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Contents / Sadržaj

Nijedno nametnuto pravilo ne može
biti ispravno, jer pravila izmišljaju ljudi koji žele da vladaju.
Osho

Iako mi ideje za urednički komentar obično ne nedostaju, ovog puta sam imao dilemu kako da se uklopim u sadašnji trenutak i istovremeno budem originalan. Kako u vremenu u kome živimo sve, nažalost, podseća na priču iz jedne pozorišne predstave, tako i ovaj komentar neće izlaziti iz okvira „teatra života". Zato ću možda ovog puta podleći stereotipu i ponovo pisati o Dogvilu naše sadašnjice.

Slično Dogvilu, i naš sadašnji životni trenutak karakterišu i oblikuju ljudi bez ikakvih ljudskih i moralnih kvaliteta. Oni su, nažalost, fasada jednog izolovanog sveta, gde je čak i poštenje ljudi prividna kategorija. A tamo gde ne postoje merila vrednosti, gde nema kulture, tamo gde poluintelektualci i politička estrada igraju beskrajnu farsu, a učeni ljudi statiraju, budućnost je sumorna i besperspektivna. Patrijarh Pavle je jednom izrekao važnu istinu: „Niko nas nije pitao kad i gde ćemo se roditi, i za to nemamo ni zasluge ni krivice. A da li ćemo postupati kao ljudi ili neljudi, to zavisi isključivo od nas".

Tamo gde su znanje i inventivnost tiranija trenutka, a lažni moral, neistina i demagogija jedini kvalitet, tu je teatar svakodnevica. Naš život je, u stvari, Dogvil, grad prototip naše stvarnosti, sa elitom koja to nije, jer je nastala pukom promenom odela, besmislenim učenjem formalnih proevropskih faza, odnosno kupovinom diploma i plagiranjem doktorskih i naučnih radova.

Ima li načina da Dogvil postane pristojniji grad? Naravno da ima, jer čovek uči samo kroz greške i napreduje samo kroz teškoće. A uistinu može postati čovek samo ako prihvati odgovornost za ono što jeste, odnosno da postane hrabar i eliminiše bilo kakav strah. Iako društvo danas živi u brojkama, najveća hrabrost je biti pojedinac i boriti se protiv estrade naše stvarnosti, boriti se protiv najstrašnijih devijacija našeg vremena i sa najgorim osobinama ljudskog roda.

Kako pobeći iz Dogvila? Jedini životni zakon je istina, za koju se treba boriti samo znanjem. A nijedan trud nije uzaludan ako ima jasan i vredan cilj. Ako se u taj cilj veruje i ako je hrabrost jedna od vrlina, svaka prepreka je savladana.

Ovaj neuobičajeni urednički komentar ću i završiti citatom velikog Osho-a, dakle onako kako sam i počeo. On daje najbolju sliku sadašnjeg trenutka i ukazuje na „sigurne" vrednosti koje promoviše „pozorišna" elita.

„Samo se idiot oseća sigurno, a čovek koji je uistinu živ uvek je nesiguran. Jedino znanje može čoveka učiniti sigurnim."

Prof. dr Slavoljub Živković

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Ultrastructural analysis of uninstrumented root canal areas following various irrigation regimens

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SUMMARY

Introduction During endodontic treatment smaller or larger areas of root canal wall remain non-instrumented. This can affect prognosis of endodontic treatment as some bacteria may be left behind. The purpose of this study was to evaluate the morphology of non-instrumented areas of the root canal wall using scanning-electron-microscopy (SEM) after completed instrumentation and various irrigation regiments.

Materials and Methods Eighteen single-rooted extracted teeth were divided into the six groups. One tooth in each group represented a control sample. In all samples only one half of the canal was instrumented using ISO 40 hand files. Control samples were subjected to an irrigation protocols without instrumentation. Irrigants used were physiological saline, 3% sodium hypochlorite and 15% of ethylene-diamine-tetra-acetate. Irrigation protocol included using each of these irrigants alone, or a combination of NaOCl and EDTA, as well as their combination with final irrigation using NaOCl or chlorhexidine. Then after, roots were sectioned longitudinally and prepared for SEM.

Results Saline irrigation left pulpal debris on uninstrumented areas of the canal wall. Irrigation with 3% NaOCl left behind canal wall with different forms of calcospherites. However, after EDTA irrigation dentin appeared as an undulating surface with open tubules without a smear layer. The combination of NaOCl and EDTA showed remnants of calcospherites and open slightly widened dentinal tubules. Final irrigation with NaOCl on the uninstrumented areas showed enlarged dentinal tubules along with dentinal erosion, while after final irrigation with CHX clean dentin and open dentinal tubules without smear layer were noted.

Conclusion From the morphological point of view, the most favorable effect of irrigation on both uninstrumented and uninstrumented canal walls was achieved after irrigation with NaOCl and EDTA or NaOCl, EDTA and chlorhexidine as the final irrigant.

Keywords: root canal instrumentation; uninstrumented root canal areas; root canal irigation; SEM

INTRODUCTION

One of the basic preconditions for successful endodontic treatment is adequate instrumentation of the root canal. However, satisfactory instrumentation and irrigation is difficult to achieve due to the very specific and complex root canal morphology, as well as limited effect of instruments [1]. Morphological variations of the root canal system and inability of endodontic instruments to reach all parts of root canal wall make cleaning of complete root canal practically impossible [1, 2]. Micro-computerized tomography has confirmed that some areas of root canal walls remain untouched after instrumentation [3-6]. These areas may contain bacteria and compromise endodontic treatment [7]. In addition, the presence of smear layer and debris as a result of instrumentation is significant clinical problem [2, 8]. This layer often contains bacteria and blocks dentinal tubules, which significantly decrease the effect of used irrigant affecting the quality of obturation and the outcome of endodontic treatment [8, 9].

Due to limited effectiveness of endodontic instruments in root canal cleaning, it is necessary to use appropriate chemical agents during and after instrumentation. Their role is to eliminate and reduce any remaining microorganisms as well as remove smear layer [8-10]. Even though there is no general consensus about removing smear layer immediately before obturation, most endodontists agree that if it is not removed, it could disintegrate and lead to microleakage due to the low quality of the bond strength between the sealer and root canal walls [9, 10].

The aim of this study was to use a SEM analysis to evaluate the morphology of uninstrumented areas of the root canal walls following mechanical instrumentation and application of various irrigation regimens.

MATERIALS AND METHODS

The material used in this research included 18 freshly extracted intact human maxillary single-rooted teeth without

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any visible damage (root caries, cracks, internal or external resorption, etc.). According to the irrigation regimens, all teeth were divided into six groups, with one tooth in each group representing a control specimen. The teeth samples were kept for eight hours in 0.5% NaOCl solution to facilitate removal of organic debris. After rinsing teeth under running water, they were immersed in saline solution and refrigerated until the beginning of the experiment.

Prior to canal instrumentation, using a diamond disc, longitudinal grooves were created on the facial and lingual surfaces of the root, without penetrating it, in order to facilitate the fracture. The crowns were amputated and discarded, while the remaining debris was removed using running water. Following pulp extirpation, one tooth from each group (two control samples) underwent different irrigation regimens only without previous instrumentation. All root canals were checked for patency and working length was determined by shortening the distance to the anatomical foramen by 1 mm. The apex was sealed with a pink wax piece.

The root canals of experimental teeth were instrumented using the *step back* technique to the instrument size 40 (NiTi files I-FLEX, IMD, USA). Only one half of each root canal, either the facial or the lingual half, was particularly marked and instrumented [11]. During the instrumentation, care was taken that endodontic instruments did not come in contact with the oposing side of the canal wall that represented the "uninstrumented" half. The control sample from each group was used for comparison of uninstrumented areas of the canal with the uninstrumented main root canal following identical irrigation regimens. The amount of the irrigant used for each irrigation regimen was identical and carefully controlled, and the total time of chemomechanical preparation was 10 min.

The following irrigation regimens were used: (I) saline; (II) 3% sodium hypochlorite (NaOCl-*Parcan,Septodont*); (III) 15% Ethylene diaminetetraacetic acid (EDTA - *Largal Ultra, Septodont*); (IV) combination of 3% NaOCl+15% EDTA; (V) combination of 3% NaOCl+15% EDTA and 3% NaOCl as the final irrigant; (VI) combination of 3% NaOCl+15% EDTA and 2% chlorhexidine (*R4, Septodont*) as the final irrigant (Table 1).

Applying mild pressure and using a spatula, the samples were fractured in half (so that a total of 36 samples were obtained) and placed in open Petri dishes to dry at the room temperature. After 24 hours, they were attached to cylindrical tooth carriers using a fixing agent (*Dotite paint xc 12 Carbon JEOL, Tokyo, Japan*), gold sputtered (using a *JFC 1100E Ion Sputter JEOL*) and analyzed using scanning electron microscopy (*SEM, JEOL-JSM-5300).*

Table 1. Irrigants and irrigation regimens of the experimental and control samples
Tabela 1. Irigacioni rastvori i protokol irigacije eksperimentalnih i kontrolnih uzoraka

