glycated hemoglobin A1c measurement less than 9 (HbA1c < 9) [16, 17]. Smokers, patients with systemic diseases or who were taking additional medication were excluded from the study. All study participants gave consent. The study was approved by University of Belgrade, Faculty of Dentistry Committee on the Ethics of Human Research (No. 36-32, April 12, 2010).

Sample collection

Each subject underwent periodontal examination and due to irreversible mobility and poor prognoses of the remaining teeth the treatment plan required extraction with fabrication of maxillary complete dentures and new mandibular complete dentures. During the extraction procedures approximately 1mm of tissue samples were obtained adjacent to the extracted teeth. For each patient, 2 samples were taken: the sample that was previously covered by partial denture (compressive sample) and the sample that was not covered by partial denture (non-compressive sample). The specimens were frozen in liquid-nitrogen and stored at -70°C until analysis.

Experimentally induced DM

Forty male Wistar rats weighing approximately 250 g each were used in this study. The rats were divided equally into 2 groups of 20 in each. The control group consisted of normal healthy rats. In another group, diabetes was induced using a single intraperitoneal injection of the pancreatic β-cell toxin monohydrate (alloxan, 140mg/kg; Sigma, St. Louis, MO, USA). Serum glucose level were measured (Accu-Chek; Roche Diagnostic, Indianapolis, IN, USA) and rats were considered to be diabetic when blood glucose levels exceeded 10 mmol/L. Experiments were started when animals were hyperglycemic for minimum 3 weeks. For each animal, 2 tissue samples were taken: the sample that was previously covered by experimental palatal plate (compressive sample) and the sample that was not covered by experimental palatal plate (non-compressive sample). The specimens were frozen in liquid-nitrogen and stored at -70°C until analysis.

All animal procedures were approved by the Ethical Committee of the Faculty of Dentistry, University of Belgrade (No. 36-32, April 12, 2010).

Preparation of experimental palatal plates and sample collection

For the animals in the group with experimental palatal plates (control group/DM group), following the procedure of Tsurioka et al [12], precision impression of the upper jaw was taken using vinyl-silicon impression material (Zhermack). A piece of wax approximately 0.6mm thickness was set on the occlusal surfaces of the molar teeth on the plaster cast. Methil-metacrylate plate (Biokril, Galenika, Serbia) extending from the first to the third molar teeth on both sides of palate was made and cured. After curing, the attached wax was removed. The palatal plate was set on the palatine mucosal surface of each animal with pressure and was fixed to the molar teeth on both sides using the same resin (Figures 1 and 2). In order to obtain animal tissue specimens, all rats were sacrificed 3 days after palatal plate wearing using an overdose of thiopental sodium. Maxillas were removed with palatal plates and tissue with/without palatal plate was prepared for VEGF quantification.

Tissue measurement of VEGF

The concentrations of VEGF in human and animal tissues (pg/mL) were measured by commercially available Enzyme-Linked Immunosorbent Assay (Human VEGFA ELISA Cell Culture Supernatant, Urine; RayBiotech and Rat VEGFA ELISA Kit for Cell Culture Supernatant, Urine; RayBiotech) according to the manufacturer's instructions [19]. Optical densities were measured at 450 nm with a microplate reader and the minimum detectable level of the test was 5.0 pg/mL.

Statistics

Results were presented as the mean ± standard deviation (SD). Comparisons between groups were done by using two-way ANOVA with repeated measures and Chi-squared test. One-way ANOVA was used to analyze group differences, and Bonferroni correction was performed for post-hoc comparisons. Independent samples Student's *t* test was performed to determine comparisons between appropriate group-time points. The statistical significance level was considered to be 5%. All data were analyzed using Stat for Windows 8, StatSoft, Inc, USA, 1984–2007.

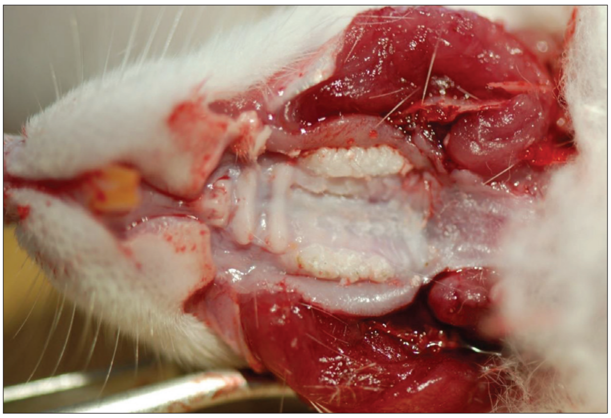

Figure 1. Palatal mucosa of rat before covering with experimental palatal plate
Slika 1. Palatinalna sluzokoža pacova pre pokrivanja eksperimentalnom palatinalnom pločom

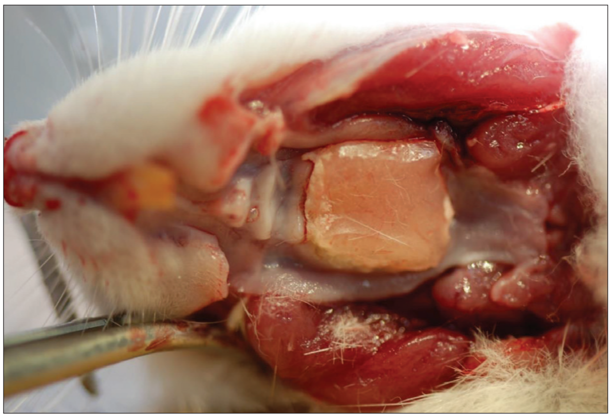

Figure 2. Experimental palatal plate on rat's maxilla
Slika 2. Eksperimentalna palatinalna ploča na maksili pacova

RESULTS

Age and gender were not considered in this study, because observed groups of participants consisted predominantly of male subjects (69%), aged between 45 and 65 years.

Table 1 shows tissue VEGF levels in non-compressive and compressive samples in healthy and DM type 2 partial denture wearers. VEGF levels in compressive samples were significantly lower in comparison to VEGF levels in non-compressive samples in both observed groups (DM type 2 and healthy). The VEGF decrease was 66% for diabetic compressive samples and 28% for compressive samples of healthy participants in comparison to non-compressive samples. DM type 2 denture wearers had significantly lower VEGF levels in compressive tissue samples comparing to healthy subjects, contrary to non-compressive samples where results showed no significant difference between healthy and DM type 2 partial denture wearers.

Table 1. Tissue VEGF in non-compressive and compressive samples in healthy and DM type 2 partial denture wearers
Tabela 1. Koncentracije VEGF u gingivi u uslovima akompresije i kompresije parcijalnom protezom kod zdravih ispitanika i ispitanika sa DM tipa 2

Participants Ispitanici	Tissue VEGF (pg/ml) Koncentracije VEGF u tkivu (pg/ml) X ± SD	
	Non-compressive samples Akompresivni deo gingive	Compressive samples Kompresivni deo gingive
Healthy (42) Zdravi (42)	37.64 ± 3.0	27.18 ± 1.0*
DM type 2 (36) DM tip 2 (36)	36.44 ± 4.9	12.58 ± 1.2***$

*p < 0.05, ***p < 0.001 – compressive vs non-compressive, $p < 0.05 – Healthy vs DM type 2 (ANOVA, Bonferroni post hoc test)
*p < 0,05, ***p < 0,001 – poređenje kompresije i akompresije, $p < 0,05 – poređenje zdravih i sa DM tipa 2 (ANOVA, Bonferonijev post hok test)

Characteristics of control rats and rats with DM referring blood glucose levels and body weight before experimental procedures, 21 days after DM induction and on the last day of experimental procedures -24th day (after 3

Table 2. Characteristics of control rats and rats with DM
Tabela 2. Karakteristike kontrolne grupe pacova i pacova sa eksperimentalno izazvanim DM

Parameters Parametri	Observation period Period posmatranja	Control rats (20) Kontrolna grupa (20)	DM rats (20) Indukovani DM (20)
	Before experimental procedures Pre eksperimentalnih postupaka	6.5 ± 1.8	6.4 ± 1.5
Blood glucose level (mmol/l) Nivo glukoze u krvi (mmol/l)			
	21st day 21. dan eksperimenta 24th day 24. dan eksperimenta	6.3 ± 1.3 6.4 ± 2.8	15.4 ± 3.2* 14.3 ± 0.2*
	Before experimental procedures Pre eksperimentalnih postupaka	245.0 ± 4.96	243.2 ± 5.0
Weight (g) Telesna masa (g)			
	21st day 21. dan eksperimenta 24th day 24. dan eksperimenta	300.0 ± 8.2 280 ± 5.6	200.0 ± 6.1* 180.0 ± 7.3*

*p < 0.01 – control vs DM (ANOVA, Bonferroni post hoc test)
*p < 0,01 – poređenje kontrolna grupe i DM (ANOVA, Bonferonijev post hok test)

Table 3. Tissue VEGF in non-compressive and compressive samples in control and DM rats
Tabela 3. Koncentracija VEGF tkiva u nekompresivnim i kompresivnim uzorcima kod kontrolnih i pacova sa eksperimentalno izazvanim DM

	Tissue VEGF (pg/ml) Koncentracije VEGF (pg/ml) X ± SD	
Animals Životinje	Non-compressive sample Nekompresivni uzorak	Compressive samples Kompresivni uzorak
Control (20) Kontrolne (20)	2.6 ± 0.1	9.56 ± 0.8*
DM (20) DM (20)	2.8 ± 0.2	6.34 ± 0.37*$

*p < 0.01 – compressive vs non-compressive, $p < 0.01 – control vs DM (ANOVA, Bonferroni post hoc test)
*p < 0,01 – poređenje kompresivnog uzorka i nekompresivnog uzorka;
$p < 0,01 – poređenje kontrolne grupe i DM (ANOVA, Bonferonijev post hok test)

days of wearing experimental palatal plate) are presented in Table 2. Results showed no significant difference in blood glucose levels and body weight before experimental procedures in observed groups. On 21st and 24th day, blood glucose level was increased and significantly higher in DM rats in comparison to healthy controls. At the 21st day, when experimental palatal plate was inserted, results showed decrease in body weight in DM animals contrary to controls and that resulted in significant difference in body weight in observed groups. On the last day of experimental procedures- after 3 days of wearing experimental palatal plate, both group of animals showed decrease of body weight, but DM rats had significantly lower body weight comparing to healthy controls.

Table 3 shows tissue VEGF levels in non-compressive and compressive samples in control and DM rats. Tissue VEGF levels are significantly increased in samples under experimental palatal plate (compressive samples), comparing to VEGF levels in tissue that is not covered by experimental palatal plate (non-compressive samples) in both observed groups. DM and control rats did not show statistical difference in tissue VEGF levels in non-compressive samples, contrary to compressive samples where VEGF levels are significantly lower in DM animals comparing to healthy controls.

DISCUSSION

Acute and chronic load during functional and para functional behaviors caused by dentures have the potential to cause deformation of underlying connective tissues [20, 21, 22]. Investigating the influence of the continuous compression of the removable partial denture on the blood flow in underlying mucosa of denture wearers, Akazawa and Sakurai [8] showed tissue ischaemia and delays in recovery of blood flow after release of compression. Tsouruka et al. reported that cells in the tissue, under experimental palatal plate, synthesize HSP70 and VEGF to maintain homeostasis [12]. However, the authors of this study identified lack of data concerning the influence of acute and chronic mechanical stress caused by denture wearing on VEGF tissue levels in diabetic conditions. To elucidate the effects of DM on acute changes of underlying oral mucosa, this study intended to stimulate the effects of denture wearing in an animal experimental model. Human model was used to show the influence of chronically mechanical stress on underlaying tissue at the cellular level in denture wearers with/without DM type 2. Following the study of Tsuruoka et al. [12], we did not consider the effects of used materials for acrylic experimental plate and partial denture on the underlying tissues. Namely, in accordance with Mori et al. [21] histopathological findings concerning heat-cured resin, Inoue et al. [23] showed minimal cytotoxic effect of 4-META/MMA-TBB, despite of monomer penetration in the tissue during polymerization.

Results of our study revealed increased tissue VEGF levels after 3 days wearing experimental palatal plate, in both observed group of animals: with and without DM. Our finding is in accordance with study of Tsuruoka et al., which found that VEGF staining was positive for vascular endothelial cells in connective tissues and for osteoblasts in periosteum of maxilla of healthy rats after 3 days wearing experimental palatal plate [12]. Having in mind studies that reported reduction of blood flow under experimental palatal plates resulting in ischaemia [8, 9], increased VEGF levels in rats tissue after 3 days wearing experimental palatal plate might be caused by hypoxic condition, a major stimuli for VEGF. Contrary to our results showing no statistically significance in tissue VEGF levels in non-compressive samples between DM and healthy animals (samples without experimental plate), there was significantly lower tissue VEGF increase under experimental palatal plate in rats with DM comparing the controls, suggesting that VEGF expression is altered under mechanical stress in diabetic conditions.

Analyzing model of chronic stress, our results showed significantly lower VEGF levels in tissue covered by partial denture (compressive samples) in comparison to VEGF levels in tissue that wasn't covered by partial denture (non-compressive samples). Mentioned decrease of tissue VEGF might suggest on oral mucosa adaptation and cellular response on mechanical stress in order to maintain oral homeostasis. Obtained result of significantly higher tissue VEGF decrease in compressive samples of DM type 2 partially denture wearers comparing to healthy subjects suggest on altered oral homeostasis in diabetic conditions. However, our results showed no statistical significance in tissue VEGF levels in non-compressive samples between DM type 2 and healthy denture wearers. Published data about tissue VEGF levels in diabetic periodontitis patients are conflicting. Contrary to Sakalioglu et al. [23] who reported significantly higher VEGF levels in gingival supernatants of diabetic periodontitis patients comparing to controls, Keles et al. [24] found no significant difference in expression levels of VEGF mRNA in gingival tissues of periodontitis patients with and without DM type 2. However, many investigations showed tissue changes and alterations under denture base in diabetic patients and animals with experimentally induced DM [25–28] comparing to healthy controls, but our results for the first time introduced changes in tissue VEGF levels as cellular response on denture wearing in diabetic conditions.

CONCLUSION

Acute mechanical stress increases concentration of VEGF in palatal mucosa, while, chronically mechanical stress, conversely, decreases tissue VEGF levels. Lower VEGF levels were related in acute and chronic compression in diabetic conditions, suggesting altered homeostasis. Our research emphasizes the importance of prosthodontists education on denture maintenance in patients with DM type 2, as it contributes to a better understanding of the cellular response and biological processes in the oral mucosa under mechanical stress.

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DISCLOSURE STATEMENT

The authors report no conflict of interest.

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Vaskularni endotelni faktor rasta kao odgovor mobilnim zubnim protezama kod pacijenata sa dijabetesom melitusom

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KRATAK SADRŽAJ

Uvod/Cilj Brojne studije ukazuju na vaskularni endotelni faktor rasta (VEGF) kao jedan od medijatora oralnih komplikacija dijabetesa melitusa tipa 2 (DM tip 2), ali nijedna nije pokazala efekte nošenja proteze na vrednosti VEGF kod dijabetičkih stanja.

Cilj ovog rada bio je da se odredi koncetracija VEGF u gingivi na mestima hroničnog i akutnog pritiska proteze kod pacijenata i eksperimentalnih životinja sa dijabetesom melitusom i bez njega.

Metode Istraživanje je sprovedeno na pacijentima i eksperimentalnim životinjama soja Vistar. U istraživanje su uključeni zdravi (42) i pacijenti sa DM tipa 2 (36), nosioci parcijalnih proteza duže od pet godina, sa indikacijom za vađenje zuba. Kod svakog pacijenta za merenje VEGF (model hroničnog pritiska) uzeta su po dva uzorka tkiva, kompresivni i akompresivni uzorak, tokom ekstrakcije zuba. Za merenje VEGF u palatinalnoj sluzokoži eksperimentalnih pacova posle tri dana nošenja eksperimentalne ploče (model akutnog pritiska) uzeta su dva uzorka gingive (pokrivene/nepokrivene eksperimentalnom pločom) kod kontrolne grupe (20) i pacova sa eksperimentalno indukovanim DM (20). Za kvantitativno merenje VEGF (pg/ml) korišćen je komercijalni ELISA kit.

Rezultati Koncentracije VEGF u sluzokoži koja nije pokrivena protezom bile su iste u kontrolnoj grupi i grupi pacijenata sa DM tipa 2. U kompresivnim uzorcima VEGF je smanjen u obe grupe, ali značajno niži kod dijabetičara u poređenju sa zdravim pacijentima. Koncentracije VEGF u sluzokoži bez palatinalne ploče bile su slične kod kontrolnih i pacova sa DM. U prisustvu palatinalne ploče koncentracije VEGF su bile povećane, međutim, značajno niže kod pacova sa DM u poređenju sa kontrolnom grupom.

Zaključak Hronični mehanički stres smanjuje VEGF za razliku od akutnog mehaničkog stresa koji povećava VEGF u palatinalnoj sluzokoži. Akutna i hronična kompresija povezane su sa nižim nivoima VEGF kod pacijenata sa DM, što ukazuje na izmenjenu homeostazu.

Ključne reči: kompresija; dijabetes melitus tip 2; proteza; sluzokoža; VEGF

UVOD

Dijabetes melitus tip 2 (DM tip 2) povezan je sa povećanom incidencom oralnih stanja kao što su: parodontopatija, usporeno zarastanje oralnih rana i ulceracija sluzokože – posebno ispod baze totalne proteze [1, 2]. Protetska rehabilitacija smanjene mastikatorne funkcije kod pacijenata sa DM je od velike važnosti, imajući u vidu značaj dobre i uravnotežene ishrane, kao deo terapije dijabetesa. Međutim, prisustvo proteze i njena kompresija mogu kompromitovati oralnu homeostazu [3]. Naime, nošenjem proteza, potporna tkiva moraju da izdrže mehanička opterećenja različitog nivoa i trajanja. Pored konstantnog opterećenja potrebnog za stabilizaciju i retenciju proteze, oralna sluzokoža mora biti otporna na različite nivoe i trajanje akutnog i hroničnog opterećenja tokom funkcionalnih i parafunkcionalnih navika [4]. Studije su pokazale morfološke i histološke promene u oralnoj sluzokoži izazvane hroničnim stresom zbog nošenja proteza kod ljudi i glodara [5, 6, 7]. Ispitivanja mehaničke kompresije na sluzokoži izazvane bazom proteze pokazala su ishemiju in vivo i višestruki poremećaj funkcija bazalnih ćelija endotela [3, 8, 9]. Pored navedenog, poznato je da mehaničke sile i kompresija indukuju lučenje rastvorljivih medijatora, uključujući citokine i faktore rasta [10, 11, 12]. Jedan od njih, vaskularni endotelni faktor rasta (VEGF), angiogenetski je signalni molekul koji izaziva ćelijske odgovore na hipoksiju [13]. Tsuruoka M. i saradnici [12] pokazali su povećanu ekspresiju VEGF ispod eksperimentalne ploče u palatinalnoj sluznici i osteoblastima u periostiumu maksile kod zdravih pacova.

Studije su pokazale izmenjene nivoe VEGF u pljuvački, gingivalnim tkivima i tečnosti gingivalnog sulkusa kod dijabetičara [14, 15], ukazujući na ovaj faktor kao jedan od ključnih medijatora oralnih komplikacija kod DM tipa 2. Međutim, malo je podataka o promenama u nivou VEGF kao ćelijskog odgovora na mehanička opterećenja izazvanog nošenjem proteze kod dijabetičara.

Na osnovu prikazanih podataka, cilj ove studije bio je 1) da se odredi nivo VEGF u tkivu na mestu hroničnog pritiska izazvanog nošenjem mobilne zubne proteze kod ispitanika sa DM tipa 2 i bez njega, i 2) da se odredi koncentracija VEGF u gingivi u uslovima akutnog pritiska ispod eksperimentalne palatinalne ploče u grupi životinja sa eksperimentalno izazvanim DM i kontrolnoj grupi životinja.

MATERIJAL I METODE

Ispitanici

Klinička studija je obuhvatala 78 ispitanika, oba pola, starosne dobi od 45 do 64 godine. U studiji su učestvovali ispitanici sa dijagnostikovanim DM tipa 2 (42) koji su lečeni na Klinici za endokrinologiju KBC „Zvezdara“ u Beogradu i ispitanici kod kojih nije dijagnostikovan DM (36) sa Klinike za stomatološku protetiku u Beogradu. Ispitanici su bili nosioci gornjih parcijalnih pločastih proteza (PPP) i donjih totalnih proteza (TP) u vremenskom periodu od pet godina, sa terminalnim stadijumom parodontopatije u prednjoj i premolarnoj maksilarnoj regiji [16, 17, 18]. Kriterijum za uključivanje ispitanika sa DM tipa 2 u istraživanje bila je istorija bolesti pacijenata sa trajanjem oboljenja od najmanje pet godina i kontrolisana glikemija određena kroz vrednosti glikoziliranog hemoglobina (HbA1C < 9) [16, 17]. Ispitanici koji su bili pušači ili su imali prisustvo drugih sistemskih bolesti zbog kojih su uzimali određene medikamente su isključeni iz studije. Uključeni su svi ispitanici koji su dali saglasnost za učešće u ovoj studiji. Studiju je odobrio Etički odbor Stomatološkog fakulteta, Univerziteta u Beogradu (br. 36-32, 12. april 2010).

Uzimanje uzoraka

Svaki ispitanik je podvrgnut parodontološkom pregledu i zbog ireverzibilne mobilnosti i loše prognoze preostalih zuba plan lečenja zahtevao je ekstrakciju zuba sa izradom totalnih proteza u gornjoj i donjoj vilici. Tokom postupka ekstrakcije uzeti su uzorci tkiva veličine približno 1 mm pored izvađenih zuba. Za svakog pacijenta uzeta su dva uzorka: uzorak koji je prethodno bio pokriven parcijalnom protezom (kompresivni uzorak) i uzorak koji nije bio pokriven parcijalnom protezom (akompresivni uzorak). Uzorci su zamrznuti u tečnom azotu i čuvani na temperaturi -70° C do analize.

Eksperimentalno indukovan DM

Ispitivanje je izvedeno na 40 mužjaka pacova soja Vistar, telesne mase oko 250 g. Pacovi su podeljeni u dve grupe od po 20 u svakoj. Kontrolnu grupu su činili zdravi pacovi. U drugoj grupi dijabetes je indukovan upotrebom jedne intraperitonealne injekcije monohidrata toksina β-ćelija pankreasa (alloxan, 140 mg/kg; Sigma, St. Louis, MO, SAD). Izmeren je nivo glukoze u serumu (Accu-Chek; Roche Diagnostic, Indianapolis, IN, SAD) i DM je dijagnostikovan kada je nivo glukoze u krvi prelazio 10 mmol/L. Eksperimenti su započeti kada su životinje bile hiperglikemične najmanje tri nedelje. Za svaku životinju uzeta su dva uzorka tkiva: uzorak koji je prethodno bio pokriven eksperimentalnom palatinalnom pločom (kompresivni uzorak) i uzorak koji nije bio pokriven eksperimentalnom palatinalnom pločom (nekompresivni uzorak). Uzorci su zamrznuti u tečnom azotu i čuvani na temperaturi -70° C do analize.

Sva ispitivanja na životinjama odobrio je Etički odbor Stomatološkog fakulteta, Univerziteta u Beogradu (br. 36-32, 12. april 2010).

Priprema eksperimentalnih palatinalnih ploča i sakupljanje uzoraka

Za životinje u grupi sa eksperimentalnim palatinalnim pločama (kontrolna grupa / DM grupa), po proceduri Tsurioka i saradnika [12], uzet je precizni otisak gornje vilice adicionim silikonom (Zhermack). Parče voska debljine 0,6 mm je postavljeno na model preko okluzalnih površina molara. Preko voska je načinjena akrilatna ploča (Biokril, Galenika, Srbija) koja je bila ekstendirana duž palatuma od prvog do trećeg molara sa obe strane vilice. Nakon polimerizacije akrilata vosak je uklonjen. U daljem postupku, palatinalna ploča je bila postavljena na nepce pod kompresijom i fiksirana za molare sa obe strane korišćenjem istog akrilata (slike 1 i 2). Radi uzimanja biološkog materijala, životinje su bile žrtvovane anestetikom zoletil u letalnoj dozi tri dana posle postavljanja palatinalne ploče. Biološki materijal mekog tkiva pacova je uzet sa kompresivnih i akompresivnih mesta na kojima je ležala palatinalna ploča posle žrtvovanja životinja i odvajanja eksperimentalnih ploča od nepca, sa pripremom za kvantifikaciju VEGF.

Detekcija tkivnog VEGF

Za kvantitativno merenje VEGF u tkivu pacijenata i životinja (pg/mL) korišćen je komercijalni Enzyme-Linked Immunosorbent Assay kit (Human VEGFA ELISA Cell Culture Supernatant, Urine; RayBiotech and Rat VEGFA ELISA Kit for Cell Culture Supernatant, Urine; RayBiotech) u skladu sa uputstvima proizvođača [19]. Optičke gustine su izmerene na 450 nm čitačem mikroploča i minimalni nivo detekcije testa bio je 5,0 pg/mL.

Statistika

Rezultati su prikazani kao srednja vrednost ± standardna devijacija (SD). Poređenja između grupa su vršena korišćenjem testom ANOVA sa ponovljenim merenjima i testom Che-squared. Test ANOVA je korišćen za analizu grupnih razlika, a test Bonferroni za post-hoc poređenja. Da bi se odredila poređenja između odgovarajućih grupnih vremenskih tačaka, korišćen je Studentov t-test. Vrednosti $p < 0,05$ su se smatrale statistički značajnim. Svi podaci su analizirani pomoću Stat for Windovs 8, StatSoft, Inc, SAD, 1984–2007.

REZULTATI

U ovoj studiji starost i pol nisu uzeti u obzir, jer su posmatrane grupe učesnika pretežno bile muške osobe (69%), starosti između 45 i 65 godina.

U Tabeli broj 1 prikazane su koncentracije VEGF iz uzoraka prekrivenih protezom (akompresivni deo), kao i iz gingive koja je bila komprimovana protezom (kompresivni deo) kod zdravih ispitanika i ispitanika sa DM tipa 2. Koncentracije VEGF u kompresivnom delu gingive bile su značajno manje, kako u grupi zdravih ispitanika, tako i kod ispitanika sa DM tipa 2, u odnosu na koncentracije VEGF iz akompresivne gingive obe grupe. Naime, u grupi zdravih ispitanika koncentracija VEGF u kompresivnom delu gingive je bila za 28%, a kod ispitanika sa DM tipa 2 za 66% manja u odnosu na koncentracije ovog faktora rasta u akompresivnom delu gingive. Ispitanici sa DM tipa 2 imali su značajno manje koncetracije VEGF u kompresivnim uzorcima gingive u odnosu na zdrave ispitanike; suprotno tome uzorci gingive bez kompresije ne pokazaju razliku u koncetraciji VEGF između zdravih ispitanika i ispitanika sa DM tipa 2.

U Tabeli 2 prikazane su karakteristike kontrolnih pacova i pacova sa DM koje se odnose na nivo glukoze u krvi i telesnu težinu pre eksperimentalnih procedura, 21 dan posle indukcije DM i poslednjeg dana eksperimentalne procedure 24. dana (posle tri dana nošenja eksperimentalne palatinalne ploče). Rezultati nisu pokazali značajnu razliku u nivou glukoze u krvi i telesnoj težini pre eksperimentalnih procedura u posmatranim grupama. Nivo glukoze u krvi je povećan i značajno veći kod pacova sa DM u poređenju sa zdravim kontrolnim grupama 21. i 24. dana. Kada je postavljena eksperimentalna palatinalna ploča, 21. dana, rezultati su pokazali smanjenje telesne težine kod životinja sa DM za razliku od kontrolne grupe, što je rezultiralo značajnom razlikom u telesnoj težini u posmatranim grupama. Poslednjeg dana eksperimenta, posle tri dana nošenja eksperimentalne ploče, obe grupe životinja su pokazale smanjenje telesne mase. Pacovi sa eksperimentalno izazvanim DM imali su značajno manju telesnu masu u odnosu na zdrave pacove.

Tabela 3 prikazuje nivoe VEGF u palatinalnoj sluznici, nekompresivnih i kompresivnih uzoraka, kod kontrolne grupe i pacova sa DM. Koncentracije VEGF u tkivu su značajno povećane u uzorcima ispod eksperimentalne palatinalne ploče (kompresivni uzorci), u poređenju sa koncentracijama VEGF u tkivu koje nije pokriveno eksperimentalnom palatinalnom pločom (nekompresivni uzorci) u obe posmatrane grupe. Zdravi pacovi, kao i pacovi sa eksperimentalno izazvanim DM, nisu pokazali statističku razliku u nivoima VEGF tkiva u nekompresivnim uzorcima, za razliku od kompresivnih uzoraka gde su nivoi VEGF značajno niži kod životinja sa eksperimentalno izazvanim DM u poređenju sa zdravim pacovima.

DISKUSIJA

Akutno i hronično opterećenje tokom funkcionalnih i parafunkcionalnih navika izazvanih protezama ima potencijal da izazove deformaciju potpornog tkiva [20, 21, 22]. Ispitujući uticaj kontinuirane kompresije mobilne parcijalne proteze na protok krvi u potpornoj sluzokoži nosilaca proteza, Akazava i Sakurai [8] pokazali su ishemiju tkiva i smanjenje protoka krvi u sluznici posle oslobađanja kompresije. Tsouruka i sar. su pokazali da ispod eksperimentalne palatinalne ploče ćelije u tkivu sintetišu HSP70 i VEGF za održavanje homeostaze [12]. Međutim, autori ove studije ne daju podatake o uticaju akutnog i hroničnog mehaničkog stresa izazvanog nošenjem proteze na koncentracije VEGF u tkivu ispitanika sa DM. Da bi se razjasnili efekti DM na akutne promene potpornog tkiva, ova studija je imala za cilj da stimuliše efekte nošenja proteza na eksperimentalnom modelu životinja. Ljudski model je korišćen da se pokaže uticaj hroničnog mehaničkog stresa na potporno tkivo, na ćelijskom nivou kod nosilaca proteza sa/bez DM tipa 2. Prateći studiju Tsuruoka i sar. [12], nismo razmatrali efekte korišćenih materijala za akrilatnu eksperimentalnu ploču i parcijalnu protezu na potporna tkiva. Naime, u skladu sa Mori i sar. [21], u studiji koju su sproveli Inoue i sar. [23], histopatološki nalazi koji se odnose na toplopolimerizujući akrilat pokazali su minimalni citotoksični efekat 4-META/MMA-TBB, uprkos penetraciji monomera u tkivo tokom polimerizacije.

Rezultati našeg istraživanja su otkrili povećanje nivoa VEGF u tkivima posle tri dana nošenja eksperimentalne palatinalne ploče, u obe posmatrane grupe životinja sa DM i bez njega. Naš nalaz je u skladu sa studijom Tsuruoka i sar. koja je otkrila da je povećana ekspresija VEGF kako u endotelnim ćelijama, tako i u vezivnom tkivu, osteoblastima i periostu gornje vilice posle tri dana nošenja palatinalne ploče kod zdravih pacova [12]. Imajući u vidu studije koje su pokazale smanjenje protoka krvi ispod eksperimentalnih palatinalnih ploča, što je praćeno ishemijom [8, 9], povećani nivoi VEGF u tkivu pacova posle tri dana nošenja eksperimentalne palatinalne ploče mogu biti uzrokovani hipoksičnim stanjem, glavnim faktorom za stimulaciju VEGF. Suprotno našim rezultatima koji ne pokazuju statističku značajnost za koncentraciju VEGF tkiva u nekompresivnim uzorcima između DM i zdravih životinja (uzorci bez eksperimentalne ploče), kod pacova sa DM došlo je do značajno manjeg povećanja koncentracije VEGF tkiva ispod eksperimentalne palatinalne ploče u poređenju sa kontrolnim grupama, što sugeriše da se ekspresija VEGF menja u uslovima mehaničkog stresa kod DM.