Groups Grupe	Irrigants Irigacioni rastvori	Irrigation regimen of the experimental samples (uninstrumented areas in uninstrumented canals)* Irigacioni protocol eksperimentalnih uzoraka (neinstrumentisane površine u instrumentisanim kanalima)*	Irrigation regimen of the control samples (uninstrumented canals)* Irigacioni protocol kontrolnih uzoraka (neinstrumentisani kanali)*
I	Saline solution	Irrigation using 3 ml saline solution after pulp extirpation and after each endodontic instrument Irigacija sa 3 ml fiziološkog rastvora posle ekstirpacije pulpe i posle svakog endodontskog instrumenta	Irrigation using 3 ml saline solution following pulp extirpation Irigacija sa 3 ml fiziološkog rastvora posle ekstirpacije pulpe
II	3% NaOCl (*Parcan, Septodont*)	Irrigation using 3 ml NaOCl following pulp extirpation and after each endodontic instrument Irigacija sa 3 ml NaOCl posle ekstirpacije pulpe i posle svakog endodontskog instrumenta	Irrigation using 3 ml NaOCl following pulp extirpation Irigacija sa 3 ml NaOCl posle ekstirpacije pulpe
III	15% EDTA (*Largal Ultra, Septodont*)	Irrigation using 3 ml saline solution following pulp extirpation and after each endodontic instrument, final irrigation with 3 ml EDTA for 60 seconds Irigacija sa 3 ml fiziološkog rastvora posle ekstirpacije pulpe i posle svakog endodontskog instrumenta; završno ispiranje sa 3 ml EDTA u trajanju od 60 sekundi	Irrigation using 3 ml saline solution following pulp extirpation, final irrigation using 3 ml EDTA for 60 seconds Irigacija sa 3 ml fiziološki rastvora posle ekstirpacije pulpe; završno ispiranje sa 3 ml EDTA u trajanju od 60 sekundi
IV	3% NaOCl (*Parcan, Septodont*) + 15% EDTA (*Largal Ultra, Septodont*)	Irrigation using 3 ml NaOCl following pulp extirpation and after each endodontic instrument; final irrigation using 3 ml EDTA for 60 seconds Irigacija sa 3 ml NaOCl posle ekstirpacije pulpe i posle svakog endodontskog instrumenta; završno ispiranje sa 3 ml EDTA u trajanju od 60 sekundi	Irrigation using 3 ml NaOCl following pulp extirpation; final irrigation using 3 ml EDTA for 60 seconds Irigacija sa 3 ml NaOCl posle ekstirpacije pulpe; završno ispiranje sa 3 ml EDTA u trajanju od 60 sekundi
V	3% NaOCl+15% EDTA and 3% NaOCl as the final irrigant / kao završni irigans	Irrigation using 3 ml NaOCl following pulp extirpation and after each endodontic instrument; flushing using 3 ml EDTA for 60 seconds, final irrigation using 3 ml NaOCl for 3 min. Irigacija sa 3 ml NaOCl posle ekstirpacije pulpe i posle svakog endodontskog instrumenta; ispiranje sa 3 ml EDTA u trajanju od 60 sekundi, završno ispiranje sa 3 ml NaOCl u trajanju od 3 min.	Irrigation using 3 ml NaOCl following pulp extirpation; flushing using 3 ml EDTA for 60 seconds, final irrigation using 3 ml NaOCl for 3 min. Irigacija sa 3 ml NaOCl posle ekstirpacije pulpe; ispiranje sa 3 ml EDTA u trajanju od 60 sekundi, završno ispiranje sa 3 ml NaOCl u trajanju od 3 min.
VI	3% NaOCl+15% EDTA and 2% chlorhexidine (*R4, Septodont*) as the final irrigant / kao završni irigans	Irrigation using 3 ml NaOCl following pulp extirpation and each endodontic instrument; flushing using 3 ml EDTA for a period of 60 seconds, final irrigation using 3 ml CHX for 3 min. Irigacija sa 3 ml NaOCl posle ekstirpacije pulpe i posle svakog endodontskog instrumenta; ispiranje sa 3 ml EDTA u trajanju od 60 sekundi, završno ispiranje sa 3 ml CHX u trajanju od 3 min.	Irrigation using 3 ml NaOCl following pulp extirpation; flushing using 3 ml EDTA for 60 seconds, final irrigation using 3 ml CHX for 3 min. Irigacija sa 3 ml NaOCl posle ekstirpacije pulpe; ispiranje sa 3 ml EDTA u trajanju od 60 sekundi, završno ispiranje sa 3 ml CHX u trajanju od 3 min.

*At the end of all the irrigation regimens, as well as after irrigation with each tested irrigant, sterile water was used [11] in the amount of 3 ml, and canal was dried with sterile paper points prior to the use of the following instrument/irrigant.

* Kod svih irigacionih protokola je na kraju, kao i posle irigacije sa svakim testiranim irigansom, korišćena sterilna voda [11] u količini od 3 ml, a kanal je sušen papirnatim poenima pre korišćenja sledećeg instrumenta/irigansa.

Table 2. SEM analysis of uninstrumented and instrumented areas in the root canal after different irrigation protocols
Tabela 2. SEM opis neinstrumentisanih i instrumentisanih površina u kanalu korena posle različitih irigacionih protokola

Groups Grupe	Irrigants Irigacioni rastvori	Uninstrumented areas in uninstrumented canals Neinstrumentisane površine u instrumentisanim kanalima	Instrumented canals Instrumentisani kanali
I	Saline solution Fiziološki rastvor	Amorphous layer of residual pulpal components found, along with predentin and pulpal debris, tubules barely visible Prisutan amorfni sloj zaostalih komponenata pulpe, predentin i pulpni debris, kanalići se jedva uočavaju	Smear layer and dentin debris, tubules not visible at all. Razmazni sloj i dentinski debris, kanalići se uopšte ne uočavaju
II	NaOCl	Absence of predentin and pulpal debris (pulpal remnants), presence of dome-shaped and ridge-shaped calcospherites, noted orifices of the tubules, rough surface of calcospherites. Odsutan predentin i pulpni debris (pulpni ostaci), prisutni kupolasti i grebenasti kalcisferiti, uočavaju se otvori kanalića, površina kalcisferita gruba	Flat surface of dentin wall, smear layer and dentin debris present, some orifices of dentinal tubules visible Ravna površina dentinskog zida, prisutan razmazni sloj i dentinski debris, nazire se po neki otvor dentinskih kanalića
III	EDTA	There are no calcospherites, pulpal debris was found, dentin present as an undulating surface, open tubules Ne uočavaju se kalcisferiti, nađeni delovi pulpnog debrisa, talasasta površina dentinskog zida, otvoreni tubuli	Flat surface of the dentin wall, removed smear layer, debris present Ravna površina dentinskog zida, uklonjen razmazni sloj, prisutan debris
IV	NaOCl + EDTA	Reduced calcospherites with smooth surface. No predentin and pulpal debris, open tubules, slightly wider, clearly visible. Smanjeni kalcisferiti sa glatkom površinom. Odsutan predentin i pulpni ostaci, kanalići otvoreni, blago prošireni, jasno uočljivi	Flat surface of dentin, removed smear layer and dentin debris, tubules wide open Ravna površina dentinskog zida, uklonjen razmazni sloj i dentinski debris, kanalići široko otvoreni
V	NaOCl + EDTA + NaOCl	Greater reduction of calcospherites in comparison to previous irrigation regimen (V). No predentin and pulpal debris. Tubules wide open, significantly enlarged, excessive erosion of intertubular dentin Veća redukcija kalcisferita u odnosu na prethodni irigacioni protokol (V). Odsutni predentin i pulpni ostaci. Kanalići široko otvoreni, znatno uvećani, izrazita erozija intertubularnog dentina	Flat surface of dentin wall, removed smear layer and dentin debris, tubular orifices, openings enlarged, excessive erosion of intertubular and peritubular dentin Ravna površina dentinskog zida, uklonjen razmazni sloj i dentinski debris, otvori kanalića uvećani, izrazita erozija intertubularnog i peritubularnog dentina
VI	NaOCl +EDTA + CHX	Calcospherites reduction. Absence of predentin and pulpal remnants, open tubules clearly visible, intertubular dentin preserved. No difference compared to irrigation regimen IV Redukcija kalcisferita. Odsutni predentin i ostaci pulpe, kanalići otvoreni, jasno uočljivi intertubularni dentin očuvan. Nije uočena razlika u odnosu na irigacioni protokol IV	Flat surface of dentin wall, removed smear layer and dentin debris, open dentinal tubules, intertubular dentin preserved Ravna površina dentinskog zida sa uklonjenim razmaznim slojem i dentinskim debrisom, otvoreni dentinski kanalići, intertubularni dentin očuvan

RESULTS

After irrigation with saline pulpal debris covering dentin of uninstrumented areas of the canal was noticed. Irrigation with 3% NaOCl left behind dentin with different forms of calcospherites. When EDTA was used alone for irrigation dentin was present as an undulating surface and open tubules without a smear layer were visible. Combination of NaOCl and EDTA for irrigation left remnants of calcospherites and open and slightly widened dentinal tubules. If NaOCl was used as the final irrigant (after NaOCl and EDTA) enlarged dentinal tubules were noted along with dentinal erosion, while if the final irrigant was CHX, clean dentin and open dentinal tubules without smear layer were noted.

SEM findings on uninstrumented and instrumented areas are shown in Table 2 and Figures 1-7. Wall morphology of uninstrumented areas in instrumented root canals did not show any differences compared to the morphology of uninstrumented canals (control samples) following all irrigation regimens.

DISCUSSION

The aim of this study was to analyze the morphology of uninstrumented areas of the root canal walls after canal instrumentation using SEM. Several studies used micro-computerized tomography to determine the presence of uninstrumented surfaces in the main root canal by calculating the area that remains intact after instrumentation (canal volume before and after instrumentation, distance between canal surface before and after instrumentation in µm, the size of a specific area, the width of the canal, taper, etc.) [3-6]. On the other hand, SEM analysis allows visualization of root canal walls, their cleanliness, dentinal tubules covered with smear layer, as well as complete dentin morphology at ultrastructural level [13-15].

In the current study the control samples included uninstrumented canals after performed irrigation regimens. That way it was possible to compare the morphology of uninstrumented canals with uninstrumented surfaces of instrumented canals. According to Peters et al. after biomechanical instrumentation, both hand or rotary, approximately 35% of the canal wall remains untouched by the instruments [16]. In addition, other studies have also confirmed the presence of uninstrumented surfaces, especially in the apical third of the root canal, where any irregularities on canal walls (grooves and depressions) prevent contact between the wall and instrument [17, 18]. Endodonic instruments are mostly designed to fit into the conical root configuration, which leaves untreated regions in oval and flat canals [16]. Beside complex canal morphology [19], limitation of instrumentation techniques

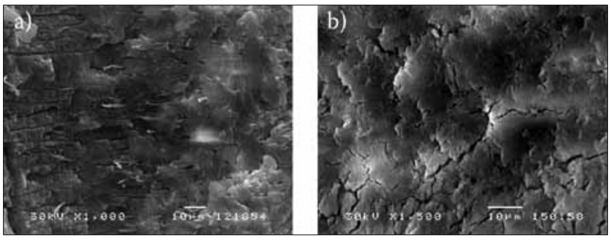

Figure 1. Saline solution. a) uninstrumented area – remnants of pulpal components, parts of odontoblasts, noticeable elongated dentinal tubules; b) instrumented area with a tree-bark model of smear layer which appears in both hand and rotary root canal instrumentation [9].