Analizirajući model hroničnog stresa, naši rezultati su pokazali značajno niže nivoe VEGF u tkivu ispod parcijalne proteze (kompresivni uzorci) u poređenju sa nivoima VEGF u tkivu koje nije bilo pokriveno parcijalnom protezom (nekompresivni uzorci). Pomenuto smanjenje tkivnog VEGF može ukazivati na adaptaciju oralne sluzokože i ćelijski odgovor na mehanički stres u cilju održavanja oralne homeostaze. Dobijeni rezultat značajno većeg smanjenja tkivnog VEGF u kompresivnim uzorcima kod nosilaca parcijalne proteze sa DM tipa 2 u poređenju sa zdravim ispitanicima ukazuje na izmenjenu oralnu homeostazu kod DM. Međutim, naši rezultati nisu pokazali statističku značajnost za nivoe VEGF tkiva u nekompresivnim uzorcima između DM tipa 2 i zdravih nosilaca proteza. Objavljeni podaci o nivoima VEGF u tkivima kod pacijenata sa DM i parodontopatijom su kontradiktorni. Za razliku od Sakaliogllua i sar. [23], koji su prijavili značajno više nivoe VEGF u uzorcima gingive kod pacijenata sa DM i klinički utvrđenom parodontopatijom u poređenju sa kontrolnom grupom, Keles i sar. [24] nisu našli značajnu razliku u ekspresiji VEGF mRNA u gingivalnim tkivima pacijenata sa parodontopatijom sa DM tipa 2 i bez njega. Međutim, mnoga istraživanja su pokazala tkivne promene ispod baze proteze kod pacijenata sa dijabetesom i životinja sa eksperimentalno izazvanim DM [25–28] u poređenju sa zdravim kontrolnim grupama. Međutim, naši rezultati su prvi put prikazali promene u nivoima VEGF u tkivima kao ćelijski odgovor na nošenje proteze kod ispitanika sa DM.

ZAKLJUČAK

Akutni mehanički stres povećava koncentraciju VEGF u palatinalnoj sluzokoži, dok hronični mehanički stres, obrnuto, smanjuje nivo VEGF u tkivu. Niže koncetracije VEGF su povezane sa akutnom i hroničnom kompresijom kod DM, što ukazuje na izmenjenu homeostazu. Naše istraživanje naglašava značaj edukacije stomatologa o održavanju proteza kod pacijenata sa DM tipa 2, jer doprinosi boljem razumevanju ćelijskog odgovora i bioloških procesa u oralnoj sluzokoži pod mehaničkim stresom.

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Evaluation of sorption and solubility of materials based on calcium aluminate

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SUMMARY

Introduction In addition to good biological properties, biomaterials should also possess appropriate physical properties in order to provide stability and longevity at the place of application. The aim of this work was to evaluate physical properties of an experimental nanostructured material based on calcium aluminate (CAL) and calcium silicate (CS).

Material and method The research used nanostructured calcium aluminate synthesized by the hydrothermal sol-gel method from the individual components of calcium aluminate ($CaO{\cdot}Al_2O_3$), calcite ($CaCO_3$) and barium sulfate ($BaSO_4$) as an X-ray contrast agent and calcium silicate (CS). The prepared material was placed in plastic molds with a diameter of 5 ± 0.1 mm and a height of 2 ± 0.1 mm. After setting time, the materials were left in an incubator at 37°C for 24 hours, and then they were removed from the mold and absorption and solubility of the materials was calculated. MTA (Angelus Londrina, Brazil) was used as a control material.

Results The lowest material solubility was recorded with MTA (0.255 mg/mm^3), followed by calcium silicate (0.267 mg/mm^3), and the highest with calcium aluminate (0.725 mg/mm^3). The difference was statistically significant between calcium aluminate and MTA ($p = 0.001901$) and between calcium aluminate and calcium silicate ($p = 0.002550$). After 28 days in deionized water, the lowest water sorption was recorded with MTA (0.347 mg/mm^3), followed by calcium silicate (0.357 mg/mm^3), and the highest water sorption was measured with calcium aluminate (0.474 mg/mm^3). Statistically significant differences were observed between calcium aluminate and MTA ($p = 0.000283$) and between calcium aluminate and calcium silicate ($p = 0.001576$).

Conclusion Material solubility and water absorption of calcium aluminate-based nanostructured material was significantly higher compared to calcium silicate (CS) and MTA.

Keywords: solubility; water sorption; calcium aluminate; calcium silicate

INTRODUCTION

Mineral trioxide aggregate (MTA) is the material of choice in many indications. It was initially developed as a cement for retrograde root canal filling and for cases of root perforation, but later due to its good clinical properties, it was also used in other indications in endodontics (pulpotomy, direct pulp capping, apexification, for incomplete root growth and for root canal filling [1]. MTA is a biocompatible material and capable of stimulating osteogenesis [2]. It is produced as a powder consisting of fine trioxide particles (tricalcium oxide, silicate oxide and bismuth oxide) and other hydrophilic particles (tricalcium silicate and tricalcium aluminate) responsible for its physical and chemical properties. However, MTA also has some poor properties. Among them, the most significant is the long setting time (which is a consequence of the chemical composition) and difficult clinical manipulation, low resistance to compression, high solubility in a moist environment, the presence and release of arsenic, as well as a high incidence of discoloration of dental structures. A long setting time (more than 3 h) carries the risk of washout immediately after placement, considering that it is placed in cavities that can be contaminated with blood and other tissue fluids [3].

In order to overcome the mentioned disadvantages, new formulations of materials based on calcium silicate and calcium aluminate have been synthesized in recent years [4-5]. Cements based on calcium aluminate, due to the reduced bonding time and associated microstructure, provide great potential in the field of biomaterials. Using nanotechnology, it is possible to overcome certain disadvantages in order to preserve good biological characteristics. Nanotechnology can improve the properties of materials, reduce their mass, increase stability and improve their functionality. One of the main questions that arise in connection with nanomaterials is the safety of their application, their biocompatibility. In recent years at the Institute for Nuclear Research in Vinča

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according to the report of V. Jokanović, a new nanostructured material based on the calcium-aluminate system was synthesized. The material was obtained by hydrothermal sol-gel method and selfexpanding combustion reaction. This mode of synthesis provides high particle activity, faster hydration and short setting time [6-7].

In previous studies *in vivo* conditions, the material based on calcium aluminate has proven biocompatibility and bioinductive potential, the induction of dentine bridge formation and the manifestation of reparative activity [7–12].

In addition to good biological properties, biomaterials should also possess appropriate physical properties to provide stability, longevity at the place of application. In addition to biocompatibility, dimensional stability of endodontic materials is of crucial importance for the success of endodontic treatment. Calcium aluminate-based material binds much faster compared to MTA, has better handling properties and reduced porosity [13, 14].

The aim of this work was to examine physical properties of an experimental nanostructured material based on calcium aluminate (CAL) and calcium silicate (CS).

MATERIAL AND METHOD

The material based on calcium aluminate and calcium silicate was mixed with distilled water in a ratio of 3:1, and the control material (MTA) was prepared according to the manufacturer's instructions. All materials were placed in plastic molds with a diameter of 5 ± 0.1 mm and a height of 2 ± 0.1 mm. For each tested material, 8 samples were made. After setting, the materials were left in an incubator at 37°C for 24 h, then the material samples were taken out of the mold and weighed on a scale (Acculab, Sartorius group, Göttingen, Germany) with an accuracy of 0.0001 g. The obtained material values are marked as initial dry mass (m1). Then the volume (V) of the bonded material samples was calculated based on the volume of the mold in which they were placed. Each sample was separately placed in plastic containers with 5 ml of distilled and demineralized water. Material samples were stored in closed plastic containers and incubated at 37°C for 28 days. After 28 days, the samples were removed from the liquid and the measured mass of the material samples was denoted as (m2). Then the samples were dried with silica gel until a constant mass was established (24 h), which was designated as the final dry mass of the material (m3). For each sample of the tested materials, the values of water sorption and solubility of the material were calculated according to the following formulas:

Water sorption (mg/mm^3) = (m2 - m3) / V

Solubility (mg/mm^3) = (m1-m3) / V [14].

The volume was calculated according to the volume of the mold in which the tested materials were placed.

- m1 – initial mass of material samples before immersion in deionized and distilled water
- m2 – mass of material samples after 28 days in deionized and distilled water
- m3 – mass of material samples after drying

The processing program was used: SPSS 22.0. The obtained results were statistically processed using the t-test of the difference (Student T-test) between the arithmetic means of two small independent samples.

RESULTS

After 28 days in deionized water, the lowest water sorption was found for MTA (0.347 mg/mm^3), followed by calcium silicate (0.357 mg/mm^3), whereas the highest values was observed in calcium aluminate (0.474 mg/mm^3) (Figure 1).

Using the Student's T-test, statistically significant differences were observed between calcium aluminate and MTA (t = -4.913661; p = 0.000283) and between calcium aluminate and calcium silicate (t =-3.908202; p = 0.001576), difference between calcium silicate and MTA was not statistically significant.

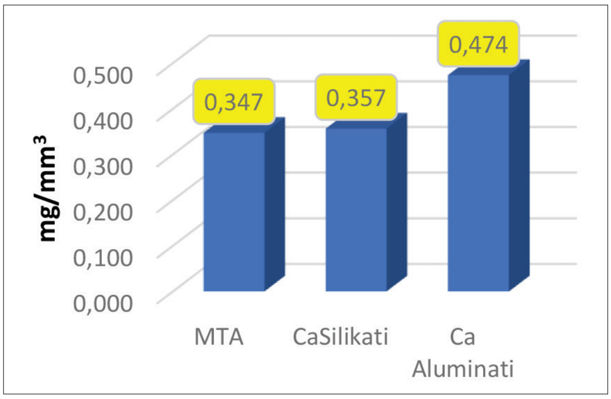

Figure 1. Average water sorption values of the tested materials after 28 days in distilled and deionized water (mg/mm^3)
Slika 1. Prosečne vrednosti sorpcije tečnosti testiranih materijala nakon 28 dana u destilovanoj i dejonizovanoj vodi (mg/mm^3)

The lowest material solubility was found for MTA (0.255 mg/mm^3), followed by calcium silicate (0.267 mg/mm^3). Calcium aluminate had the highest solubility (0.725 mg/mm^3) (Figure 2). Statistically significant difference was found between calcium aluminate and MTA (t= -4.539258; p = 0.001901), as well as between calcium aluminate and calcium silicate (t = -4.318967; p =0, 002550). The difference between MTA and calcium silicate was not statistically significant.

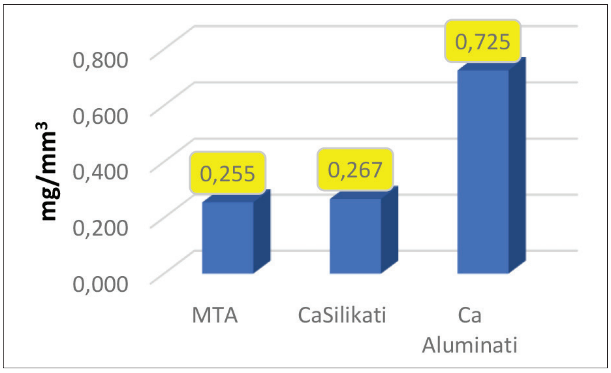

Figure 2. Average solubility values of the tested materials after 28 days in distilled and deionized water (mg/mm^3)
Slika 2. Prosečne vrednosti rastvorljivosti testiranih materijala posle 28 dana u destilovanoj i dejonizovanoj vodi (mg/mm^3)

DISCUSSION

The conditions in which the solubility and porosity of materials (*in vitro*) are evaluated differ in terms of the size of the samples, the amount and type of liquid in which the samples are immersed, the length of the experiment and the type of liquid used [15-16]. In this research, samples of the tested materials were immersed in a liquid (distilled and deionized water) and kept for 28 days, which is in accordance with the methodology of Gandolfi et al, which evaluated the solubility and porosity of the material over a longer period [17, 18].

The water sorption results obtained in this study after 28 days in liquid showed the lowest water sorption for the MTA material. In the case of calcium silicate material, the measured values were slightly higher, while the highest water sorption was observed in calcium aluminate. This result could be explained by the size of the particles in the composition of the material. The nanoparticles in the calcium aluminate formulation are smaller, therefore they are exposed to a larger surface area, which can lead to a more pronounced contact between the particles of the material and the surface on which they react. A larger reactive surface may lead to a consequent higher liquid absorption, that is, a more pronounced contact of the liquid with this material, which results in a higher porosity of the material [19].

The obtained results indicate the hydrophilic nature of all tested cements, considering the fact that high sorption (porosity) of the material was recorded. Porosity is a characteristic of all dental cements that are prepared by mixing powder and liquid and is a consequence of the incorporation of air bubbles during mixing. With calcium aluminate cements, higher values of water absorption were recorded compared to MTA and calcium silicate, which indicates the fact that the material has a highly porous structure.

Solubility and porosity of materials are properties of materials that can directly affect their stability, integrity and durability [20]. It is known that solubility and porosity depends on the ratio of liquid and cement during the preparation [21, 22], on the type of X-ray contrast agent in the composition of the material [23], as well as on the pH value of the environment [24].

Friedland and Rosado claim that all dental cements prepared with water have some degree of porosity due to the incorporation of microscopic air bubbles during the cement mixing process. The same authors claim that the presence of amorphous pores and capillary structure in the composition of MTA is another important cause of the porosity of this material [21].

Luz et al. claim that the porosity of calcium aluminate cement decreases over time, which may be related to the continuity of formation and the deposition of hydrate phases in the newly created pores [25, 26].

Ivone Regina de Oliveira et al. claim that the key to the physical properties is the X-ray contrast medium. The mentioned authors suggest the addition of 15%ZnO:10%Bi_2O_3 in the composition of calcium aluminate as the most suitable agent that can achieve the best compromise between good physical and mechanical properties and X-ray contrast. They indicate that the addition of 15%ZnO:10%Bi_2O_3 results in a decrease in porosity [27, 28, 29]. The same authors claim that Bi_2O_3 increases the porosity and reduces the mechanical strength of calcium aluminate cement whose particles are of different sizes and elongated shapes.

Garcia et al. evaluated solubility and porosity of materials based on calcium aluminate EB (EndoBinder) with three different radiopacifiers agents: bismuth oxide ($B_{i2}O_3$), zinc oxide (ZnO) and zirconium oxide (ZrO_2). EndoBinder showed similar behavior to MTA, regardless of X-ray contrast. The authors claim that the long time of MTA binding leads to its instability, to greater solubility and disintegration, and as a result there is a greater release of components present in the cement itself. The solubility of MTA was 5.74% for gray and 6.65% for white, and these values are above the limit values of (3%) proposed by the specifications [13].

Parreira et al. in their study (2016) claim that the addition of ZnO, as well as hydroxyapatite in calcium aluminate cement formulations, results in a reduction in the porosity level of the tested samples and a reduction in pores after contact with simulated body fluids. ZnO is a biomaterial capable of inducing mineralization processes [30].

However, some authors think that the high solubility of the material can be a benefit, from a biological and physico-chemical point of view, because more calcium ions are released into the surrounding tissue, just as a high pH can cause a greater antibacterial effect [30]. Therefore, applied *in vivo* conditions, these cements become a source of calcium and hydroxyl ions with consequent bioactivity and antimicrobial effect.

Studies have shown that calcium ions are the main component detected in solubility, showing that cement solubility is an important phenomenon in the release of calcium and hydroxyl ions into the periodontal tissue, which can affect reparative processes [31].

However, it should be mentioned that in clinical conditions only a small part of the material comes into contact with tissue fluids, compared to laboratory tests where the entire material sample is immersed in liquid, therefore the osmotic effect is more pronounced.

It must be considered that the measurement of differences in the weight of cement samples may also record decay processes that may not be the result of dissolution. For example, material particles may fall out of the cement structure during storage of the material in liquid over time [32].

CONCLUSION

The solubility and water absorption of the experimental nanostructured material based on calcium aluminate was significantly higher compared to calcium silicates and MTA.

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Ispitivanje sorpcije i rastvorljivosti materijala na bazi kalcijum-aluminata

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KRATAK SADRŽAJ

Uvod Pored dobrih bioloških osobina, biomaterijali treba da poseduju i odgovarajuća fizička svojstva kako bi obezbedili stabilnost, odnosno dugotrajnost na mestu aplikacije.

Cilj ovog rada je bio da se ispitaju fizička svojstva eksperimentalnog nanostrukturnog materijala na bazi kalcijum-aluminata i kalcijum-silikata.

Materijal i metod rada U istraživanju je korišćen nanostrukturni kalcijum-aluminat sintetisan hidrotermalnom sol-gel metodom od pojedinačnih komponenata kalcijum-aluminata ($CaO{\cdot}Al_2O_3$), kalcita ($CaCO_3$) i barijum-sulfata ($BaSO_4$) kao rendgenskog kontrastnog sredstva i kalcijum-silikata. Zamešani materijal je postavljen u plastične kalupe prečnika 5 ± 0,1 mm i visine 2 ± 0,1 mm. Nakon vezivanja materijali su ostavljeni u inkubatoru na 37°C tokom 24 h, a potom su izvađeni iz kalupa i proverene su apsorpcija i rastvorljivost materijala. Kao kontrolni materijal korišćen je MTA (Angelus Londrina, Brazil).

Rezultati Najmanja rastvorljivost materijala zabeležena je kod MTA (0,255 mg/mm^3), zatim kod kalcijum-silikata (0,267 mg/mm^3), a najveća kod kalcijum-aluminata (0,725 mg/mm^3). Razlika je bila statistički značajna između kalcijum-aluminata i MTA ($p = 0,001901$) i između kalcijum-aluminata i kalcijum-silikata ($p = 0,002550$). Nakon 28 dana u dejonizujućoj vodi, najmanja sorpcija tečnosti zabeležena je kod MTA (0,347 mg/mm^3), potom kod kalcijum-silikata (0,357 mg/mm^3), a najveća sorpcija tečnosti izmerena je kod kalcijum-aluminata (0,474 mg/mm^3). Statistički značajne razlike uočene su između kalcijum-aluminata i MTA ($p = 0,000283$) i između kalcijum-aluminata i kalcijum-silikata ($p = 0,001576$).

Zaključak Rastvorljivost materijala i apsorpcija tečnosti kod nanostrukturnog materijala na bazi kalcijum-aluminata je bila značajno veća u poređenju sa kalcijum-silikatom i MTA.

Ključne reči: rastvorljivost; apsorpcija; kalcijum-aluminat; kalcijum-silikat

UVOD

Mineralni trioksidni agregat (MTA) danas je materijal izbora u brojim indikacijama. U početku je razvijen kao cement za retrogradno punjenje kanala i za slučajeve kod perforacije korena zuba, ali je kasnije zbog svojih dobrih kliničkih osobina primenjivan i u drugim indikacijama u endodonciji (pulpotomija, direktno prekrivanje pulpe, apeksifikacije, kod nezavršenog rasta korena i za punjenje kanala korena zuba) [1]. MTA je biokompatibilan materijal i sposoban da stimuliše osteogenezu [2]. Proizvodi se kao prah koji se sastoji od finih čestica trioksida (trikalcijev oksid, silikatni oksid i bizmutov oksid), te ostalih hidrofilnih čestica (trikalcijev silikat i trikalcijev aluminat) odgovornih za njegova fizička i hemijska svojstva. Međutim, MTA poseduje i neke loše osobine. Među njima su najznačajnije dugo vreme stvrdnjavanja (koje je posledica hemijskog sastava) i otežana klinička manipulacija, odnosno niska otpornost na kompresiju, visoka rastvorljivost u vlažnoj sredini, prisustvo i oslobađanje arsena, kao i visoka incidenca prebojavanja dentalnih struktura. Dugo vreme vezivanja (više od 3 h) nosi rizik od ispiranja materijala sa mesta aplikacije s obzirom na to da se postavlja u kavitete koji mogu biti kontaminirani krvlju i drugim tkivnim fluidima [3].

U cilju prevazilaženja navedenih problema, poslednjih godina sintetisane su brojne nove formulacije materijala na bazi kalcijum-silikata (KS) i kalcijum-aluminata (KAL) [4]. Cementi na bazi KAL zbog smanjenog vremena vezivanja i povezane mikrostrukture obezbeđuju veliki potencijal na polju biomaterijala. Upotrebom nanotehnologije moguće je prevazići pomenute nedostatke prilikom sintetisanja materijala u cilju očuvanja dobrih bioloških karakteristika. Nanotehnologijom se mogu poboljšati osobine materijala, smanjiti njihova masa, povećati stabilnost i unaprediti njihova funkcionalnost. Jedno od glavnih pitanja koje se postavlja u vezi sa nanomaterijalima je bezbednost njihove primene, tj. njihova biokompatibilnost. Poslednjih godina na Institutu za nuklearna istraživanja u Vinči, prema izveštaju V. Jokanovića, sintetisan je novi nanostrukturni materijal na bazi KAL sistema dobijen hidrotermalnom sol-gel metodom i samoširećom reakcijom sagorevanja. Ovakav način sinteze obezbeđuje visoku aktivnost čestica, bržu hidrataciju i kratko vreme vezivanja [6].

U dosadašnjim ispitivanjima u *in vivo* uslovima materijal na bazi KAL je pokazao biokompatibilnost i bioinduktivni potencijal, tj. indukciju stvaranja dentinskog mosta i ispoljavanje reparatorne aktivnosti [8–12].

Pored dobrih bioloških osobina, biomaterijali treba da poseduju i odgovarajuća fizička svojstva kako bi obezbedili stabilnost, odnosno dugotrajnost na mestu aplikacije. Pored biokompatibilnosti, dimenzionalna stabilnost endodontskih materijala od krucijalnog je značaja za uspeh endodontskog tretmana. Materijal na bazi KAL vezuje se dosta brže u poređenju sa MTA, poseduje bolja svojstva rukovanja i redukovanu poroznost [13, 14].

Cilj ovog rada je da se ispitaju fizička svojstva eksperimentalnog nanostrukturnog materijala na bazi KAL i KS.

MATERIJAL I METOD RADA

Materijal na bazi KAL i KS su zamešani sa destilovanom vodom u odnosu 3 : 1, a kontrolni materijal (MTA) zamešan je prema uputstvu proizvođača. Svi materijali su postavljeni u plastične kalupe prečnika 5 ± 0,1 mm i visine 2 ± 0,1 mm. Za svaki testirani materijal napravljeno je po osam uzoraka. Nakon vezivanja materijali su ostavljeni u inkubatoru na 37°C tokom 24 h, potom su uzorci materijala izvađeni iz kalupa i izmereni na vagici (Acculab, Sartorius group, Getingen, Germany) sa preciznošću od 0,0001 g. Dobijene vrednosti materijala označene su kao inicijalna suva masa (m1). Zatim je izračunata zapremina (V) uzoraka vezanih materijala na osnovu zapremine kalupa (valjka) u kome su postavljeni. Svaki uzorak posebno je postavljen u plastične kontejnere sa 5 ml destilovane i demineralizovne vode. Uzorci materijala su čuvani u zatvorenim plastičnim kontejnerima i inkubirani na 37°C tokom 28 dana. Posle 28 dana uzorci su izvađeni iz tečnosti i izmerena masa uzoraka materijala označena je kao m2. Zatim su uzorci isušeni gelom silika do uspostavljanja konstantne mase (24 h), koja je označena kao finalna suva masa materijala (m3). Potom su za svaki uzorak testiranih materijala izračunate vrednosti sorpcije tečnosti i rastvorljivosti materijala prema sledećim formulama:

Sorpcija tečnosti (mg/mm^3) = (m2 - m3) / V

Rastvorljivost (mg/mm^3) = (m1 - m3) / V [14].

Volumen je izračunat prema zapremini kalupa u kome su postavljeni testirani materijali.

m1 – inicijalna masa uzoraka materijala pre potapanja u dejonizujuću i destilovanu vodu

m2 – masa uzoraka materijala posle 28 dana u dejonizujućoj i destilovanoj vodi

m3 – masa uzoraka materijala nakon isušivanja

Korišćen je program za obradu SPSS 22.0 Dobijeni rezultati su statistički obrađeni primenom t-testa razlike (Studentov t-test) između aritmetičkih sredina dva mala nezavisna uzorka.

REZULTATI

Posle 28 dana u dejonizujućoj vodi, najmanja sorpcija tečnosti zabeležena je kod MTA (0,347 mg/mm^3), potom kod KS (0,357 mg/mm^3), dok je najveća sorpcija tečnosti izmerena kod KAL (0,474 mg/mm^3) (Grafikon 1).

Primenom Studentovog t-testa uočene su statistički značajne razlike između KAL i MTA ($t = -4{,}913661$; $p = 0{,}000283$) i između KAL i KS ($t = -3{,}908202$; $p = 0{,}001576$), dok razlika između KS i MTA nije bila statistički značajna.

Najmanja rastvorljivost materijala zabeležena je kod MTA (0,255 mg/mm^3), zatim kod KS (0,267 mg/mm^3). Kod KAL je uočena najveća rastvorljivost (0,725 mg/mm^3) (Grafikon 2). Utvrđena je statistički značajna razlika između KAL i MTA ($t = -4{,}539258$; $p = 0{,}001901$), kao i između KAL i KS ($t = -4{,}318967$; $p = 0{,}002550$). Razlika između MTA i KS nije bila statistički značajna.

DISKUSIJA

Uslovi u kojima se procenjuje rastvorljivost i poroznost materijala (*in vitro*) razlikuju se u pogledu veličine uzoraka, količine i vrste tečnosti u koju se uzorci potapaju, odnosno od dužine trajanja eksperimenta i vrste tečnosti koja se koristi [16]. U ovom istraživanju uzorci testiranih materijala potopljeni su u tečnost (destilovanu i dejonizujuću vodu) i čuvani su tokom 28 dana, što je u skladu sa metodologijom Gandolfija i sar., čime su praćene rastvorljivost i poroznost materijala u dužem vremenskom intervalu [17, 18].

Rezultati sorpcije tečnosti dobijeni u ovoj studiji nakon 28 dana u tečnosti pokazali su najmanju sorpciju tečnosti kod materijala MTA. Kod materijala KS izmerene vrednosti bile su nešto veće, dok je najveća sorpcija tečnosti uočena kod KAL. Ovakav rezultat bi se mogao objasniti veličinom čestica u sastavu materijala. Nanočestice u formulaciji KAL su manje, samim tim su izložene većoj površini, što može da dovede do izraženijeg kontakta između čestica materijala i površine na koju deluju. Veća reaktivna površina može da dovode do posledično većeg upijanja tečnosti, odnosno izraženijeg kontakta tečnosti sa ovim materijalom, što rezultira većom poroznošću materijala [19].

Dobijeni rezultati ukazuju na hidrofilnu osobinu svih testiranih cemenata, s obzirom na činjenicu da je zabeležena visoka sorpcija, odnosno poroznost materijala. Poroznost je osobina svih dentalnih cemenata koji se pripremaju mešanjem praha i tečnosti i posledica je inkorporiranja mehurića vazduha prilikom mešanja. Kod KAL cementa zabežene su veće vrednosti apsorpcije tečnosti, odnosno poroznosti u odnosu na MTA i KS, što ukazuje na činjenicu da materijal poseduje visoko poroznu strukturu.

Rastvorljivost i poroznost materijala su osobine materijala koje direktno mogu uticati na njihovu stabilnost, integritet i trajnost [20]. Poznato je da rastvorljivost i poroznost zavise od količine tečnosti koja se koristi prilikom pripreme cementa [21, 22], od vrste rendgenskog kontrasnog sredstva u kompoziciji materijala [23], kao i od vrednosti pH sredine [24].

Fridland i Rosado tvrde da svi dentalni cementi pripremljeni sa vodom imaju određen stepen poroznosti zbog inkorporacije mikroskopskih mehurića vazduha za vreme postupka mešanja cemenata. Isti autori tvrde da je prisutnost amorfnih pora i kapilarne strukture u kompoziciji MTA još jedan važan uzrok poroznosti ovog materijala [21].

Luz i saradnici tvrde da se poroznost KAL cementa smanjuje tokom vremena, što može da bude povezanao sa kontinuitetom formiranja i taloženjem hidratnih faza u novostvorenim porama [25, 26].

Ivone Regina de Oliveira i sardnici tvrde da je ključ fizičkih osobina rendgensko kontrastno sredstvo. Navedeni autori predlažu dodatak 15% ZnO : 10% Bi_2O_3 u kompoziciji KAL kao najpogodnije sredstvo koje može postići najbolji kompromis između dobrih fizičkih i mehaničkih svojstava i rendgenske kontrastnosti. Oni ukazuju da dodatkom 15% ZnO : 10% Bi_2O_3 dolazi do smanjenja poroznosti [27, 28, 29].

Isti autori tvrde da Bi_2O_3 povećava poroznost i smanjuje mehaničku čvrstoću KAL cementa čije su čestice različite veličine i izduženog oblika.

Garcia i saradnici su u svojoj studiji proveravali rastvorljivosti i poroznosti materijala na bazi KAL EB (EndoBinder) sa tri različita radiokontrastna sredstva: bizmut-oksid (Bi_2O_3), cink-oksid (ZnO) i cirkonijum-oksid (ZrO_2). EndoBinder je pokazao slično ponašanje kao MTA, bez obzira na rendgensku kontrastnost. Autori tvrde da dugo vreme vezivanja MTA-a dovodi do njegove nestabilnosti, odnosno do veće rastvorljivosti i dezintegracije, a posledično nastaje veće otpuštanje komponenata prisutnih u samom cementu. Rastvorljivost MTA iznosila je 5,74% za sivi, odnosno 6,65% za beli, i ove vrednosti su iznad graničnih vrednosti od (3%) predloženih specifikacijama [13].

Parreira i saradnici 2016. godine u svojoj studiji tvrde da dodatkom ZnO, kao i hidroksiapatita u formulacijama KAL cemenata dolazi do smanjenja nivoa poroznosti testiranih uzoraka i smanjenja pora posle kontakta sa simuliranim telesnim tečnostima. ZnO je biomaterijal sposoban da izazove mineralizacione procese [30].

Međutim, neki autori su mišljenja da velika rastvorljivost materijala može da bude i prednost, gledajući sa biološke i fizičko-hemijske tačke gledišta, jer dolazi do otpuštanja više kalcijumovih jona u okolno tkivo, isto kao što visok pH može da izazove veći antibakterijski efekat [30]. Samim tim, primenjeni u *in vivo* uslovima ovi cementi postaju izvor kalcijumovih i hidroksilnih jona sa posledičnom bioaktivnošću i antimikrobnim dejstvom.

Studije su pokazale da su joni kalcijuma glavna komponenta detektovana kod rastvorljivosti, pokazujući da je rastvorljivost cementa važan fenomen u otpuštanju kalcijumovih i hidroksilnih jona u periodontalno tkivo, što može da utiče na reparacijske procese [31].

Međutim, treba napomenuti da u kliničkim uslovima samo manji deo materijala dolazi u kontakt sa tkivnim fluidima, za razliku od laboratorijskih testova gde se čitav uzorak materijala potapa u tečnost, samim tim i osmotski efekat je izraženiji.

Štaviše, mora se uzeti u obzir da merenje razlika u težini uzoraka cementa takođe može zabeležiti procese raspadanja koji možda nisu rezultat rastvaranja. Na primer, čestice materijala mogu ispasti iz cementne strukture tokom skladištenja materijala u tečnosti tokom vremena [32].

ZAKLJUČAK

Rastvorljivost i apsorpcija tečnosti eksperimentalnog nanostrukturnog materijala na bazi KAL bile su značajno veće u poređenju su KS i MTA.

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Application of atomic force microscopy in investigations of dental alloy surfaces

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SUMMARY

Introduction The aim of this informative paper is to show the importance and key role of atomic force microscopy, on one hand, in quantitative determination of the physicochemical changes on the surfaces of dental materials due to their exposure to acids and, on the other hand, in quantification of changes in the physicochemical properties of the surfaces of dental alloys after their processing and the consequences of the processing itself on the quality and applicability of the same.

Methods Atomic force microscopy was used to obtain data quantitatively describing nanoscale changes in Co-Cr dental alloys caused by exposure to formic acid over a 7-day period and to describe the effects of electropolishing and black brush polishing on the surface roughness of dental alloys.

Results Analysis of the topography and roughness of the surfaces of alloys treated with a black brush and electropolishing shows that both methods are applicable in dentistry and leave defects of insufficient size for microorganisms. Treatment of the studied alloys with formic acid leads to an increase in surface roughness, indicating the presence of corrosion processes, especially in the areas of interdentritic nanocrystals.

Conclusion The aforementioned effects of processing and treatment of Co-Cr alloys can be followed in great detail using the technique of atomic force microscopy, especially by analysing the surface topography and its roughness. The method is of extraordinary importance for the evaluation of the use of Co-Cr alloys in dentistry.

Keywords: atomic force microscopy; surface roughness; Co-Cr dental alloys; formic acid; electropolishing

INTRODUCTION

Co-Cr alloys are materials that have been used for long time as dental materials due to their exceptional mechanical properties, especially their strength [1]. Without going into the description of the structure of the alloys themselves, one of their essential properties is their surface roughness [2]. In order to be used as dental materials, Co-Cr alloys require processing, which includes their polishing [3, 4]. In addition, the surface of dental alloys is extremely important for their application, because the surface roughness depends on the interaction of the surface with oral fluid, formation of dental plaque, and interaction with microorganisms and fluids in the oral cavity. As a result of interaction with oral fluids, chemical reactions and corrosion occur, which can alter the physical and chemical properties of dental alloy surfaces and thus limit their use.