Slika 1. Fiziološki rastvor. a) neinstrumentisana površina – zaostale komponente pulpe, delovi odontoblasta, uočljivi uzdužno presečeni dentinski kanalići; b) instrumentisana površina sa tree-bark modelom razmaznog sloja koji se pojavljuje i kod ručne i kod mašinske obrade kanala korena [12].

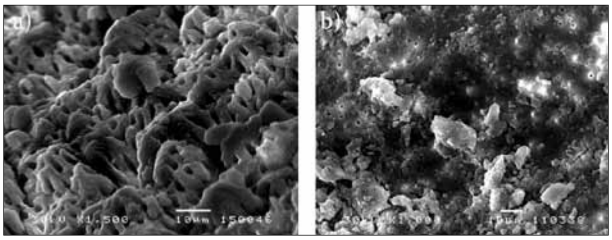

Figure 2. NaOCl. a) uninstrumented area – dome-shaped calcospherites with a grainy, uneven surface; b) instrumented area – smear layer covers dentin, with barely visible tubular orifices

Slika 2. NaOCl. a) neinstrumentisana površina – kupolasti kalcisferiti sa sitno zrnastom, neravnom površinom; b) instrumentisana površina – razmazni sloj pokriva dentinsku površinu, jedva uočljivi otvori dentinskih kanalića

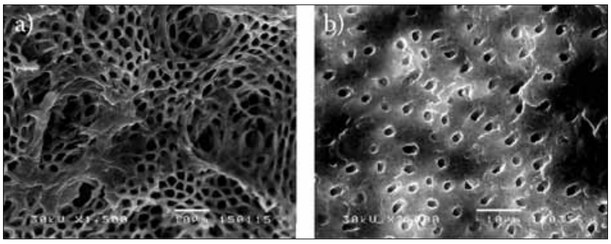

Figure 3. EDTA. a) uninstrumented area – groovy surface of dentin with no calcospherites; b) instrumented area – removed smear layer, but with presence of debris

Slika 3. EDTA. a) neinstrumentisana površina – talasasta površina dentina bez kalcisferita; b) instrumentisana površina – uklonjen razmazni sloj, ali prisutan debris

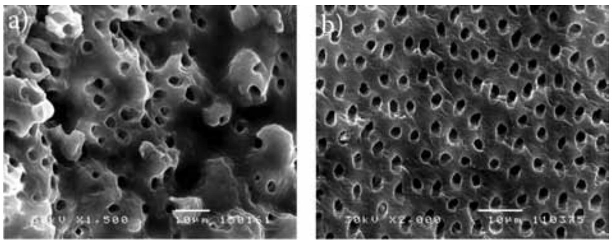

Figure 4. NaOCl + EDTA. a) uninstrumented area – a reduction in calcospherites, no organic debris; b) instrumented area – root canal wall with removed debris and smear layer

Slika 4. NaOCl + EDTA. a) neinstrumentisana površina – redukcija kalcisferita, nema organskog debrisa; b) instrumentisana površina – zid kanala korena sa uklonjenim debrisom i razmaznim slojem

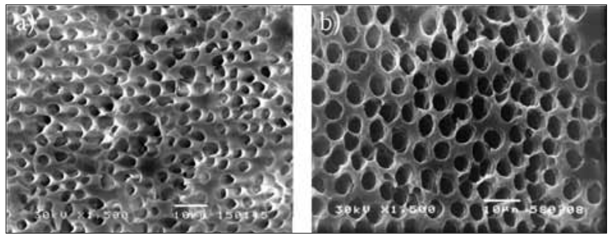

Figure 5. NaOCl + EDTA + NaOCl. a) uninstrumented area – apronounced reduction in calcospherites with a funnel-like widening on dentinal tubules, no organic debris; b) instrumented area **–** root canal wall with removed debris and smear layer, but with intratubular dentin which has worn away. Dentin erosion in some areas connects two or more orifices of the dentin tubules.

Slika 5. NaOCl + EDTA + NaOCl. a) neinstrumentisana površina – izrazita redukcija kalcisferita sa levkasto proširenim dentinskim kanalićima, odsutan organski debris; b) instrumentisana površina – zid kanala korena sa uklonjenim debrisom i razmaznim slojem, ali i sa istanjenim intratubularnim dentinom. Dentinska erozija na nekim mestima spaja dva ili više otvora dentinskih kanalića.

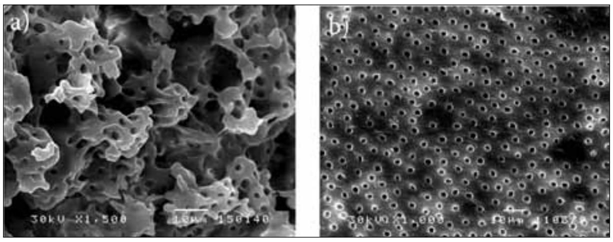

Figure 6. NaOCl + EDTA + CHX. a) uninstrumented area-moderately reduced calcospherites, some of which have retained their dome-shaped form; b) instrumented area – root canal wall with removed debris and smear layer, no erosion of intratubular and peritubular dentin.

Slika 6. NaOCl + EDTA + CHX. a) neinstrumentisana površina – umereno redukovni kalcisferiti, pojedini su zadržali kupolasti oblik; b) instrumentisana površina – zid kanala korena sa uklonjenim debrisom i razmaznim slojem, nema erozije intratubularnog i peritubularnog dentina.

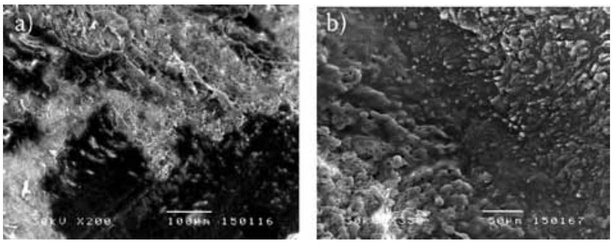

Figure 7. a) The darker areas on the micrography represent instrumented surfaces; b) An ultrastructural appearance of the uninstrumented root canal following irrigation using NaOCl solution, magnified at 350×. On dentin walls dome-shaped and ridge-shaped calcospherites are noted.

Slika 7. a) tamna polja na mikrografiji predstavljaju instrumentisane površine; b) ultrastrukturni izgled neinstrumentisanog kanala korena posle irigacije sa NaOCl na uvećanju 350×. Na dentinskim zidovima se uočavaju kupolasti i grebenasti kalcisferiti.

[20], instrument taper [21] or file alloy properties [22] add to impossibility to instrument all canal walls.

In the current study we assumed that uninstrumented surfaces in the main root canal actually exist, which is why biomechanical instrumentation was performed with the intention of leaving half of the root canal uninstrumented. On the other hand, instrumented areas of the root canal showed surfaces with expected morphology and more-less clean wall surfaces following certain irrigation regimens as reported in other studies [13-15]. In our study we analyzed only the coronal and middle third of the canal, while the apical third was excluded due to its complexity and possible presence of a smear layer even after irrigation that could influence the interpretation of obtained results.

Uninstrumented areas of the canal were difficult to notice prior to irrigation with NaOCl that removed organic debris and exposed conical and wedge-shaped calcospherites. Structures that were found on uninstrumented areas included pulpal tissue remnants, odontoblastic extensions, but no smear layer was found. In the current study 3% NaOCl solution was used and completely removed organic debris. In studies where canals were irrigated with 0.5% NaOCl solution, dentin of uninstrumented areas was not completely cleaned of organic debris [23].

According to the findings of many studies, NaOCl irrigation is exceptionally important because it dissolves organic tissue very efficiently. Even though it has an inadequate surface tension and cannot reach narrow canals, NaOCl can effectively clean uninstrumented areas of the main canal that consist of predentin, necrotic pulpal tissue and a bacterial biofilm [7, 24].

Following irrigation regimens IV, V, and VI (NaOCl+EDTA; NaOCl+EDTA+NaOCl; NaOCl +EDTA+CHX) uninstrumented surfaces showed more or less reduced calcospherites that was also confirmed in other studies. However, some studies have not found calcospherites after the same irrigation regimens [11,18].

In the current study, following irrigation regimen V (NaOCl+EDTA+NaOCl), erosion of intertubular and peritubular dentin occurred on both uninstrumented and instrumented surfaces. Most likely NaOCl was not able to prevent demineralizing effect of EDTA on peritubular and intertubular dentin due to its slow degradation [25]. In addition, there was an interaction between EDTA and NaOCl that manifested in sudden decrease in the amount of free chlorine causing loss of NaOCl activity and inability to dissolve soft tissue within the canal [26]. In our study no organic debris was noted after this irrigation regimen, but many authors do not recommend the use of NaOCl as the final irrigant (after EDTA) due to possible dentinal erosion [25, 27].

The literature reports interaction between irrigants that can be manifested as mutual inactivation, coloring of dentin or creation of harmful precipitation [28-30]. Therefore, flushing canals with sterile water between each irrigant is recommended, as well as drying the canal prior to introduction of a new irrigant [26, 29]. In the current study, these recommendations were followed in order to prevent any unwanted interactions between the irrigants and obtain desired result [11].

Following irrigation regimen VI (NaOCl+EDTA+CHX) no dentinal erosion was noted. According to the literature, when EDTA and CHX come into contact EDTA anion is neutralized with CHX cation and there is no further reduction in dentin [30]. In addition, antimicrobial effect of CHX against *Enterococcus faecalis* and *Candida albicans* as well as its substantivity (prolonged effect) support its use as the final irrigant in endodontic treatment [24].

CONCLUSION

Taking into consideration limitations of all *in vitro* studies, the following can be concluded:

The morphology of uninstrumented areas of main root canal is similar to the morphology of those parts of the canal endodontic instruments cannot reach (narrowings, lateral canals, anastomosis, invagination of the root canal, etc.).

The presence of uninstrumented areas in the root canal during endodontic instrumentation is inevitable due to the complex morphology of the canals and indicates the importance of irrigants use during instrumentation.

Even though this was not the primary aim of this study, the most favorable effect of irrigation (including instrumented and uninstrumented areas of the canal) was noted following the irrigation regimen: NaOCl+EDTA, or even better using NaOCl+EDTA+CHX as the final irrigant.

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Ultrastrukturna analiza neinstrumentisanih površina u kanalu korena posle različitih irigacionih protokola

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KRATAK SADRŽAJ

Uvod Tokom endodontske terapije manje ili veće površine kanala korena zuba ostaju neinstrumentisane i mogu sadržavati bakterije, što može dovesti do neuspešnog ishoda. Cilj rada je bio da se skening-elekron-mikroskopskom (SEM) analizom proceni morfologija neistrumentisanih delova zidova kanala posle instrumentacije i primene različitih irigacionih protokola.