Atomic force microscopy is one of the techniques that can be used to determine topography of surfaces at the nanoscale. Therefore, it is of crucial importance in assessing the quality of dental materials, especially their surfaces [5]. Without going into the description of the basic working principle of the technique itself, an important parameter determined by this method, in addition to topography, is the surface roughness parameter, which indicates the size of surface defects suitable for microorganism uptake [6].

In this informative paper, the approach to the problems of determining the topography and roughness of the previously studied surfaces of Co-Cr alloys and their changes that occurred after the processing of the alloys themselves by electropolishing and polishing with a black brush is described, focusing on the use of atomic force microscopy as a key technique for obtaining arguments important for the application of the alloy in dentistry [4]. In addition, a detailed analysis of the topography of Co-Cr alloy surfaces before and after corrosion induced by formic acid treatment over a 7-day period is presented and explained.

THE EFFECT OF POLISHING ON THE TOPOGRAPHY AND SURFACE ROUGHNESS OF CO-CR ALLOYS

The preparation and processing of the Co-Cr alloys themselves have been described in previous studies [3, 4]. Briefly, after casting, the alloy samples were cleaned in a vacuum casting machine at 1420°C, sandblasted, and then polished with a black brush and electropolishing. After that, they were additionally polished with a rotating brush for 15 minutes. The Co-Cr alloys were taped to a metal disk and imaged in contact mode (Nanoscope III with control program, Bruker Instruments, Santa Barbara, USA). Imaging was performed with a Bruker NP probe whose nominal probe constant is k_{nom} = 0.32 N/m. The location of interest for surface imaging is selected with an optical camera. The topographies of dental alloy surfaces

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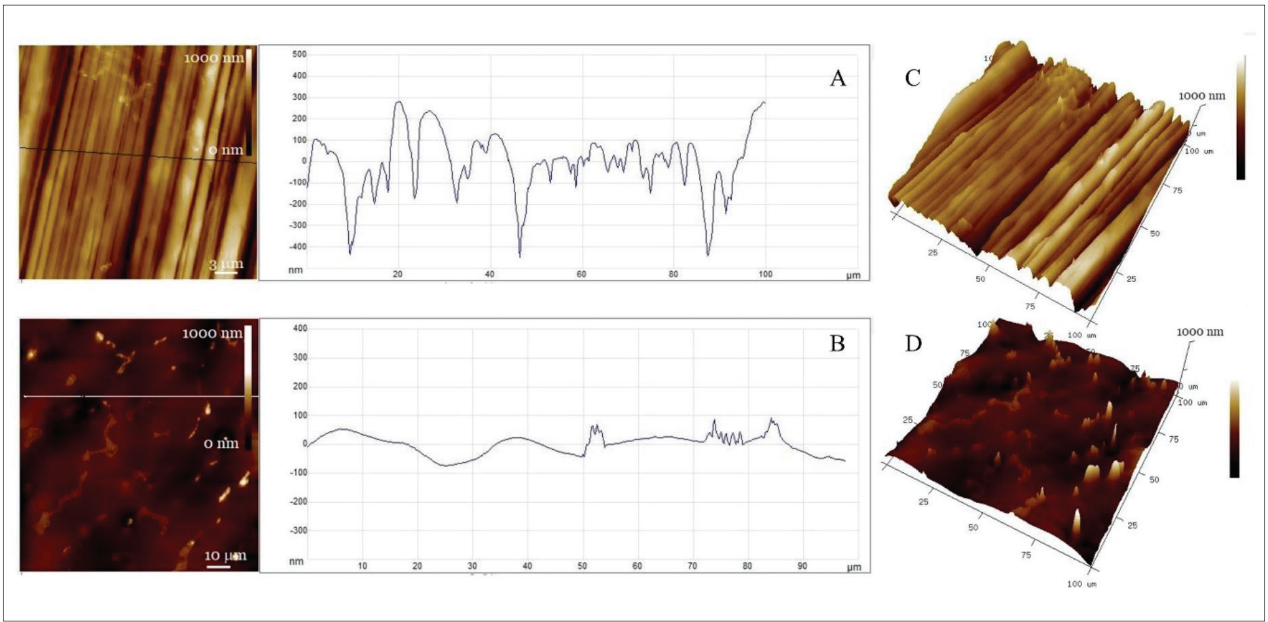

Figure 1. 2D topographic view of the heights and cross-sectional profile along the marked line of the 30 × 30 μm² Co-Cr alloy surface treated with a black brush (A) and electropolishing (B). 3D view of the heights of the Co-Cr alloy treated with a black brush (C) and electropolishing (D). The vertical scale is 1000 nm.
Slika 1. 2D topografski prikaz visine i profila poprečnog preseka duž označene linije legure Co-Cr površine 30 × 30 μm² obrađene rotirajućom četkom (A) i elektropoliranjem (B). 3D prikaz visina legure Co-Cr obrađene crnom četkom (C) i elektropoliranjem (D). Vertikalna skala je 1000 nm.

polished with black brush and electropolishing, their cross-sectional profiles, and 3D topographic views of heights are shown in Figure 1.

As can be seen in Figures 1C and 1D, the three-dimensional topographic images of the heights corresponding to the two different polishing methods are completely different. After polishing, the fine dendritic wave structure and the resulting interdendritic nanocrystals on the surface of the Co-Cr alloy are damaged by newly formed dominant scratches distributed at different spacing and scratch depths, reaching values of up to 400 nm. In contrast to the surface treated with a black brush, the surface of Co-Cr alloy that has been electropolished is dominated by dendrites with relatively smoothed wavy surfaces and interdentritic areas with clusters of nanocrystals emerging from the smoothed surface with an average height of 100-200 nm. For the application of polished Co-Cr alloys, it is important that the surface in direct contact with liquids is as smooth and flat as possible, with the lowest values of roughness parameters. For this reason, an analysis of the cross-section profile of the two polished Co-Cr alloys was performed, as shown in Figures 1A and 1B. This type of analysis must be performed on multiple surfaces to determine the mean and standard deviation. To gain insight into the smoothness of alloy surfaces with a higher degree than local along the section line, roughness analysis was performed on larger alloy surfaces (30×30 μm² and 100×100 μm²) in addition to the cross-section profile, and the values of the roughness parameters of one analysis are listed in Table 1. It is common to consider R_a (roughness parameter), R_q (root mean square of roughness) and Z range (the largest vertical distance between the highest and lowest measured position on the surface).

Table 1. Roughness parameters for Co-Cr dental alloys treated with a black brush and by electropolishing
Tabela 1. Vrednosti parametara hrapavosti za dentalne legure Co-Cr pre i posle tretmana mravljom kiselinom

Polishing treatment Tretman poliranjem	Analyzed surface area / μm² Analizirana površina / μm²	^aR_a/nm	^bR_q/nm	^{c}Z range/nm
Black brush Crna	30 × 30	84	112	684
	100 ×100	115	154	1236
Electropolishing Elektropoliranje	30 × 30	22	33	358
	100 × 100	44	60	877

^aR_a – average roughness; ^bR_q – the root mean square of the roughness; ^{c}Z range – the greatest vertical distance between the highest and the lowest measured position on the surface
^aR_a – srednja hrapavost; ^bR_q – koren srednjeg kvadrata hrapavosti; ^{c}Z raspon – najveća vertikalna udaljenost između najviše i najniže izmerene pozicije na površini

It should be noted that roughness values increase with increasing size of the surface under investigation. This is especially important when analyzing inhomogeneous surfaces. Therefore, it is always better to analyze as large a surface area of the alloys as possible to get a realistic view of the topography and roughness of the alloys. Since the values of the structures emerging from the wavy surface and the depth of the scratches are smaller than the dimensions of microorganisms (1–4 μm) in the oral cavity, the aforementioned polishing effects are not representing an obstacle to the use of both treated alloys in dentistry.

Finally, the procedure for analyzing surfaces of Co-Cr-treated alloys is presented, which is necessary to verify whether the surface properties of the alloy are satisfactory.

In conclusion, the method and technique necessary and sufficient to evaluate the validity of processing alloys by various methods for their safe use in dentistry is described here.

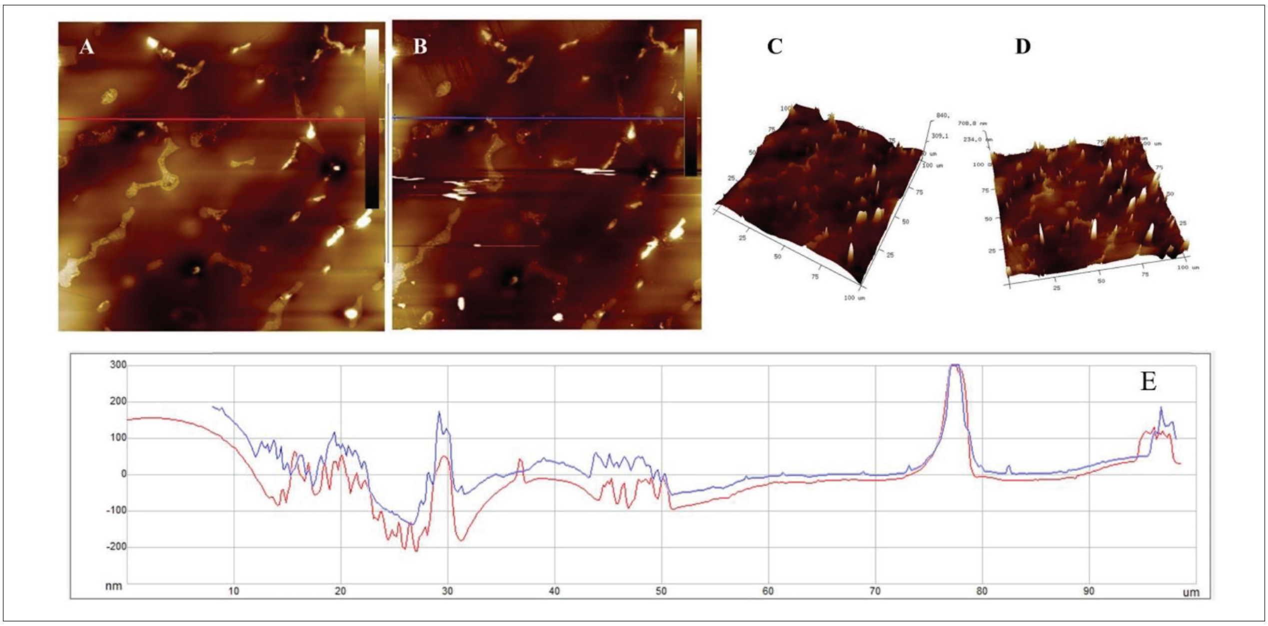

Figure 2. 2D and 3D topographic representation of the heights of Co-Cr alloy with a surface area of 100 × 100 μm² before (A and C) and after 7-day treatment with formic acid (B and D), and cross-sectional profile along the marked lines (E) before (red) and after (blue).
Slika 2. 2D i 3D topografski prikaz visina legure Co-Cr površine 100 × 100 μm² pre (A i C) i posle sedmodnevnog tretmana mravljom kiselinom (B i D), kao i profil poprečnog preseka duž označenih linija (E) pre (crveno) i posle (plavo). Vertikalna skala je 1000 nm.

EFFECT OF CORROSION ON TOPOGRAPHY AND SURFACE ROUGHNESS OF CO-CR ALLOYS

Having described the effect of polishing on topography and surface roughness, a very important information that atomic force microscopy can and does provide is the change in the surface due to corrosion caused by treatment with formic acid. It is critical that identical areas are imaged before and after acid treatment. Indeed, whenever alloy surfaces are treated, they must be removed from the unit, the treatment itself performed, and the alloys returned for imaging. Therefore, it is necessary to develop a method that ensures the analysis of completely identical locations on the alloy surfaces, which was the case in this study.

Briefly, the alloy samples were imaged at four different locations before treatment, and the size of the imaged area varied (100×100 μm²; 30×30 μm²; and 10×10 μm²). Figure 2 (A and B) and Figure 2 (C and D) show an example of 2-D and 3-D topographic representation of the surface heights of Co-Cr alloys before and after 7-day treatment with formic acid.

Topographic images of the Co-Cr alloy surface show typical dendritic structures with relatively smooth, undulating surfaces and interdentritic regions with clusters of nanocrystals.

The apparently identical 2D and 3D topographic surface height images of Co-Cr alloys before and after formic acid treatment, shown in Figure 2(A) and (B) and 2(C) and (D), are examined in more detail by analyzing the profile of the cross-section shown in Figure 2(E). The cross-sectional profile of the surface of the Co-Cr alloy before treatment is shown in red, while the cross-sectional profile after the 7-day treatment with formic acid is shown in blue. It is immediately noticeable that the effect of formic acid affects the interdentritic regions of the Co-Cr alloy surface the most. The height of the nanocrystals emerging from the smoothed surfaces averages between 100 and 200 nm before and after treatment. However, what is immediately noticeable is that the contours of the nanocrystals are rounded before treatment, while they are lower, sharper, and rougher after treatment, suggesting that a corrosion process is involved. Detailed analysis showed that about 2.5% of the nanocrystals were corroded at their height. In this way, it is very practical for the practical application of the cross-section method in analyzing the corrosion of dental materials. However, in order to obtain a general picture of the surface processes, it is necessary to analyze the roughness of the surfaces of Co-Cr alloys before and after treatment with formic acid. The results presented in Table 2 for a selected surface (100×100 μm²) show that the roughness of the Co-Cr surface increased by 9% in R_a, 24% in R_q, and 142% in Z, indicating that the corrosion process after treatment with formic acid is significant. The results of extensive investigations are reported in the literature [4].

Table 2. Roughness parameter values for Co-Cr dental alloys before and after treatment with formic acid
Tabela 2. Vrednosti parametara hrapavosti za dentalne legure Co-Cr pre i posle tretmana mravljom kiselinom

Treatment Tretman	Analyzed surface area / μm² Analizirana površina / μm²	R_a/nm	Rq/nm	Z range/nm
-	100 × 100	63.4	81.7	1028
7 days 7 dana	100 × 100	69.1	101	2487

CONCLUSION

Atomic force microscopy is a very powerful method for the analysis of all dental materials, especially Co-Cr dental alloys. It allows imaging of various surfaces and visualization of the topography of surfaces in two and three dimensions. Moreover, it allows not only a detailed insight into the physicochemical properties of surfaces, especially surface roughness, but also the monitoring of processes taking place on the surfaces themselves at the nanoscale.

This paper presents the application of atomic force microscopy to the research already conducted on dental materials, which is not only useful but also necessary for the application of dental materials in dentistry. Based on the section profile of the examined surface along a line, insight into local surface properties such as roughness is obtained, while roughness analysis is applicable to larger surfaces and is a useful parameter for monitoring the quality of the dental materials themselves.

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Primena mikroskopije atomskih sila u istraživanjima površina dentalnih legura

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KRATAK SADRŽAJ

Uvod Cilj ovog informativnog rada je da prikaže važnost i ključnu ulogu mikroskopije atomskih sila, s jedne strane, u kvantitativnom određivanju fizičko-hemijskih promena na površinama dentalnih materijala usled njihove izloženosti kiselinama, a s druge strane u kvantifikovanju promena fizičko-hemijskih svojstava površina dentalnih legura posle njihove obrade, te posledicama same obrade na njihov kvalitet i primenjivost.

Metode Mikroskopijom atomskih sila dobijeni su podaci koji kvantitativno opisuju promene na nanoskali na dentalnim legurama Co-Cr nastale usled izloženosti delovanju mravlje kiseline kroz period od sedam dana, a opisani su i efekti elektropoliranja i poliranja crnom rotirajućom četkom na hrapavost površina.

Rezultati Analiza topografije i hrapavosti površina legura obrađenih rotirajućom crnom četkom nakon elektropoliranja ili samo elektropoliranjem pokazuje da su obe metode primenjive u stomatologiji ostavljajući defekte dimenzija nedostatnih za adherenciju mikroorganiama. Izlaganje analiziranih legura mravljoj kiselini dovodi do povećanja hrapavosti površina ukazujući na prisutnost procesa korozije, naročito na područjima interdentritskih nanokristala.

Zaključak Navedeni efekti obrade i tretmana legura Co-Cr vrlo se detaljno mogu pratiti tehnikom mikroskopije atomskih sila, specifično analizom topografije površina i analizom njene hrapavosti. Metoda pokazuje izuzetnu važnost za procenu primene legura Co-Cr, a takođe i drugih legura u dentalnoj medicini.