Materijal i metode rada Osamnaest jednokorenih ekstrahovanih zuba je podeljeno u šest grupa. Jedan zub iz svake grupe je predstavljao kontrolni uzorak. Eksperimentalni uzorci su preparisani do instrumenta veličine ISO40. Kod svakog zuba je instrumentisana samo jedna polovina kanala. Kontrolni uzorci su podvrgnuti samo irigacionim protokolima bez preparacije. Osim fiziološkog rastvora, 3% natrijum-hipohlorita i 15% etilen-diamin-tetra-acetata, koji su primenjeni samostalno, eksperimentalni uzorci su irigirani i sa kombinacijom NaOCl i EDTA, kao i sa kombinacijom istih iriganasa, pri čemu je kao završni irigans korišćen ili NaOCl ili hlorheksidin. Korenovi su uzdužno presečeni i pripremljeni za SEM.

Rezultati Posle irigacije sa fiziološkim rastvorom u neinstrumentisanim delovima kanala uočen je dentin pokriven debrisom, posle irigacije sa NaOCl dentin sa različitim oblicima kalcisferita. Posle irigacije sa EDTA uočena je talasasta površina dentinskog zida sa otvorenim tubulima bez razmaznog sloja. Irigacija kombinacijom NaOCl i EDTA je pokazala ostatke kalcisferita i otvorene, blago proširene dentinske tubule. Završno ispiranje sa NaOCl na neinstrumentisanim delovima dentina pokazuje proširene dentinske tubule i eroziju dentina, a završno ispiranje sa CHX čist dentin i otvorene dentinske tubule bez razmaznog sloja.

Zaključak Sa morfološkog aspekta, najpovoljniji efekat irigacije (i kod instrumentisanih i kod neinstrumentisanih delova kanala) ustanovljen je posle irigacionih protokola sa: NaOCl i EDTA i NaOCl, EDTA i hlorheksidinom kao završnim irigansom.

Ključne reči: preparacija kanala; neinstrumentisane površine kanala; irigacija kanala; SEM

UVOD

Jedan od osnovnih preduslova za uspeh endodontskog lečenja je adekvatna preparacija kanala korena zuba. Međutim, instrumentaciju i irigaciju je uglavnom teško realizovati zbog vrlo specifične i kompleksne kanalne morfologije, ali i ograničenog efekta instrumenata u nepristupačnom i ograničenom prostoru [1].

Morfološke varijacije kanalnog sistema i nemogućnost endodontskog instrumenta da dopre do svih delova zidova kanala korena potpuno čišćenje kanala čine praktično nemogućim [1, 2]. Nekoliko studija je primenom mikrokompjuterizovane tomografije potvrdilo da tokom preparacije kanala deo zidova ostaje potpuno neobrađen [3-6]. Na ovim nepristupačnim površinama zidova kanala mogu se zadržavati bakterije i time ugroziti i kompromitovati endodontsko lečenje [7].

Osim toga, i prisustvo razmaznog sloja i debrisa kao posledica instrumentacije i sečenja dentina predstavlja značajan klinički problem [2, 8]. Ovaj sloj često sadrži bakterije i blokira dentinske tubule, čime značajno umanjuje efekat sredstava za irigaciju, odnosno značajno utiče na kvalitet opturacije i ishod endodontskog lečenja [8, 9].

Zbog ograničene efikasnosti endodontskih instrumenata u čišćenju kanala neophodno je tokom i posle instrumentacije obavezno koristiti i odgovarajuća hemijska sredstva. Njihova uloga je u eliminaciji i redukciji zaostalih mikroorganizama, odnosno u efikasnom čišćenju kanalnog sistema, kao i rastvaranju i uklanjanju razmaznog sloja [8-10].

Iako ne postoji opšta saglasnost oko uklanjanja razmaznog sloja neposredno pre opturacije, najveći broj endodonata je saglasan da, ukoliko se on ne ukloni, vremenom može doći do njegove dezintegracije i povećanog kruničnog mikropropuštanja, usled slabijeg kvaliteta veze materijala za opturaciju sa zidovima kanala korena [9, 10].

Cilj ovog rada je bio da se SEM analizom proceni morfologija neinstrumentisanih delova zidova kanala korena posle mehaničke instrumentacije i primene različitih protokola za irigaciju.

MATERIJAL I METODE RADA

Kao materijal u ovom istaživanju korišćeno je 18 sveže ekstrahovanih intaktnih jednokorenih humanih zuba bez vidljivih oštećenja (karijes korena, pukotine, unutrašnje ili spoljašnje resorpcije itd.). U odnosu na irigacioni protokol, svi zubi su podeljeni u šest grupa, pri čemu je po jedan zub iz svake predstavljao kontrolu. Uzorci zuba su čuvani osam časova u 0,5% NaOCl da bi se lakše uklonili ostaci organskog tkiva. Potom su čišćeni, ispirani tekućom vodom, potopljeni u fiziološki rastvor i čuvani u frižideru do početka eksperimenta.

Pre instrumentacije kanala pažljivo su dijamantskim diskom napravljeni žlebovi po uzdužnoj osovini korena (vestibularno i oralno), ali bez kontakta sa kanalom, kako bi se kasnije olakšalo razdvajanje polovina. Krunice zuba su uklonjene, a debris nastao prilikom presecanja je ispran u tekućoj vodi.

Posle uklanjanja sadržaja kanala pulp ekstirpatorima, po jedan zub (dva kontrolna uzorka) iz svake grupe je podvrgnut samo irigacionim protokolima bez prethodne preparacije.

Na korenovima ostalih zuba izvršena je provera prohodnosti, a radna dužina preparacije je određivana skraćivanjem za 1 mm od dužine endodontske igle kada vrh igle dosegne apeksni otvor. Apeks svakog uzorka zuba je potom zapečaćen kuglicom roze voska.

Korenovi eksprimentalnih zuba su preparisani *step back* tehnikom instrumentima do dijametra ISO40 (NiTi I-FLEX, IMD, USA). Posebno je obeležena i instrumentisana samo jedna polovina kanala korena (vestibularna ili oralna) [11]. Pri preparaciji se vodilo računa da endodontski instrumenti ne dođu u kontakt sa drugim delom kanala koji je predstavljao „neinstrumentisanu" polovinu kanala korena. Kontroloni uzorak iz svake grupe je služio za poređenje neinstrumentisanih delova kanala sa neinstrumentisanim kanalom korena posle identičnih irigacionih protokola.

Količina irigansa koja je korišćena sa svaki irigacioni protokol bila je identična i pažljivo kontrolisana, kao i ukupno vreme hemomehaničke obrade svakog kanala (10 min).

U eksperimentu je korišćeno šest protokola ispiranja: (I) samo fiziološki rastvor; (II) samo 3% natrijum-hipohlorit (NaOCl-*Parcan, Septodont*); (III) samo 15% etilen-diamin-tetraacetat (EDTA – *Largal Ultra, Septodont*); (IV) kombinacija 3% NaOCl+15% EDTA; (V) kombinacija 3% NaOCl+15% EDTA i 3% NaOCl kao završni irigans; (VI) kombinacija 3% NaOCl+15% EDTA i 2% hlorheksidin (*R4, Septodont*) kao završni irigans. (Tabela 1).

Uz blagi pritisak, uzorci su pomoću špatule podeljeni na polovine (tako je dobijeno 36 uzoraka) i stavljeni u otvorene Petrijeve šolje da bi se sušili na sobnoj temperaturi. Posle 24 časa uzorci su pričvršćeni za cilindrične nosače sredstvom za fiksiranje (*Dotite paint xc 12 Carbon JEOL, Tokyo, Japan*), naparavani tankim slojem zlata po površini (u uređaju *JFC 1100E Ion Sputter JEOL*) i analizirani skening elektronskim mikroskopom (*SEM, JEOL-JSM-5300).*

REZULTATI

Dobijeni rezultati SEM analize su pokazali da je u neinstrumentisanim delovima kanala posle irigacije sa fiziološkim rastvorom uočen dentin pokriven pulpnim debrisom, posle irigacije sa 3% rastvorom NaOCl uočen dentin sa različitim oblicima kalcisferita, a posle irigacije sa EDTA uočena je talasasta površina dentinskog zida i otvoreni tubuli bez razmaznog sloja. Kada je korišćena kombinacija NaOCl i EDTA, uočeni su ostaci kalcisferita i otvoreni i blago prošireni dentinski tubuli. Kada je kao završni irigans korišćen NaOCl na neinstrumentisanim delovima dentina, uočeni su uvećani dentinski tubuli i erozija dentina, a kada je kao završni irigans korišćen CHX – čist dentin i otvoreni dentinski tubuli bez razmaznog sloja.

Rezultati SEM neinstrumentisanih i instrumentisanih površina su prikazani u tabeli 2 i na mikrografijama od 1 do 10.

Morfologija zidova neinstrumentisanih delova preparisanog kanala korena nije pokazivala razlike u odnosu na morfologiju neinstrumentisanih kanala (kontrolne uzorke) posle svih irigacionih protokola.

DISKUSIJA

U ovom istraživanju je bio cilj da se SEM-om ispita morfologija neinstrumentisanih delova zidova kanala korena tokom obrade kanala. Nekoliko studija je metodom mikrokompjuterizovane tomografije ustanovilo prisustvo neinstrumentisanih polja u glavnom korenskom kanalu izračunavanjem površine koja ostaje netaknuta tokom tretmana (meren je volumen kanala pre i posle instrumentacije, rastojanje između površine kanala pre i posle preparacije u µm, površina određenog polja, debljina kanala, koničnost itd.) [3-6]. Za razliku od toga, SEM analizom je omogućena bolja vizuelizacija kvaliteta čišćenja zidova kanala korena, pokrivenost dentinskih tubula razmaznim slojem, kao i kompletna morfologija dentina na ultrastrukturnom nivou [13-15].

Ovo istraživanje je koristilo kao kontrolne uzorke neinstrumentisane zidove kanala koji su predstavljali model ultrastrukture dentinskog zida posle određenih irigacionih protokola. Na ovaj način je bilo moguće porediti morfologiju neinstrumentisanih kanala sa neinstrumentisanim površinama preparisanih kanala. Prema Petersu i sar., u toku procesa širenja i oblikovanja, bilo mašinskim ili ručnim instrumentima, oko 35% površine kanala ostaje neinstrumentisano [16]. Takođe, i druge studije potvrđuju prisustvo neinstrumentisanih polja, naročito u apeksnoj trećini u kojoj nepravilnosti na zidovima kanala (žlebovi i udubljenja) sprečavaju kontakt između zida i instrumenata [17, 18]. Endodontski instrumenti su uglavnom dizajnirani tako da se uklope u konusnu konfiguraciju korena, što ovalne ili spljoštene kanale ostavlja sa nepreparisanim poljima [16]. Drugi autori postojanje neinstrumentisanih regija opravdavaju pre svega kompleksnom kanalnom morfologijom [19], ograničenošću instrumentacionih tehnika [20], koničnošću instrumenata [21] ili osobinama legure od koje su izrađeni instrumenti [22].

U ovom istraživanju se pošlo od pretpostavke da neinstrumentisane površine u glavnom korenskom kanalu zaista postoje, zbog čega je mehanička instrumentacija urađena sa namerom da se polovina zida korenskog kanala ostavi bez mehaničke obrade. S druge strane, instrumentisani delovi u kanalu korena su pokazali površinu sa očekivanom morfologijom manje ili više čistih zidova posle određenih irigacionih protokola, kako je objavljeno i u drugim studijama [13-15]. U ovom istraživanju su analizirane samo cervikalna i srednja trećina kanala, dok je izostavljena apeksna trećina, koja bi zbog svoje kompleksnosti i mogućeg prisustva razmaznog sloja i posle irigacionih protokola mogla uticati na tumačenje dobijenih rezultata.

Neinstrumentisane oblasti zidova kanala je bilo teško uočiti pre irigacije sa NaOCl, koji je uklonio organske ostatke i prikazao kupolaste ili grebenaste kalcisferite. Na neinstrumentisanim površinama su nađene strukture koje su zbog svoje pozicije ukazivale na ostatke pulpnog tkiva, ili čak delove odontoblastičnih produžetaka, ali na njima nije bilo razmaznog sloja. U ovom istraživanju je korišćen 3% NaOCl, koji je kompletno uklonio organske ostatke. U studijama gde je kanal korena irigiran sa 0,5% NaOCl, površine dentinskog zida neinstrumentisanih oblasti nisu bile u potpunosti očišćene od organskih ostataka [23].

Prema nalazima većine studija, korišćenje NaOCl tokom instrumentacije je izuzetno važno jer ovaj irigans dobro rastvara organsko tkivo u kanalnom sistemu korena. Iako ima neadekvatan površinski napon i ne može da prodre do uskih i akcesornih kanala, NaOCl efikasno „čisti" neistrumentisane delove glavnog kanala, koji se sastoje od predentina, nekrotičnog pulpnog tkiva i bakterijskog biofilma [7, 24].

Posle irigacionih protokola IV (NaOCl i EDTA), V (NaOCl + EDTA i NaOCl kao završni irigans) i VI (NaOCl + EDTA i CHX kao završni irigans), na neinstrumentisanim površinama nađeni su manje ili više redukovani kalcisferiti, što je u skladu sa drugim istraživanjima, iako u nekim studijama posle identičnih protokola irigacije kalcisferiti nisu ni uočeni [11, 18].

U ovom istraživanju, posle irigacionog protokola V, gde je kao završni irigans korišćen NaOCl, došlo je do erozije intertubularnog i peritubularnog dentina i kod neinstrumentisanih i kod instrumentisanih polja. Ovo se može objasniti time što

NaOCl nije mogao da spreči demineralizujuće dejstvo EDTA na peritubularni i intertubularni dentin jer dovodi do veoma spore degradacije ovog helatora [25]. Pored toga, postoji interakcija između EDTA i NaOCl, koja se ogleda u naglom smanjenju količine slobodnog hlora odmah pri kontaktu ovih iriganasa, što može da ima za posledicu gubitak aktivnosti NaOCl i nemogućnost rastvaranja mekog tkiva unutar kanala [26]. U ovom istraživanju nije primećen ni organski debris posle primene ovog protokola, ali mnogi autori ne preporučuju NaOCl kao finalni irigans (posle EDTA) zbog moguće erozije dentina [25, 27].

Literaturni podaci ukazuju i na postojanje interakcije između iriganasa, koje se mogu ispoljiti međusobnom inaktivacijom, prebojavanjem dentina ili stvaranjem štetnih precipitata [28-30], zbog čega se preporučuje ispiranje kanala sa destilovanom (sterilnom) vodom između svakog irigansa i sušenje kanala pre uvođenja novog rastvora za irigaciju [26, 29, 30]. U protokolu ovog istraživanja su uvažene ove preporuke, kako bi se sprečile neželjene interakcije između iriganasa i pritom dobio adekvatan rezultat [11].

Posle irigacionog protokola VI (NaOCl + EDTA i CHX kao završni irigans) nije uočena erozija dentina. Prema podacima iz literature, pri kontaktu EDTA i CHX dolazi do neutralizacije anjonskog EDTA pomoću katjonskog CHX, zbog čega nema dalje redukcije dentina [30]. Pored toga, antimikrobni efekat CHX (*enteroccocus faecalis* i *Candida albicans*), kao i osobina supstantivnosti ovog irigansa (protrahirani efekat), opravdavaju njegovu upotrebu kao završnog irigansa u endodontskom tretmanu [24].

ZAKLJUČAK

Uprkos ograničenjima karakteristišnim za sva *in vitro* istraživanja, na osnovu dobijenih rezultata može se zaključiti sledeće:

- Morfologija neinstrumentisanih delova glavnog korenskog kanala je slična morfologiji onih delova kanala do kojih endodontski instrumenti ne mogu dopreti (suženja, bočni kanali, anastomoze, invaginacije zidova korena itd.).
- Prisustvo neinstrumentisanih površina kanala korena tokom endodontske instrumentacije je neizbežno zbog kompleksne morfologije kanala i ukazuje na važnost adekvatne primene hemijskih sredstava i dezinfekcije svih oblasti kanalnog sistema.
- Iako to nije bio primarni cilj istraživanja, najpovoljniji efekat irigacije (i kod instrumentisanih i kod neinstrumentisanih delova kanala) uočen je posle primene irigacionih protokola sa: NaOCl i EDTA, odnosno NaOCl, EDTA i CHX kao završnim irigansom.

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Linear measurements of facial morphology using automatic aproach

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SUMMARY

Introduction Clinical extraoral examination prior to orthodontic treatment includes face analysis (front and profile). Development of computer technology has increased efficacy and simplified this process through automating several steps of the analysis. The aim of this paper was to examine the possibility of automatic determining of linear measurements based on the facial image of a patient.

Material and Methods Based on the set of 20 patients in NHP (Natural Head Position) position, three sets of measurements were conducted. Trained orthodontist performed positioning of predefined points on the image of the patient two times with one week apart, after which the points were automatically determined using customized computer software. Based on the position of the points, measurements for bizygomatic distance, upper and lower facial height and full facial height were computed. Three sets of measurements were compared and statistically analyzed.

Results showed that computer software produced measurements comparable to measurements obtained by a trained orthodontist. Statistical analysis included calculating mean values and standard deviations, as well as paired two-tailed T-test. Differences between measurements ranged from 0.03% to 0.6% suggesting that automatic method can be successfully used.

Conclusions The results of this research suggest that it is possible to ease, accelerate and automate work of the orthodontist on the image analysis using suitable software without significant differences in measured values.

Keywords: computer analysis; standard analysis; frontal head photography

INTRODUCTION

Human face analysis represents both art and science. In order to assess the characteristics of human face, various measurements are used, such as: anthropometry, cephalometry and photogrammetry. Application of photogrammetry in orthodontics was first suggested by Stoner who compared profiles before and after the orthodontic treatment with ideal profiles [1]. Taking into consideration ethnic characteristics, gender, and age, it becomes clear that one culture's concept of beautiful and acceptable can be seen differently in other cultures [2, 3, 4]. By measuring facial soft tissues, as well as dentofacial ratios, standard reference values for different populations were determined [5, 6].

A number of orthodontic patients is interested in orthodontic therapy due to aesthetic disharmony. Such problems are easiest to observe and analyze on photographs [7]. Photographs represent auxiliary diagnostic method in orthodontics. They are used for proper diagnosis, treatment planning and treatment progress, tracking changes during growth and development, as well as an aid in communication with the patient. Photographs are also used for planning surgical treatment in orthodontics. Photographs have to be taken in standardized conditions, including predefined distance from lens to the patient, as well as standardized head position during imaging [8]. Photographs can also be used for educational purposes and for research. Digital technology has become an important element of clinical activities in orthodontic documentation. Digital extraoral and intraoral photographs can be imported into the software and presented together on the screen [9]. Photographs are part of proper documentation and visual reference for tracking changes in growth period. They are more understandable and self-explanatory to patients compared to radiographic images. Making measurements from photographs is less invasive procedure for the patient and long lasting record that can be accessed at a later time [10, 11].

Determining positions of anthropological points and angles is necessary for precise determining deviation of normal. Anthropological analysis allows obtaining valuable information of the face characteristics. Proper choice and precisely determined reference points, regardless of method of analysis (manually or by a software) is of utmost importance for obtaining the exact measurements of angular and linear values [12].

The aim of this paper was to assess usability of computer software for automated quantifying of linear measurements of frontal face photography of the patient.

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MATERIAL AND METHODS

Measurements were performed on the set of 20 frontal face images in NHP (Natural Head Position) collected from various sources: 4 photographs from FEI set [13], 7 photographs of students of the University of Banja Luka and 9 publically accessible photographs of the models (in total 8 female and 12 male images). In order to make conditions more realistic for the computer software, dimensions of the photographs, position and relative face size were not the same on all photos.

Measurement points were chosen to fulfill two criteria: 1) most often used points in orthodontic analysis and 2) those that could be determined on two-dimensional image. Measurement procedure and data analysis were conducted based on the analysis performed by Bland and Douglas [14].

Reference measurement was done as described: positions of the following points on every image were determined by an orthodontist: N (Nasion), Sn (Subnasale), Gn (Gnation) and left and right Zg (Zigion). Measurements were repeated after one week. Based on those two measurements, the average position of every point was calculated and used as a third set of data. Figure 1 demonstrates the work window of used computer program.