Ključne reči: mikroskopija atomskih sila; hrapavost površina; dentalne legure Co-Cr; mravlja kiselina; elektropoliranje

UVOD

Legure Co-Cr su materijali koji se zbog svojih izuzetnih mehaničkih svojstava, naročito zbog svoje čvrstoće, već dugo koriste kao stomatološki materijali [1]. Ne ulazeći u opis strukture samih legura, jedno od njihovih bitnih svojstava jeste njihova površinska hrapavost [2]. Da bi se mogle upotrebiti kao stomatološki materijal, legure Co-Cr moraju se obraditi, što uključuje i njihovo poliranje [3, 4]. Nadalje, sama površina dentalnih legura od izuzetne je važnosti za njihovu primenu jer o hrapavosti njihove površine zavisi interakcija površine s oralnim tečnostima, formiranje dentalnog plaka, te interakcija s mikroorganizmima i tečnostima prisutnim u oralnoj šupljini. Usled interakcije s oralnim tečnostima dolazi do hemijskih reakcija i korozije koje mogu promeniti fizičko-hemijska svojstva površina dentalnih legura i na taj način ograničiti njihovu primenu.

Mikroskopija atomskih sila jedna je od tehnika kojom se može odrediti topografija površina na nanoskali. Stoga je njena uloga od ključne važnosti u proceni kvaliteta dentalnih materijala, posebno njihovih površina [5]. Bez ulaženja u opis osnovnog principa rada same tehnike, osim topografije, važan parametar koji se određuje ovom metodom jeste parametar hrapavosti površine, koji ukazuje na veličinu površinskih defekata pogodnih za smeštaj mikroorganizama [6].

U ovom informativnom radu opisaće se pristup problemima određivanja topografije i hrapavosti već istraživanih površina legura Co-Cr i njihovih promena nastalih posle obrade samih legura elektropoliranjem i poliranjem rotirajućom crnom četkom s naglaskom na primeni mikroskopije atomskih sila kao ključne tehnike u dobijanju argumenata važnih za primenu legura u stomatologiji [4]. Nadalje, biće prikazana i objašnjena detaljna analiza topografije površina legura Co-Cr pre i posle korozije inducirane uranjanjem u mravlju kiselinu u periodu od sedam dana.

EFEKAT POLIRANJA NA TOPOGRAFIJU I HRAPAVOST POVRŠINA LEGURA CO-CR

Postupak pripreme i obrade samih legura Co-Cr opisan je u ranijim studijima. Ukratko, posle izlivanja pomoću mašine za vakuumsko livenje pri 1420° C, uzorci legure su očišćeni od uložne mase i posle toga peskareni i zatim elektropolirani. Nakon toga su dodatno polirani rotirajućom četkom u trajanju od 15 minuta. Legure Co-Cr su zalepljene lepljivom trakom za metalni disk te oslikane u kontaktnom načinu rada AFMa (nanoskopom III sa kontrolnim programom, Bruker Instruments, Santa Barbara, SAD), a snimanje je izvedeno sondom Bruker NP, čija je nominalna konstanta probe k_{nom} = 0,32 N/m. Lokacija od interesa za oslikavanje površina odabrana je optičkom kamerom. Topografije površina dentalnih legura elektropolirane su i polirane crnom rotirajućom četkom i njihovi profili poprečnog preseka i 3D topografski prikazi visina prikazani su na Slici 1.

Kao što se vidi iz slika 1C i 1D, trodimenzionalna topografska slika visina kojima odgovaraju dve različite metode poliranja potpuno je različita. Fina dentritska valovita struktura i izranjajući interdentritski nanokristali na površini legure Co-Cr posle elektropoliranja narušeni su novoformiranim dominantnim brazdama međusobno raspoređenim na različitim udaljenostima, te dubinama brazda koje dosežu vrednosti i do 400 nm. Za razliku od površine obrađene rotirajućom četkom, površinom legure Co-Cr koja je podvrgnuta samo elektropoliranju dominiraju dendriti s relativno zaglađenim valovitim površinama i interdentritskim područjima s nakupinama nanokristala koja izranjaju iz zaglađene površine s visinama u proseku do 100 do 200 nm. Za primenu poliranih legura Co-Cr važno je da je površina koja dolazi direktno u kontakt s tečnostima što glađa i ravnija, sa što manjim vrednostima parametara hrapavosti. Zato je analiziran profil poprečnog preseka obe polirane legure Co-Cr, koji je prikazan na slikama 1A i 1B. Ovakvu analizu potrebno je uraditi na više površina i dobiti srednju vrednost i standardno odstupanje. Da bi se dobio uvid u glatkoću površina legura višeg stupnja od lokalnog uzduž linije preseka, osim profila poprečnog preseka, analizirana je hrapavost na većim površinama legure ($30 \times 30\ \mu m^2$ i $100 \times 100\ \mu m^2$), a vrednosti parametara hrapavosti jedne analize prikazane su u Tabeli 1. Uobičajeno je da se razmatraju parametri R_a (parametar hrapavosti), R_q (koren srednjeg kvadrata hrapavosti) i Z raspon (najveća vertikalna udaljenost između najviše i najniže izmerene pozicije na površini).

Ovde treba imati na umu da vrednost hrapavosti površina raste s povećanjem analizirane površine. To je naročito važno kad se analiziraju površine koje nisu homogene. Zato je uvek bolje analizirati maksimalno moguće veliku površinu legura kako bi se dobio realan uvid u topografiju i hrapavost legura.

Budući da su vrednosti struktura koje izranjaju iz valovite površine i dubina brazda manje od dimenzija mikroorganizama (1–4 μm) u oralnoj šupljini, navedeni efekti poliranja ne predstavljaju prepreku za upotrebu obe metode u obradi legura u stomatologiji.

Zaključno, prikazan je postupak analize površina obrađenih legura Co-Cr koji je nužan za ispitivanje da li su površinska svojstva legure zadovoljavajuća.

Zaključno, ovde su opisani način i tehnika koji su nužni i dovoljni za procenu valjanosti obrade legura različitim metodama za njihovu sigurnu primenu u stomatologiji.

EFEKAT KOROZIJE NA TOPOGRAFIJU I HRAPAVOST POVRŠINA LEGURA CO-CR

Posle opisanog efekta poliranja na topografiju i hrapavost površina, vrlo važnu informaciju koju daje mikroskopija atomskih sila jeste njihova promena usled korozije inducirane tretmanom kiselinama, mravljom i octenom. Pritom je od ključne važnosti da se oslikaju identična područja od interesa pre i posle tretmana kiselinama. Naime, bilo koji tretman površina legura zahteva njihovo uklanjanje s uređaja, sam tretman i ponovno vraćanje legura za oslikavanje. Zato je potrebno razviti metodologiju kojom bi se osigurala analiza potpuno identičnih lokacija na površinama legura, što je u ovom istraživanju i bio slučaj.

Ukratko, uzorci legura pre tretmana oslikani su na četiri različite pozicije, dok je veličina oslikanog područja varirala ($100 \times 100\ \mu m^2$, $30 \times 30\ \mu m^2$ i $10 \times 10\ \mu m^2$). Primer topografskog 2D i 3D prikaza visine površine legura Co-Cr pre i posle sedmodnevnog tretmana mravljom kiselinom prikazan je na Slici 2 (A i B) i 2 (C i D).

Topografske slike površine legure Co-Cr pokazuju tipične dendritske strukture s relativno zaglađenim valovitim površinama te interdendritskim područjima s nakupinama nanokristala.

Naizgled identične topografske 2D i 3D slike visina površina legura Co-Cr pre i posle tretmana mravljom kiselinom prikazanih na Slici 2 (A) i (B) i 2(C) i ((D) proučavaju se detaljno analizom njihovog profila poprečnog preseka prikazanog na Slici 2(E). Na slici je crvenom bojom naznačen profil poprečnog preseka površine legure Co-Cr pre tretmana, dok je plavom bojom naznačen profil poprečnog preseka posle sedmodnevnog tretmana mravljom kiselinom. Odmah se uočava da je delovanje mravlje kiseline najviše uticalo na interdendritska područja površine legure Co-Cr. Visina nanokristala koja izranjaju iz zaglađenih površina kreću se u proseku od 100 do 200 nm pre i posle tretmana. Međutim, odmah se uočava da su obrisi nanokristala pre tretmana zaobljeni, dok su posle tretmana niži, zaoštreni i hrapavi sugerišući da se radi o procesu korozije. Detaljnom analizom se ustanovilo da je korodiralo oko 2,5% nanokristala s obzirom na visinu. Na ovaj način je vrlo praktično pokazana primena metode poprečnog preseka u analizi korozije dentalnih materijala. No, želeći da se dobije generalna slika površinskih procesa, nužno je i ovde analizirati hrapavosti površina legura Co-Cr pre i posle tretmana s mravljom kiselinom. Rezultati prikazani u Tabeli 2 na jednoj odabranoj površini ($100 \times 100\ \mu m^2$) pokazuju da se hrapavost površine Co-Cr povećala u R_a za 9%, R_q za 24% te *Z* rasponu za 142%, što ukazuje na to da je posle tretmana mravljom kiselinom proces korozije značajan. Rezultati opsežnog istraživanja navedeni su u literaturi [4].

ZAKLJUČAK

Mikroskopija atomskih sila vrlo je snažna metoda za analizu svih dentalnih materijala, a specifično i dentalnih legura Co-Cr. Ona omogućuje oslikavanje različitih površina i vizualizaciju topografije površina u dve i tri dimenzije. Osim navedenog, ona omogućuje ne samo detaljan uvid u fizičko-hemijska svojstva površina, posebno hrapavost površina, nego i praćenje procesa koji se događaju na samim površinama na nanoskali.

Ovaj rad predstavlja primenu mikroskopije atomskih sila na već sprovedenim istraživanjima stomatoloških materijala, a koja su ne samo korisna nego i neophodna za primenu stomatoloških materijala u stomatologiji. Pomoću profila preseka analizirane površine duž jedne linije dobija se uvid u lokalna svojstva površina kao što su hrapavost, dok je analiza hrapavosti primenjiva na većim površinama i koristan je parametar praćenja kvaliteta samih dentalnih materijala.

sciendo

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Split crest technique - an effective method in dental implantology for narrow ridges: a case report

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SUMMARY

Introduction Implant placement with simultaneous bone augmentation presents procedure with high risk of complications. In those situations, one of the surgical procedures with promising outcomes is the split crest technique.
Case report A 69-year-old edentulous patient was referred to the Clinic of Oral Surgery, at the Faculty of Dentistry in Pancevo. The width of the mandibular alveolar ridge in the inter-canine region was 2 mm. For the ridge splitting and expansion, a special split and expansion kit was used (Esset Kit®, Osstem, South Korea). The procedure started with crestal remover carbide cylindrical bur of 7 mm diameter for flattening of the alveolar ridge to the width of 4 mm. Then, a sharp lance drill was used to mark the places for implant osteotomies and placement. After that, 1.8 mm twist drill was used to prepare a bed of 10 mm depth, and a 13 mm saw was directed vertically. Set of expansion drills were used to expand the alveolar ridge gradually. Two implants 3.5x10 mm each (TSIII SA®, Osstem, South Korea) were inserted in the region of lower canines and the space between split buccal and lingual bone lamellae remained empty. Initial stability of inserted implants was assessed by implant stability quotient (ISQ). The values for implant stability were high for both inserted implants (98 and 93). After the surgical procedure, a control CBCT was done. The wound healed uneventfully.
Conclusion Split crest technique seems to be a predictable alternative for implant placement in narrow alveolar ridges.
Keywords: split crest technique; ridge splitting; dental implants

INTRODUCTION

Dental implants present excellent solutions for replacement of missing teeth and rehabilitation of edentulous jaws. In some patients, especially those with long-standing edentulism and unstable dentures, residual alveolar ridges can become sharp and narrow. In those situations, placement of implants proves challenging without bone augmentation procedures, which are costly and traumatic for patients, with risks of complications [1, 2]. One of the surgical procedures which shows promising outcomes is the split crest technique.

CASE REPORT

A 69-year-old male patient was referred to the Clinic of Oral Surgery, at the Faculty of Dentistry in Pancevo, with edentulous lower jaw, with the aim to find the best solution for his unstable and troublesome lower complete denture. Clinical examination revealed narrow residual alveolar ridge with limited width of keratinized mucosa and shallow vestibules. Additionally, the 3D CBCT investigation, showed extremely narrow residual bone in the inter-foraminal region, and low height in posterior parts of the mandible. On the base of clinical and radiographic examination, the treatment plan was made, which included the split crest technique for horizontal ridge augmentation and placement of two intraosseous implants for an implant-supported complete overdenture. The plan was explained to the patient in details and informed consent was obtained.

Surgical procedure

Local infiltration anesthesia, using 4% articaine with adrenaline 1:100 000, was injected supraperiostally in the vestibulum and as submucous injection lingually in the frontal region of the edentulous mandible. Mid-crestal incision was performed in the inter-canine region, with minimal relieving incisions of 3 mm at the end of the mid-crestal incision (Figure 1). The full thickness mucoperiosteal flap was elevated in order to access the residual alveolar bone. The width of the alveolar ridge was measured clinically with a UNC-15 periodontal probe (2mm) and on the CBCT scan on cross-section images

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Figure 1. Mid-crestal incision in the inter-canine region, with minimal relieving incisions of 3 mm at the end of the mid-crestal incision
Slika 1. Rez po sredini grebena u međuočnjačkom regionu, sa minimalnim relaksacijama od 3 mm na kraju reza

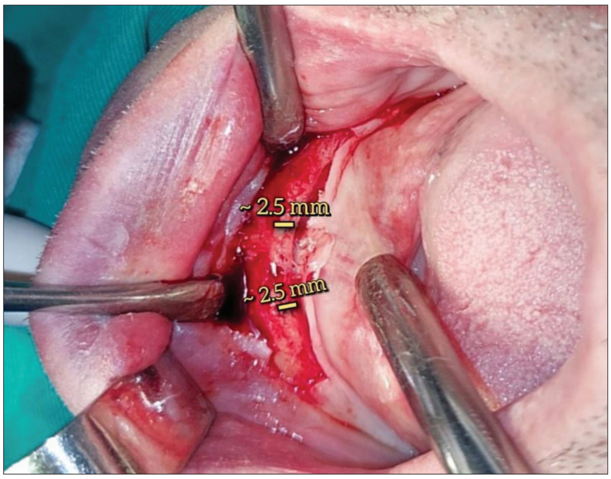

Figure 2. The full thickness mucoperiosteal flap was raised and the width of the alveolar ridge was measured.
Slika 2. Mukoperiostealni režanj pune debljine je podignut i izmerena je širina alveolarnog grebena.

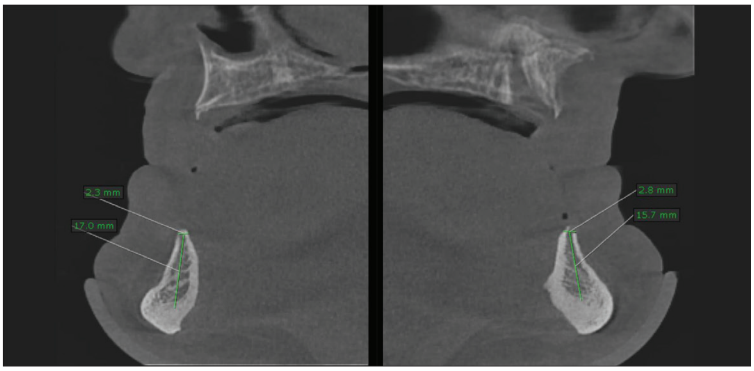

Figure 3. CBCT measurements of the width of alveolar bone
Slika 3. CBCT merenja širine alveolarne kosti

(2.3 mm) (Figures 2 and 3). For the ridge splitting and expansion, a special split and expansion kit was used (Esset Kit®, Osstem, South Korea). First, crestal remover carbide cylindrical bur of 7 mm diameter and speed of 1500 rpm, was used for flattening of the alveolar ridge to the width of 4 mm (Figure 4). Then, a sharp lance drill was used to mark the places for implant osteotomies and placement. After that, 1.8 mm twist drill with average speed of 1500 rpm was used to prepare a bed of 10 mm depth, and a 13 mm saw was directed vertically to the depth of 5 mm and then mesial and distal centrally, alongside the alveolar ridge (Figure 5). Set of expansion drills 1.6/2.8 and 2.2/3.6 mm with 50Ncm/35rpm were used to expand the alveolar ridge gradually (Figure 6). Two implants 3.5x10mm each (TSIII SA®, Osstem, South Korea) were inserted with 50Ncm/35rpm in the region of lower canines (Figure 7) and the space between split buccal and lingual bone lamellae was not filled with any bone substitutes (Figure 8). Initial stability of inserted implants was assessed by implant stability quotient (ISQ) measured using the special apparatus (Osstel mentor®) and smart pags previously fixed to dental implant body. The values for implant stability were high for both inserted implants

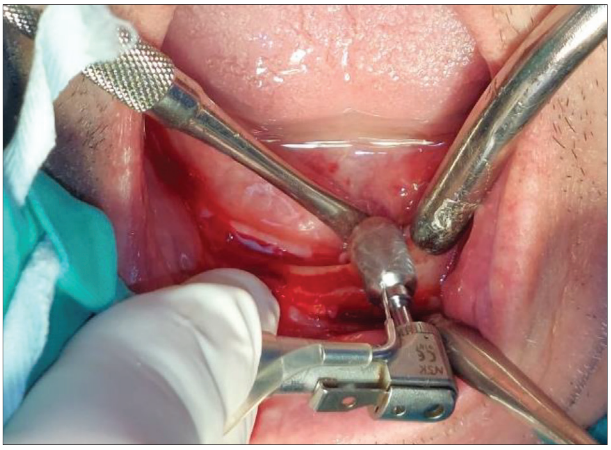

Figure 4. Flattening of the alveolar ridge to the width of 4 mm with crestal remover carbide cylindrical bur
Slika 4. Ravnanje alveolarnog grebena do širine 4 mm sa karbidnim cilindričnim borerom za uklanjanje grebena

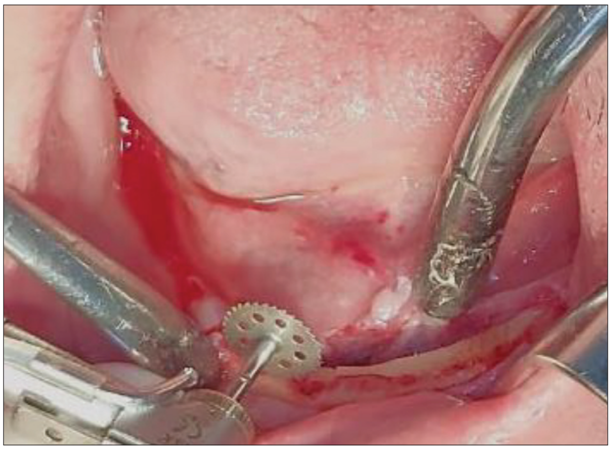

Figure 5. A 13mm saw was directed vertically to the depth of 5 mm and then mesial and distal centrally, alongside the alveolar ridge
Slika 5. Testera od 13 mm je usmerena vertikalno do dubine od 5 mm, a zatim mezijalno i distalno centralno, duž alveolarnog grebena

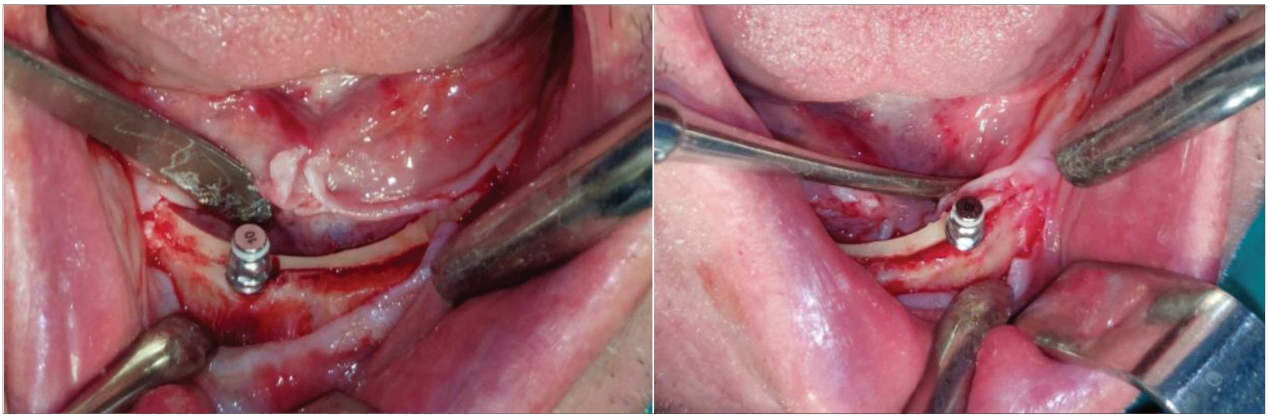

Figure 6. Expansion drills were used to expand the alveolar ridge gradually
Slika 6. Ekspanzione bušilice su korišćene za postepeno širenje alveolarnog grebena

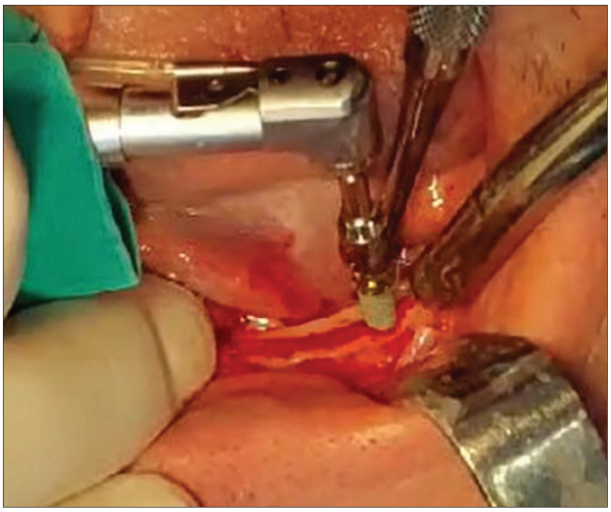

Figure 7. Implants insertion in the region of lower canines
Slika 7. Ugradnja implantata u predelu donjih očnjaka

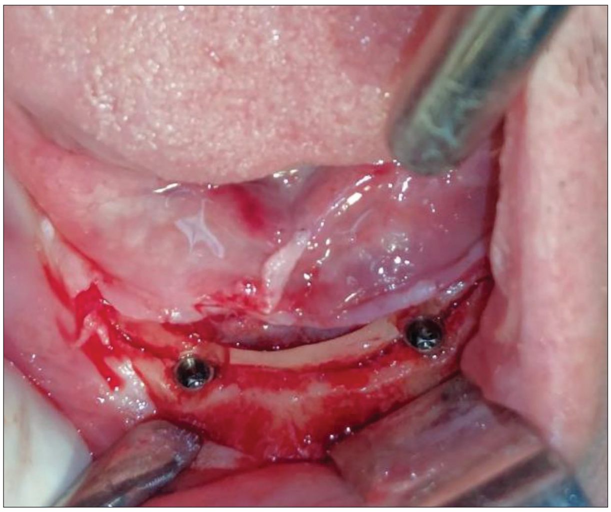

Figure 8. Two inserted implants with no bone damage
Slika 8. Dva ugrađena implantata bez oštećenja kosti

Figure 9. The measurement of implant stability quotient (ISQ)
Slika 9. Merenje koeficijenta stabilnosti implantata (ISQ)

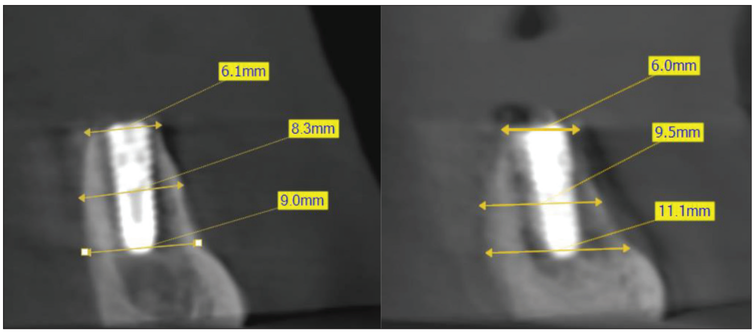

Figure 10. Ideal implant position was confirmed on cross-section CBCT
Slika 10. Idealan položaj implantata potvrđen je na CBCT preseku

(98 and 93) (Figure 9). After the surgical procedure, a control CBCT was done, and ideal implant position was confirmed on cross-section (Figure 10). Postoperative measures included systemic antibiotics (Amoxicillin caps. 500mg x 3 per day for 7 days) and analgesics. Sutures were removed after 7 days. The wound healed uneventfully.

DISCUSSION

Narrow alveolar ridges present a clinical challenge for dental implants insertion. In such cases, common procedure includes simultaneous implant placement with bone grafting and collagen membrane coverage [3, 4]. Bone grafting is associated with many possible complications and high cost [5]. One of the best solutions in such cases for the patient may be split cast technique with simultaneous implant placement [6]. Split cast technique with special expansion kit allows clinician to avoid vertical osteotomy and prevent cortical bone breakage. With this approach, alveolar splitting is done gradually, with special expanders which expand the bone by self-tapping. This was confirmed in our patient where the buccal bone remained intact after bone expansion. This is in agreement with previous studies [7, 8]. Strong initial stability of inserted dental implants is obtained by elasticity of the expanded bone, as was the case in our patient (ISQ 98 and 93). Very high ISQ allows immediate loading of the inserted implants [9]. Additionally, 3-wall bony defect permits fast ingrowth of stem cells toward implant surface and excellent healing, without need for additional bone grafting. Scipioni et al. 1994 demonstrated 98% of implants success rate for over the 5 years with ridge splitting [10]. Bone possesses viscoelastic properties, so when more trabecular bone is present, the more viscoelastic nature of the alveolar ridge is expected. Some authors showed better success rate with ridge splitting in the maxilla than in the mandible [11].

Split crest with expansion technique seems to be a safe and simple procedure in cases with narrow alveolar ridges.

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Tehnika ekspanzije alveolarnog grebena – efikasna metoda u dentalnoj implantologiji za uske grebene: prikaz slučaja

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KRATAK SADRŽAJ

Uvod Ugradnja implantata uz istovremeno presađivanje kosti predstavlja proceduru sa visokim rizikom od komplikacija. U tim situacijama jedna od hirurških procedura sa obećavajućim ishodima je tehnika podeljenog (ekspanzije) grebena.

Prikaz slučaja Pacijent bez zuba, star 69 godina, upućen je na Kliniku za oralnu hirurgiju Stomatološkog fakulteta u Pančevu. Širina alveolarnog grebena donje vilice u međuočnjačkom regionu bila je 2 mm. Za cepanje i proširenje grebena korišćen je poseban komplet za cepanje i proširenje (Esset Kit®, Osstem, Južna Koreja). Procedura je započeta karbidnim cilindričnim svrdlom prečnika 7 mm za izravnavanje alveolarnog grebena do širine 4 mm. Zatim je korišćena oštra bušilica za označavanje mesta za osteotomiju i postavljanje implantata. Posle toga je korišćeno spiralno svrdlo od 1,8 mm za pripremu ležišta dubine 10 mm, a testera od 13 mm je usmerena vertikalno. Za postepeno širenje alveolarnog grebena korišćen je set ekspanzionih borera. Dva implantata po 3,5 × 10 mm (TSIII SA®, Osstem, Južna Koreja) umetnuta su u predelu donjih očnjaka i prostor između podeljenih bukalnih i jezičnih koštanih lamela nije bio ispunjen nikakvim koštanim zamenama. Inicijalna stabilnost ugrađenih implantata procenjena je koeficijentom stabilnosti implantata (ISQ). Vrednosti ISQ bile su visoke za oba ugrađena implantata (98 i 93). Nakon hirurškog zahvata urađen je kontrolni CBCT. Rana je zarasla bez problema.

Zaključak Čini se da tehnika ekspanzije grebena predstavlja predvidljivu alternativu za postavljanje implantata u uske alveolarne grebene.

Ključne reči: tehnika *split crest*; ekspanzija alveolarnog grebena; zubni implantati

UVOD

Zubni implantati predstavljaju odlično rešenje za nadoknadu nedostajućih zuba i rehabilitaciju bezubih vilica. Kod nekih pacijenata, posebno onih sa dugotrajnom bezubošću i nestabilnim protezama, rezidualni alveolarni grebeni mogu postati oštri i uski. U takvim situacijama ugradnja implantata se pokazuje kao izazovna, bez procedura koštane augmentacije, koje su skupe i traumatične za pacijente, uz rizik od komplikacija [1, 2]. Jedna od hirurških procedura koja pokazuje obećavajuće rezultate je tehnika podeljenog grebena.

PRIKAZ SLUČAJA

Pacijent star 69 godina upućen je na Kliniku za oralnu hirurgiju Stomatološkog fakulteta u Pančevu sa bezubom donjom vilicom, sa ciljem da pronađe najbolje rešenje za svoju nestabilnu i problematičnu donju totalnu protezu. Kliničkim pregledom utvrđen je uski rezidualni alveolarni greben sa ograničenom širinom keratinizovane sluzokože i plitkim vestibulima. Pored toga, 3D kompjuterizovana tomografija s konusnim snopom (CBCT) pokazala je izuzetno usku kost alveolarnog grebena u interforaminalnoj regiji i nisku visinu u zadnjim delovima donje vilice. Na osnovu kliničkog i radiografskog pregleda napravljen je plan lečenja koji je obuhvatao tehniku podeljenog grebena za horizontalnu augmentaciju grebena i ugradnju dva intrakoštana implantata za totalnu protezu na implantatima. Plan je detaljno objašnjen pacijentu i dobijen je informisani pristanak.

HIRURŠKA PROCEDURA

Lokalna infiltraciona anestezija, primenom 4% artikaina sa adrenalinom 1 : 100 000, ubrizgana je supraperiostalno u vestibulum i kao submukozna injekcija lingvalno u frontalni region bezube donje vilice. Rez po sredini grebena je urađen u interkaninom regionu, sa minimalnim relaksacionim urezima od 3 mm na kraju reza (Slika 1). Mukoperiostalni režanj pune debljine je podignut da bi se pristupilo rezidualnoj alveolarnoj kosti. Širina alveolarnog grebena je merena klinički pomoću parodontalne sonde UNC-15 (2 mm) i na CBCT skeniranju na slikama poprečnog preseka (2,3 mm) (slike 2 i 3). Za cepanje i proširenje grebena korišćen je specijalni komplet za cepanje i proširenje (Esset Kit®, Osstem, Južna Koreja). Za ravnanje alveolarnog grebena do širine od 4 mm prvo je korišćen cilindrični borer od karbida prečnika 7 mm i brzine 1500 obrtaja u minuti (Slika 4). Zatim je korišćeno oštro svrdlo za označavanje mesta za osteotomiju i postavljanje implantata. Nakon toga, korišćeno je spiralno svrdlo od 1,8 mm sa prosečnom brzinom od 1500 obrtaja u minuti za pripremu ležišta dubine 10 mm, a testera od 13 mm je usmerena vertikalno na dubinu od 5 mm, a zatim mezijalno i distalno centralno, uz alveolarni greben (Slika 5). Za postepeno širenje alveolarnog grebena korišćen je set ekspanzionih borera 1,6/2,8 i 2,2/3,6 mm sa 50 Ncm/35 rpm (Slika 6). Dva implantata po 3,5 × 10 mm (TSIII SA®, Osstem, Južna Koreja) ugrađena su sa 50 Ncm/35 rpm u predelu donjih očnjaka (Slika 7) i prostor između razdvojene bukalne i jezične koštane lamele nije bio ispunjen nikakvim zamenicima za kosti (Slika 8). Početna stabilnost ugrađenih implantata procenjena je koeficijentom stabilnosti implantata (ISQ) izmerenim pomoću specijalnog aparata (Osstel mentor®) i nastavaka (smart pag), koji su prethodno fiksirani na telo zubnog implantata. Vrednosti stabilnosti implantata bile su visoke za oba umetnuta implantata (98 i 93) (Slika 9). Nakon hirurškog zahvata urađen je kontrolni CBCT i na poprečnom preseku je potvrđen idealan položaj implantata (Slika 10). Postoperativne mere su uključivale sistemske antibiotike (Amokicillin caps. 500 mg × 3 na dan tokom sedam dana) i analgetike. Šavovi su uklonjeni nakon sedam dana. Rana je zarasla bez problema.