After the initial manual measurements were done, the same points' positions were determined automatically by custom-made computer program. The program uses OpenCV (Open Computer Vision) [15] library for face detection, *dlib* [16] library and the predictor [17] that enables efficient detection of 68 reference points shown on the Figure 2. Based on those points, it is possible to determine positions of dependent points as well as required measurements. As all measurements were made in pixels, which real size varies between photographs, the program also calculates normalized values by dividing measured vertical distances (upper and lower facial height and total facial height) with bizygomatic distance (facial width). This approach required establishing correct position of Zg points first and whether the calculated values of bizygomatic distance were close to individually measured.

Figure 1. Work window of the computer software
Slika 1. Radni prozor kompjuterskog programa

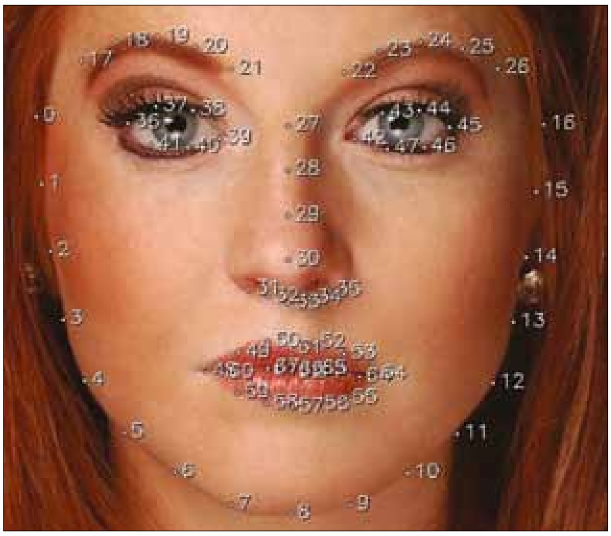

Figure 2. Positions of automatically determined 68 points
Slika 2. Automatski određeno 68 tačaka

Table 1. Summary of measurements
Tabela 1. Sumarni pregled rezultata merenja

Measurement Merena veličina	Orthodontist Ortodont		Program		T-Test p value T-Test P vrednost	Orthodontist 1 Ortodont 1		Orthodontist 2 Ortodont 2	
	Mean Value Srednja vrednost	St. dev.	Mean Value Srednja vrednost	St. dev.		Mean Value Srednja vrednost	St. dev.	Mean Value Srednja vrednost	St. dev.
Bizygomatic distance Bizigomatično rastojanje	241.38	37.89	241.45	37.06	0.92	241.20	37.72	241.55	38.10
Upper facial height Nosni sprat	94.65	15.33	95.00	16.05	0.67	94.20	15.22	95.10	15.62
Lower facial height Dentalni sprat	117.40	25.96	116.70	23.76	0.35	117.30	25.96	117.50	26.00
Facial height Visina lica	212.05	38.96	211.70	38.42	0.50	211.50	38.67	212.60	39.33
Norm. upper facial height Norm. nosni sprat	39.30	3.03	39.33	2.35	0.92	39.15	3.15	39.44	3.05
Norm. lower facial height Norm. dentalni sprat	48.34	4.92	48.11	4.34	0.47	48.31	4.79	48.36	5.09
Norm. facial height Norm. visina lica	87.64	5.45	87.45	5.27	0.56	87.46	5.16	87.81	5.82

Obtained values were statistically analyzed and mean values and standard deviations were calculated for direct and normalized values. Paired two-tailed T-test was used and conclusions were made.

RESULTS

Table 1 shows summary overview of measured values with mean values, standard deviations and p values of paired two-tailed T-test of average values measured by the orthodontist and computer. Differences of mean values for directly measured values ranged from 0.03% for bizygomatic distance, over 0.17% for facial height, 0.37% for upper facial height and 0.6% for lower facial height. Differences of means for normalized values ranged from 0.08% for upper facial height, over 0.22% for facial height to 0.48% for lower facial height. P values strongly suggested association between manually and automatically obtained measurements for every parameter.

Table 2 shows differences of mean values obtained for every measured parameters using manual (average of two measurements) and computer approach. Mean values for 4 parameters were smaller for automatic measurements, while values for 3 parameters were smaller for manual measurements.

Figures 3 to 6 give graphical representation of measured values as follows: blue line represents the mean values of two manual measurements, marks "+" and "x" represent individual manual measurements and red circles represent automatic measurements. There is no qualitative difference between automatic and manual measurements for observed set of samples, which is in agreement with values in Tables 1 and 2. There is a high level of correlation of measured values for bizygomatic distance obtained manually and automatically (Figure 3). Figure 4 shows measurements for facial height acquired manually and by the computer software. Differences in obtained values are minimal. Based on the Figure 5 that represents measurements for lower facial height, it can be seen that the differences are small while relatively larger discrepancies are present only with extreme values. Also, differences between manually and automatically obtained

Table 2. Differences of mean values
Tabela 2. Odstupanja srednjih vrednosti merenja

Measurement Merenje	Program	Orthodontist Ortodont
Bizygomatic distance Bizigomatično rastojanje	0.08	0.18
Upper facial height Nosni sprat	0.35	0.45
Lower facial height Dentalni sprat	-0.70	0.10
Facial height Visina lica	-0.35	0.55
Norm. upper facial height Norm. nosni sprat	0.03	0.15
Norm. lower facial height Norm. dentalni sprat	-0.22	0.03
Norm. facial height Norm. visina lica	-0.19	0.17

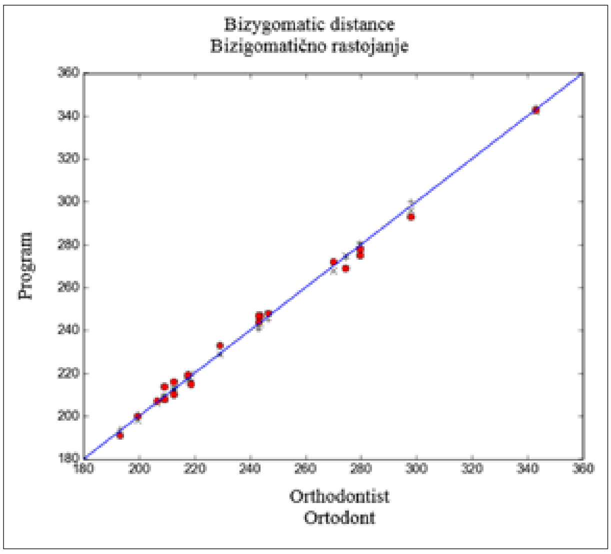

Figure 3. Bizygomatic distance
Slika 3. Vrednosti merenja za bizigomatično rastojanje

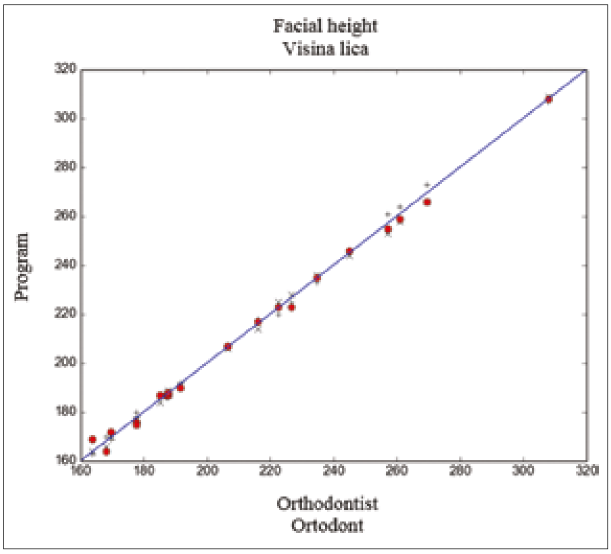

Figure 4. Facial height
Slika 4. Vrednosti merenja za visinu lica

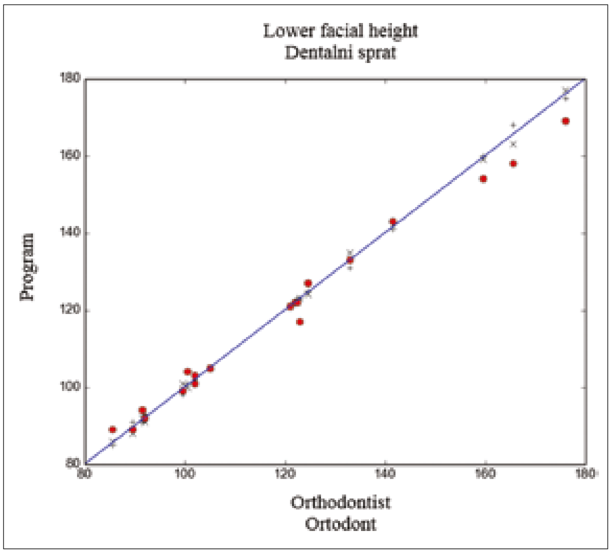

Figure 5. Lower facial height
Slika 5. Vrednosti merenja za dentalni sprat

values for upper facial height are minimal, except for extreme values of measured parameters (Figure 6).

DISCUSSION

In clinical practice, but also in significant number of papers, one can observe the trend of automation of extraoral examination through the use of digital imaging and software that allows automatic calculation of various values based on reference points determined by an orthodontist [18-20]. Moshkelgosha et al. performed statistical analysis based on 27 points and 43 calculated values on the sample of 110 patients [18]. Aksu et al. analyzed the reliability of reference points in photogrammetry based on 9 measurements taken directly on 100 patients, as well as measurements determined by computer using facial images. After statistical analysis they concluded that reliability depends on gender and measured parameters [19]. Milutinović et al. compared standard and computer aided method of analysis of profile cephalogram on the sample of 32 patients. They found no statistically significant difference between the measurements obtained by the two methods with significant advantages in efficacy with computerized method [20].

On the other side, there is a widespread use of 3D scanners that enable generating and analysis of three-dimensional models of the patient's face with the possibility of automatic determining of the reference points for further analysis [21].

Over the course of multiple years of research Deli et al. identified problems related to automatic determining of the facial indicators. When reference indicators were projected on the faces or structured light approach used, the biggest problem has been shown to be low contrast between indicators and the skin of the patient [22]. Therefore, they concluded that the use of photogrammetry and multiple standard cameras were better solution compared to other approaches such as laser scanning and structured light [23]. The whole system included five precisely positioned digital cameras, predefined background with coded markers and light system. Differences from reference measurements were under 1% that was considered acceptable.

As indicator positioning directly on face is not an optimal approach, Loconsole et al. developed a method based on Microsoft Kinect device and custom made software for automatic determining of 11 points on the face [24]. During the research they used three methods of measurements – manually with digital two-pronged orthodontic caliper, automatic measurement based on the single recording and automatic measurement based on 100 successive recordings of the same face by averaging the values. Based on performed analysis they concluded that proposed method provided good results for nasion, subansale and left and right chelion. They identified problems with automatic measurements of the points that included palpation in manual measurements as well as the points that could not be precisely positioned in two-dimensional image (e.g. tip of the nose).

The proposed method for automatic determining of linear measurements of the face provided results comparable to measurements generated by standard manual method with no statistically significant difference. Therefore, it can be concluded that the use of automation in this case did not deteriorate the exactness of measurements, where on the other hand computer program was much faster in measuring than an orthodontist. The software is capable to position the points, calculate dependant parameters and analyze them for the time that human operator need just to select the image for analysis – about one second. Also, taking digital images is far cheaper and faster than generating 3D model of the face. Also patients are more familiar with digital imaging than 3D scanning.

Keeping in mind rapid advances in information technologies and mobile computing systems, it is expected in the near future to enable the smartphone or tablet for rapid determining of facial parameters of the patient and analysis of obtained data with improvements in interaction and cooperation with the patient.

CONCLUSION

The results of this research suggest that it is possible to ease, accelerate and automate the work of an orthodontist in the image analysis by using adequate supporting software without the significant differences in measured values.

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Automatsko određivanje linearnih veličina lica uz pomoć računara

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KRATAK SADRŽAJ

Uvod Obavezan deo kliničkog ekstraoralnog pregleda ortodontskog pacijenta je analiza lica (anfas i profil). Razvojem računarske tehnike omogućeno je povećanje efikasnosti i olakšavanje rada ortodonata, ali i uvođenje automatizacije pojedinih koraka u ortodontskoj analizi. Cilj ovog rada je bio da se ispita mogućnost upotrebe programa za automatsko određivanje linearnih veličina lica na osnovu anfas fotografije pacijenta.

Materijal i metode rada Na uzorku od 20 fotografija ispitanika u NHP (Natural Head Position) položaju urađeno je tri vrste merenja. Obučeni ortodont je najpre odredio dva puta pozicije definisanih tačaka na digitalnoj fotografiji ispitanika sa pauzom od sedam dana, a nakon toga su pozicije ovih tačaka određene automatski upotrebom namenski razvijenog računarskog programa. Na osnovu dobijenih pozicija tačaka izračunate su vrednosti za bizigomatično rastojanje, visinu nosnog i dentalnog sprata, kao i za visinu lica. Dobijene vrednosti za tri vrste merenja su potom upoređivane i statistički obrađene.

Rezultati Statističkom obradom i upoređivanjem dobijenih rezultata uočeno je da namenski razvijen računarski program daje vrednosti uporedive sa vrednostima merenja obučenog ortodonta. Statistička obrada je uključivala računanje srednjih vrednosti i standardnih devijacija, kao i sprovođenje uparenog obostranog T-testa. Odstupanja merenih veličina su se kretala od 0,03% do 0,6%, što predstavlja zadovoljavajući rezultat i sugeriše na opravdanu upotrebu automatske metode merenja.

Zaključak Rezultati ovog istraživanja sugerišu da je moguće olakšati, ubrzati i automatizovati rad ortodonata na analizi fotografija pacijenata upotrebom pogodnih programa bez značajnih odstupanja u vrednostima merenih veličina.

Ključne reči: računarska i standardna analiza; anfas snimak glave

UVOD

Analiza ljudskog lica predstavlja određenu vrstu umetnosti i nauke. Za procenu karakteristika ljudskog lica koriste se različita merenja kao što su: antropometrija, kefalometrija i fotogrametrija. Primenu fotogrametrije u ortodonciji je prvi put predložio Stoner, koji je poredio profile pre i posle ortodontskog tretmana sa idealnim profilima [1]. Uzimajući u obzir etničke karakteristike i pol, kao i promene koje nastaju zbog starosti, postaje jasno da ono što se smatra lepim i prihvatljivim kao norma za neke kulture, može biti različito za druge [2, 3, 4,]. Merenjem lica, mekih tkiva, kao i dentofacijalnih odnosa, utvrđene su normalne referentne vrednosti u različitim populacijama [5, 6].

Najveći broj ortodontskih pacijenata zainteresovan je za ortodontsku terapiju zbog postojanja estetske disharmonije. Takvi problemi se najlakše uočavaju i analiziraju na fotografijama [7].

Fotografija predstavlja pomoćno dijagnostičko sredstvo u ortodontskoj dijagnostici. Ona pomaže pravilnoj dijagnostici, pomaže prilikom donošenja odluke o planu terapije, vrlo je pouzdana za praćenje toka terapije, za praćenje promena koje nastaju tokom rasta i razvoja, te kao pomoć u komunikaciji sa pacijentom. Fotografija se u ortodonciji koristi i pri planiranju ortodontsko-hirurškog lečenja pacijenta. Fotografije moraju biti pravljene pod standardizovanim uslovima, što podrazumeva određeno rastojanje pacijenta od objektiva, te standardizovan položaj glave prilikom fotografisanja [8]. Pored ovoga, fotografije se mogu upotrebljavati u nastavne svrhe i kao temelj za dalja istraživanja. Digitalna tehnologija iz dana u dan postaje sve važniji element u kliničkim aktivnostima, tako da i u ortodonciji raste upotreba digitalne tehnologije za ortodontske dokumente. Digitalne ortodontske ekstraoralne i intraoralne fotografije mogu biti odmah ubačene u programe i biti prikazane zajedno na ekranu [9]. Fotografija predstavlja mnogo konvencionalniju dokumentaciju i vizuelnu referencu za praćenje promena koje nastaju tokom rasta i razvoja. Pacijentima su pristupačnije i puno razumljivije od radiografskih snimaka. Merenja na fotografijama predstavljaju jednostavniju proceduru za pacijenta, a takođe pružaju trajni zapis lica kojem se može pristupiti i kasnije [10, 11].

Merenja određenih antropoloških tačaka i uglova na licu je neophodno za precizno određivanje odstupanja od normalnog. Obradom vrednosti nakon antropološke analize dobijaju se podaci koji predstavljaju vredne informacije o karakteristikama lica. Pravilan odabir i precizno određivanje referentnih tačaka, bilo da se analiza radi standardno ili računarski, od presudnog je značaja za tačnost dobijenih ugaonih i linearnih vrednosti [12].

Cilj ovog rada je bio da se ispita opravdanosti upotrebe programa za automatsko određivanje linearnih veličina lica dobijenih na osnovu anfas fotografije pacijenta.

MATERIJAL I METODE

Merenja su izvršena na skupu od dvadeset frontalnih fotografija ispitanika u NHP (Natural Head Position) položaju prikupljenih iz više izvora: četiri fotografije iz FEI skupa [13], sedam fotografija studenata Univerziteta u Banjoj Luci, te devet javno dostupnih fotografija modela, od čega je na fotografijama prisutno osam žena i 12 muškaraca. U cilju ostvarivanja realnih uslova za rad programa za automatsko određivanje tačaka, dimenzije fotografija, pozicija i relativna veličina lica nisu bile iste na svim fotografijama.

Tačke za merenje su odabrane tako da zadovoljavaju dva kriterijuma: najčešće korišćene tačke u ortodontskoj analizi lica, odnosno tačke koje je moguće odrediti na osnovu dvodimenzionalne fotografije.

Procedura merenja i analiza rezultata su provedeni na osnovu analiza obavljenih od strane Blenda i Daglasa [14].

Referentno merenje je generisano na sledeći način: specijalista ortopedije vilica je upotrebom namenskog programa označio sledeće tačke na svakoj fotografiji: N (Nasion), Sn (Subnasale), Gn (Gnation), te levi i desni Zg (Zigion). Merenje je ponovljeno nakon sedam dana. Na osnovu ova dva merenja je izračunata srednja vrednost pozicije za svaku tačku, što predstavlja treću grupu podataka (Slika 1).

Nakon provedenih manuelnih merenja, iste tačke su određene automatski upotrebom namenski razvijenog programa. Program za funkcionisanje koristi OpenCV (Open Computer Vision) [15] biblioteku za detekciju lica na slici, *dlib* [16] biblioteku i prediktor [17], koji omogućava efikasnu detekciju 68 referentnih tačaka (Slika 2). Na osnovu tih tačaka je moguće odrediti lokacije zavisnih tačaka, kao i tražene veličine. Kako su vrednosti svih veličina izražene u tačkama čija realna veličina varira od slike do slike, pored direktnih vrednosti su izračunate i normalizovane vrednosti, koje su dobijene tako što je izvršeno deljenje izračunatih vertikalnih veličina (visina lica i spratova lica) bizigomatičnim rastojanjem (širinom lica). Ovaj pristup je diktirao da je pre svih ostalih analiza neophodno bilo proveriti da li je lokacija Zg tačaka pravilno određena i da li su izračunate vrednosti bizigomatičnog rastojanja bliske individualno merenim veličinama.

Dobijene vrednosti su nakon toga statistički obrađene i izračunate su srednje vrednosti i standardne devijacije za direktne i normalizovane vrednosti. Nakon toga je proveden i upareni dvostrani T-test.

REZULTATI MERENJA

U tabeli 1 prikazan je sumarni pregled merenih veličina sa srednjim vrednostima, standardnim devijacijama i vrednostima uparenog obostranog T-testa (p) sprovedenog na prosečnim vrednostima dobijenim od strane ortodonta i računara.

Kod direktno merenih vrednosti odstupanja srednjih vrednosti su se kretala između 0,03% za bizigomatično rastojanje, preko 0,17% za visinu lica i 0,37% za nosni sprat, odnosno 0,6% za dentalni sprat. Odstupanja srednjih vrednosti od normalizovanih vrednosti parametara su se kretala od 0,08% za nosni sprat, preko 0,22% za visinu lica i do 0,48% za dentalni sprat.

Dobijene vrijednosti (p) snažno sugerišu povezanost ručno merenih i automatski dobijenih vrednosti za svaki od merenih parametara.

Odstupanja srednjih vrednosti dobijenih ručnim merenjem i upotrebom programa za svaku od merenih veličina u odnosu na srednju vrednost dva ručna merenja prikazana su u tabeli 2.