DISKUSIJA

Uski alveolarni grebeni predstavljaju klinički izazov za ugradnju zubnih implantata. U takvim slučajevima uobičajena procedura uključuje istovremeno postavljanje implantata sa presađivanjem kosti i pokrivanjem kolagenom membranom [3, 4]. Koštana augmentacija povezana je sa mnogim mogućim komplikacijama i visokim troškovima [5]. Jedno od najboljih rešenja u ovakvim slučajevima za pacijenta može biti ekspanzija grebena uz istovremenu ugradnju implantata [6]. Cepanje grebena sa specijalnim kompletom za proširenje omogućava kliničaru da izbegne vertikalnu osteotomiju i spreči lomljenje kortikalne kosti. Ovim pristupom alveolarno širenje grebena se vrši postepeno, specijalnim ekspanderima koji samourezivanjem proširuju kost. Ovo je potvrđeno kod našeg pacijenta, kod koga je bukalna kost ostala netaknuta posle proširenja kosti, i u skladu je sa prethodnim studijama [7, 8]. Snažna početna stabilnost ugrađenih dentalnih implantata postiže se elastičnošću ekspandirane kosti, kao što je to bio slučaj i kod našeg pacijenta (ISQ 98 i 93). Veoma visok ISQ omogućava imedijatno opterećenje ugrađenih implantata [9]. Pored toga, koštani defekt sa tri zida omogućava brzo urastanje matičnih ćelija ka površini implantata i odlično zarastanje, bez potrebe za dodatnim nadoknadama kosti. Scipioni i sar. [10] 1994. pokazali su 98% uspešnosti implantata tokom pet godina sa tehnikom cepanja i ekspanzije alveolarnog grebena. Kost poseduje visokoelastična svojstva, tako da kada je prisutno više trabekularne kosti, očekuje se viskoelastičnija priroda alveolarnog grebena. Neki autori su pokazali bolji uspeh ove tehnike u maksili nego u mandibuli [11].

ZAKLJUČAK

Središnja osteotomija alveolarnog grebena sa tehnikom ekspanzije pokazala se kao siguran i jednostavan postupak u slučajevima sa uskim alveolarnim grebenima.

Da li ste pažljivo čitali radove?

1. Pre sterilizacije endodontske instrumente treba:
   a) samo oprati
   b) samo osušiti
   c) očistiti od ostataka

2. Tehnika ekspanzije alveolarnog grebena e realizovana kod:
   a) pacijenta uzrasta 50 godina
   b) pacijenta uzrasta 60 godina
   c) pacijenta uzrasta 69 godina

3. Rastvorljivost kalcijum-aluminata je bila:
   a) veća nego kod kalcijum-silikata
   b) manja nego kod kalcijum-silikata
   c) identična sa kalcijum-silikatima

4. Najveća rastvorljivost zabeležena je kod:
   a) kalcijum-aluminata
   b) kalcijum-silikata
   c) MTA

5. Koncentracije VEGF kod pacova bez pal ploče bile su:
   a) iste kod kontrolnih i pacova sa dijabetesom
   b) slične kod kontrolnih i pacova sa dijabetesom
   c) identične kod kontrolnih i pacova sa dijabetesom

6. Koncentracije VEGF su određivane:
   a) kod pacijenata
   b) kod eksperimentalih životinja
   c) kod pacijenata i eksperimentalnih životinja

7. Tehnika ekspanzije alveolarnog grebena je realizovana kod:
   a) pacijenta
   b) pacijentkinje
   c) deteta

8. Sorpcija tečnosti kod kalcijum-aluminata je iznosila:
   a) 0,357
   b) 0,474
   c) 0,347

9. Najmanja rastvorljivost zabeležena je kod materijala:
   a) MTA
   b) kalcijum-silikata
   c) kalcijum-aluminata

10. Kod pacijenata koji su nosili proteze uzimana su:
    a) dva uzorka gingive
    b) tri uzorka gingive
    c) pet uzoraka gingive

11. Koncentracija VEGF je određivana u gingivi na mestima:
    a) akutnog pritiska proteze
    b) hroničnog pritiska proteze
    c) akutnog i hroničnog pritiska proteze

12. Endodontski instrumenti su čišćeni od ostataka:
    a) paste jodoforma
    b) paste kalcijum-hidroksida
    c) paste za opturaciju kanala

13. Tehnika ekspanzije alveolarnog grebena je efikasna metoda:
    a) u dentalnoj implantologiji
    b) u ortodonciji
    c) u endodonciji

14. Sorpcija tečnosti kod kalcijum-silikata je iznosila:
    a) 0,357
    b) 0,474
    c) 0,347

15. Kao kontrolni materijal u istraživanju fizičkih svojstava kalcijum-aluminata korišćen je:
    a) kalcijum-silikat
    b) kalcijum-hidroksid
    c) MTA

16. Koncentracija VEGF kod pacijenta sa protezama je određivana:
    a) nakon jednog dana nošenja proteze
    b) nakon tri dana nošenja proteze
    c) nakon pet dana nošenja proteze

17. VEGF je:
    a) medijator oralnih komplikacija dijabetesa tipa 2
    b) medijator oralnih komplikacija dijabetesa tipa 1
    c) medijator oralnih komplikacija bubrežne insuficijencije

18. Pasta kalcijum-hidroksida je uklanjana sa:
    a) 35 instrumenata
    b) 42 instrumenta
    c) 50 instrumenata

19. Tehnika ekspanzije alveolarnog grebena je efikasna metoda:
    a) za široke grebene
    b) za uske grebene
    c) za široke i uske grebene

20. Najveća sorpcija tečnosti je zabeležena kod:
    a) MTA
    b) kalcijum-aluminata
    c) kalcijum-silikata

21. U istraživanju su ispitivana:
    a) fizička svojstva kalcijum-aluminata
    b) biološka svojstva kalcijum-aluminata
    c) hemijska svojstva kalcijum-aluminata

22. Kvantitativno merenje VEGF je određivano:
    a) EUSA testom
    b) PH metrom
    c) merenjem koncentracije imunoglobulina

23. Najefikasniji protokol uklanjanja kalcijum-hidroksida sastojao se u kombinovanom protokolu:
    a) mehaničkog čišćenja sunđerom natopljenim hlorheksidin-glukonatom
    b) mehaničkog čišćenja sunđerom natopljenim alkoholom
    c) mehaničkog čišćenja sunđerom natopljenim hlorfenolom

24. Pasta kalcijum-hidroksida je uklanjana iz:
    a) vitalnih zuba
    b) ekstrahovanih zuba
    c) akrilatnih modela zuba

25. Izlaganje legure Co–Cr kiselinama ukazuje na:
    a) prisustvo korozije
    b) odsustvo korozije
    c) odsustvo hrapavosti

26. Najmanja sorpcija tečnosti je zabeležena kod:
    a) MTA
    b) kalcijum-aluminata
    c) kalcijum-silikata

27. Niži nivo VEGF ukazuje na:
    a) identičnu homeostazu
    b) izmenjenu homeostazu
    c) nepromenjenu homeostazu

28. Koncentracija VEGF je određivana kod:
    a) 36 pacijenata sa parcijalnim protezama
    b) 42 pacijenta sa parcijalnim protezama
    c) 28 pacijenata sa parcijalnim protezama

29. Kombinovani protokol je bio značajno efikasniji od:
    a) hemijskog
    b) ultrazvučnog
    c) fizičkog

30. Uklanjanje paste kalcijum-hidroksida iz kanala realizovano je protokolom dekontaminacije primenom:
    a) tri metode
    b) četiri metode
    c) pet metoda

31. Izlaganje Co–Cr kiselinama:
    a) povećava hrapavost
    b) ne utiče na hrapavost
    c) smanjuje hrapavost

32. Sorpcija tečnosti kod testiranih materijala je proveravana:
    a) posle 7 dana
    b) posle 14 dana
    c) posle 28 dana

33. Akutna i hronična kompresija gingive povezane su sa:
    a) nižim nivoima VEGF kod pacijenata sa dijabetesom
    b) višim nivoima VEGF kod pacijenata sa dijabetesom
    c) identičnim nivoima VEGF kod pacijenata sa dijabetesom

34. Koncentracija VEGF je određivana kod:
    a) 42 zdrava pacijenta
    b) 36 zdravih pacijenata
    c) 28 zdravih pacijenata

35. Kombinovani protokol je ostvario efikasno čišćenje:
    a) sa potpuno čistim površinama
    b) sa delimično čistim površinama
    c) sa delimično zaostalim rezidualnim materijama

36. Kontaminirani endodontski instrumenti su posmatrani:
    a) lupom
    b) svetlosnim mikroskopom
    c) SEM-om

37. Legura Co–Cr je izlagana dejstvu kiseline:
    a) dva dana
    b) sedam dana
    c) 14 dana

38. Rastvorljivost kalcijum-silikata je iznosila:
    a) 0,267
    b) 0,725
    c) 0, 255

39. Koncentracije VEGF u prisustvu palati ploče bile su:
    a) niže kod pacova sa dijabetesom
    b) više kod pacova sa dijabetesom
    c) identične kod pacova sa dijabetesom

40. Koncentracija VEGF je određivana kod:
    a) pacova
    b) kunića
    c) vijetnamskih svinja

41. Hemijska metoda čišćenja je bila efikasnija od ultrazvučne?
    a) Da
    b) Ne
    c) Samo u tri slučaja

42. Procena efikasnosti čišćenja je procenjivana na osnovu:
    a) boje zaostale materije
    b) veličine partikula materije
    c) količine rezidualne materije

43. Legure Co–Cr su izlagane dejstvu:
    a) mravlje kiseline
    b) fosforne kiseline
    c) hlorovodonične kiseline

44. Rastvorljivost kalcijum-aluminata je iznosila:
    a) 0,725
    b) 0,255
    c) 0,267

45. U prisustvu pala ploče koncentracije VEGF su bile:
    a) smanjene
    b) povećane
    c) identične

47. Kombinovani protokol čišćenja je bio efikasniji u odnosu na:
    a) mehanički
    b) ultrazvučni
    c) ultrazvučni i hemijski

46. Koncentracija VGEF je određivana kod eksperimentalih životinja:
    a) sa dijabetesom
    b) bez dijabetesa
    c) sa dijabetesom i bez dijabetesa

48. Dekontaminacija endodontskih instrumenata je realizovana:
    a) samo mehanički
    b) samo hemijski
    c) primenom četiri protokola

49. Uloga mikroskopije atomskih sila je korišćena u analizi promena na:
    a) površinama restaurativnih materijala
    b) površinama dentalnih legura
    c) površinama materijala za opturaciju

50. U dekontaminaciji endodontskih instrumenata korišćen je i ultrazvuk?
    a) Da
    b) Ne
    c) Samo u kombinovanom protokolu

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PREVOD NA SRPSKI JEZIK. Na posebnoj stranici dokumenta priložiti naslov rada na srpskom jeziku, puna imena i prezimena autora (bez titula) indeksirana brojevima, zvaničan naziv ustanova u kojima autori rade, mesto i državu. Na sledećoj stranici dokumenta priložiti sažetak (100–250 reči) s ključnim rečima (3–6), prevod naziva priloga (tabela, grafikona, slika, shema) i celokupni tekst u njima i legendu.

STRUKTURA RADA. Svi podnaslovi se pišu velikim masnim slovima (bold). Originalni rad, metaanaliza, prethodno i kratko saopštenje obavezno treba da imaju sledeće podnaslove: Uvod (Cilj rada navesti kao poslednji pasus Uvoda), Metode rada, Rezultati, Diskusija, Zaključak, Literatura. Pregled literature čine: Uvod, odgovarajući podnaslovi, Zaključak, Literatura. Prvoimenovani autor metaanalize i preglednog rada mora da navede bar pet autocitata (kao autor ili koautor) radova publikovanih u časopisima s recenzijom. Koautori, ukoliko ih ima, moraju da navedu bar jedan autocitat radova takođe publikovanih u časopisima s recenzijom. Prikaz slučaja ili bolesnika čine: Uvod (Cilj rada navesti kao poslednji pasus Uvoda), Prikaz bolesnika, Diskusija, Literatura. Ne treba koristiti imena bolesnika, inicijale, niti brojeve istorija bolesti, naročito u ilustracijama. Prikazi bolesnika ne smeju imati više od pet autora. Priloge (tabele, grafikone, slike itd.) postaviti na kraj rukopisa, a u samom telu teksta jasno naznačiti mesto koje se odnosi na dati prilog. Krajnja pozicija priloga biće određena u toku pripreme rada za publikovanje.

SKRAĆENICE. Koristiti samo kada je neophodno, i to za veoma dugačke nazive hemijskih jedinjenja, odnosno nazive koji su kao skraćenice već prepoznatljivi (standardne skraćenice, kao npr. DNK, sida, HIV, ATP). Za svaku skraćenicu pun termin treba navesti pri prvom navođenju u tekstu, sem ako nije standardna jedinica mere. Ne koristiti skraćenice u naslovu. Izbegavati korišćenje skraćenica u sažetku, ali ako su neophodne, svaku skraćenicu objasniti pri prvom navođenju u tekstu.

DECIMALNI BROJEVI. U tekstu rada na engleskom jeziku, u tabelama, na grafikonima i drugim prilozima decimalne brojeve pisati sa tačkom (npr. 12.5 ± 3.8), a u tekstu na srpskom jeziku sa zarezom (npr. 12,5 ± 3,8). Kad god je to moguće, broj zaokružiti na jednu decimalu.

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OBIM RADOVA. Celokupni rukopis rada – koji čine naslovna strana, sažetak, tekst rada, spisak literature, svi prilozi, odnosno potpisi za njih i legenda (tabele, slike, grafikoni, sheme, crteži), naslovna strana i sažetak na srpskom jeziku – mora iznositi za originalni rad, prethodno i kratko saopštenje, rad izistorije medicine i pregled literature do 5.000 reči, a za prikaz bolesnika, rad za praksu, edukativni članak i rad za rubriku „Jezik medicine“ do 3.000 reči; radovi za ostale rubrike mogu imati najviše 1.500 reči.

PRILOZI RADU su tabele i slike (fotografije, crteži, sheme, grafikoni).

TABELE. Svaka tabela treba da bude sama po sebi lako razumljiva. Naslov treba otkucati iznad tabele, a objašnjenja ispod nje. Tabele se označavaju arapskim brojevima prema redosledu navođenja u tekstu. Tabele crtati isključivo u programu Word, kroz meni Table–Insert–Table, uz definisanje tačnog broja kolona i redova koji će činiti mrežu tabele. Desnim klikom na mišu – pomoću opcija Merge Cells i Split Cells – spajati, odnosno deliti ćelije. Kucati fontom Times New Roman, veličinom slova 12 pt, s jednostrukim proredom i bez uvlačenja teksta. Korišćene skraćenice u tabeli treba objasniti u legendi ispod tabele. Ukoliko je rukopis na srpskom jeziku, priložiti nazive tabela i legendu na oba jezika. Takođe, u jednu tabelu, u okviru iste ćelije, uneti i tekst na srpskom i tekst na engleskom jeziku (nikako ne praviti dve tabele sa dva jezika!).

SLIKE. Slike su svi oblici grafičkih priloga i kao „slike“ u SGS se objavljuju fotografije, crteži, sheme i grafikoni. Slike označavaju se arapskim brojevima prema redosledu navođenja u tekstu. Primaju se isključivo digitalne fotografije (crno-bele ili u boji) rezolucije najmanje 300 dpi i formata zapisa tiff ili jpg (male, mutne i slike lošeg kvaliteta neće se prihvatati za štampanje!). Ukoliko autori ne poseduju ili nisu u mogućnosti da dostave digitalne fotografije, onda originalne slike treba skenirati u rezoluciji 300 dpi i u originalnoj veličini. Ukoliko je rad neophodno ilustrovati sa više slika, u radu će ih biti objavljeno nekoliko, a ostale će biti u e-verziji članka kao PowerPoint prezentacija (svaka slika mora biti numerisana i imati legendu). Ukoliko je rukopis na srpskom jeziku, priložiti nazive slika i legendu na oba jezika.

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SHEME (CRTEŽI). Crteži i sheme se dostavljaju u jpg ili tiff formatu. Sheme se mogu crtati i u programu CorelDraw ili Adobe Illustrator (programi za rad sa vektorima, krivama). Svi podaci na shemi kucaju se u fontu Times New Roman, veličina slova 10 pt. Korišćene skraćenice na shemi treba objasniti u legendi ispod sheme. Ukoliko je rukopis na srpskom jeziku, priložiti nazive shema i legendu na oba jezika.

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ACKNOWLEDGMENT. List all those individuals having contributed to preparation of the article but having not met the criteria of authorship, such as individuals providing technical assistance, assistance in writing the paper or running the department securing general support. Financial aid and support in the form of sponsorship, grants, donations, etc., should be mentioned too.

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