Odstupanja srednjih vrednosti dobijenih ručnim merenjem i automatskim merenjem su u četiri slučaja manja za automatski merene vrednosti, a u tri slučaja manja kod ručno merenih vrednosti.

Na slikama 3 do 6 grafički su prikazane dobijene vrednosti merenja. Tako plava linija predstavlja srednje vrednosti ručnih merenja, oznake „+" i „x" predstavljaju pojedinačna ručna merenja, dok crveni krugovi predstavljaju automatski dobijene vrednosti. Sa slika je vidljivo da ne postoji kvalitativna razlika između automatski i ručno dobijenih vrednosti za posmatrani skup uzoraka.

Slika 3 pokazuje da postoji visok stepen poklapanja vrednosti bizigomatičnog rastojanja dobijenih ručnim merenjem i automatskim metodom, dok su na slici 4 prikazane vrednosti za visinu lica dobijene ručnim i računarskim merenjem sa minimalnim odstupanjima.

Vrednosti merenja za dentalni sprat su ukazala na mala odstupanja, dok su veća odstupanja zabeležena samo za ekstremne veličine (Slika 5).

Odstupanja između ručno i automatski dobijenih vrednosti za nosni sprat su takođe bila minimalna, osim za ekstremne vrednosti (Slika 6).

DISKUSIJA

U kliničkoj praksi je poslednjih godina u značajnom broju povećana primena računara u procesu ekstraoralnog kliničkog pregleda primenom digitalnih fotografija i programa koji omogućavaju merenje i izračunavanje potrebnih vrednosti na osnovu referentnih tačaka [18, 19, 20]. Moshkelgosha i saradnici su na osnovu računara i merenja 27 tačaka izračunavali 43 vrednosti kod 110 ispitanika [18]. Aksu, Kaya i Kocadereli su ispitivali pouzdanost referentnih udaljenosti na fotogrametrijama na osnovu merenja devet veličina direktno na licima 100 ispitanika, odnosno pomoću računara na fotografijama. Na osnovu statističke obrade rezultata zaključili su da pouzdanost merenja varira u zavisnosti od pola ispitanika i merenih veličina [19]. Milutinović i saradnici su upoređivali standardni i računarski metod analize profilnog telerendgen snimka glave na uzorku od 32 ispitanika. Na osnovu merenja i obrade rezultata zaključili su da nema statistički značajne razlike u vrednostima, ali uz značajne prednosti računarske metode [20].

S druge strane, upotreba 3D skenera omogućava generisanje i analizu trodimenzionalnih modela lica pacijenta na osnovu određivanja referentnih tačaka i vrednosti za analizu [21].

Višegodišnjim istraživanjima, Deli i saradnici su identifikovali osnovne probleme vezane za automatsko određivanje markera na licu pacijenta. Pri upotrebi projektovane mreže referentnih markera na lice pacijenta ili upotrebe strukturisanog svetla, najznačajniji problem je slab kontrast između markera i kože lica pacijenta [22]. U toku razvoja sistema autori su zaključili da je upotreba fotogrametrije i većeg broja standardnih fotografskih aparata pogodnije rešenje u odnosu na lasersko skeniranje ili upotrebu strukturisanog svetla [23]. Sam sistem se sastoji od skupa od pet digitalnih foto-aparata raspoređenih u prostoru, pozadine sa kodiranim markerima i sistema za osvetljenje. Odstupanja od referentnih merenja su lako prihvatljiva u praksi, jer su iznosila do 1%.

Kako postavljanje markera na lice pacijenta nije optimalan pristup, Loconsole i saradnici su razvili metod za upotrebu Microsoft Kinect uređaja i namenski razvijenih softvera za automatsko određivanje 11 tačaka na licu [24]. U okviru istraživanja korišćena su tri metoda merenja – ručno (upotrebom digitalnog dvokrakog ortodontskog šestara), automatskog merenja na osnovu jednog snimka i automatskog merenja na osnovu 100 sukcesivnih snimaka istog lica. Autori su zaključili da predloženi metod daje dobre rezultate za *nasion*, *subnasale*, *stomion*, te levi i desni *chelion*. Identifikovani su problemi prisutni pri automatskom merenju tačaka koje nije moguće precizno pozicionirati u prostoru na osnovu dvodimenzionalne slike (npr. vrh nosa).

Primenom programa za automatsko određivanje linearnih veličina lica dobijene su vrednosti koje su uporedive sa vrednostima koje je generisao standardni metod merenja i nisu zabeležena statistički bitna odstupanja. Upotreba računara utiče na tačnost podataka, ali je brzina i efikasnost ove metode mnogo veća. Naime, program je u stanju da izvrši određivanje tačaka, da izračuna izvedene vrednosti i izvrši analizu istih za vreme koje je ljudskom operateru potrebno samo za odabir fotografije za analizu – reda veličine jednog sekunda. Takođe, prednost opisanog rešenja u odnosu na 3D modele je u tome što je pravljenje digitalnih fotografija daleko jeftinije i brže od generisanja 3D modela lica pacijenta. Ne sme se zanemariti ni činjenica da pacijentima fotografisanje ne predstavlja nepoznanicu, dok je proces trodimenzionalnog skeniranja uglavnom nešto potpuno novo.

Imajući u vidu brzi razvoj informacionih tehnologija i prenosnih računarskih uređaja, u skorijoj budućnosti se može očekivati i upotreba mobilnog telefona ili tablet uređaja za brzo određivanje parametara lica i analizu dobijenih rezultata uz efikasniju interakciju i saradnju sa pacijentom.

ZAKLJUČAK

Dobijeni rezultati ovog istraživanja sugerišu da primena programa za automatsko određivanje linearnih veličina lica znatno olakšava, ubrzava rad ortodonata u analizi fotografija pacijenata. Upotreba odgovarajućih programa ne dovodi do značajnih odstupanja u vrednostima merenja traženih parametara.

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Structural characteristics and mechanisms of fluorapatite mechanochemical synthesis

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SUMMARY

This paper analyzes mechanisms of fluorapatite mechanochemical synthesis and its structural characteristics. Several studies of Jokanovic et al. published in appropriate journals and the book"Nanomedicine, the biggest challenge of the 21st century" are the base for this article. Characteristics of obtained materials show numerous biological advantages associated with the specific structural design of material during the process of synthesis.

X-ray diffraction (XRD) and infrared spectroscopy with Fourier transform (FTIR) were used for studying the processes of fluorapatite synthesis.

Keywords: fluoroapatite; mechanochemical synthesis; X-ray diffraction; infrared spectroscopy; low-temperature treatment

INTRODUCTION

Fluorapatite (FA), chemical formula $Ca_5(PO_4)_3F$, or $Ca_{10}(PO_4)_6F_2$ is the most stable, least soluble, and the hardest calcium orthophos mineral (Mohs hardness scale 5). Such characteristics of fluoroapatite are associated to the specific position of F- ions in the center of the Ca_2 triangle and its crystal structure. The synthesis techniques are similar to those of hydroxyapatite, but it should be noted that synthesis of fluoroapatite involves the presence of F- ions, which are transmitted into synthesis through CaF, NaF or NH_4F. Compared to hydroxyapatite (HA), which is Ca-deficient, there are no data to suggest Ca-deficiency of fluoroapatite. The chemical formula of fluorapatite is $Ca_{10}(PO_4)_6(F,OH)_2$ as the most frequent modification of OH^- ions by F^- ions is not complete. Among all human calcified tissues, the greatest concentration of fluorapatite is found in bones, and the lowest in enamel. However, even where there is the largest concentration of fluorapatite, the amount of fluoride is usually reduced related to stoichiometric quantities. Due to its low solubility (degradation rate), it is rarely used as a bone substitute.

Due to mechanical stability, its solubility is reduced and proliferation of bone tissue is improved. Hydroxyapatite / fluorapatite (FHA / FA) has been used as clinical restorative material in the recent years [1, 2]. In addition, FHA and HA / FA, are used in biomedicine as carriers of drugs and catalysts or absorbents [3, 4].

Compared to HA [1], FHA / FA has better thermal and chemical stability [5, 6]. When a certain number of OH groups in the HA matrix is replaced by F^- ions, thermal and chemical stability of FHA / FA ceramics increases significantly. Theoretically, the ratio F: OH ≥ 1 within the chain OH (in the FHA structure) would be sufficient to arrange HA crystals, stabilizing their structure due to alternating schedule of F^- ions among OH^- ions.

In practice, materials that contain F^- ions are widely used for dental restorations as they prevent tooth decay and reduce bacterial activity in an acidic environment. In addition, F^- ions themselves favour mineralization and crystallization of calcium phosphate during bone formation [7]. Furthermore, *in vitro* studies FHA / FA have indicated its slow dissolution, better deposition layer as with hydroxyapatite, better adsorption of the protein [6-8], and similar or better cell attachment compared to pure HA [7, 9] as well as improved activity of alkaline phosphatase [6].

It has also ben shown that the presence of fluoride affects the increase in quantity and quality of bone in body [5]. Fluoride ion is used to treat osteoporosis because bone mass increases with the application of F^- ions [9]. F^- ions also stimulate the activity of osteoblasts, both *in vitro* and *in vivo*. In addition, the mineral phase of enamel consists of HA (95 - 97%) with from 0.04 to 0.07 wt. % Fluoride. A dose of about 1.5-4 mg of fluoride per day significantly reduces the risk of tooth decay [5]. In addition to FHA and FHA phase, materials like CaF_2 are also important in dentistry, because they can be used as reservoir of labile fluoride in caries prevention [10-14].

Some studies have shown that dual delivery system of (F^- and Ca^{2+} ions) is necessary to allow homogeneous nucleation and formation of very small crystals of CaF_2 in the mouth. These amounts are very efficient in increasing the deposition and retention of labile F^- ions in the mouth, while at the same time remineralization effect increases

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without consequent increasing of F-content [15-23]. According to the research of Jokanovic et al. [24] it was described for the first time not only a specific method of synthesis of fluorapatite, but also a synthesis of combined system encapsulated in surface-active substance polyethylene vinyl acetate / versatate, which is a potential source of labile CaF_2 phase. This is very important in order to maintain a balance of F ions content and to improve chemical and mechanical stability of the tooth.

For the synthesis of FHA / FA using precipitation, different methods are used like sol-gel, hydrolysis, hydrothermal method and solid phase reactions. They include appropriate ion exchange between the reactants that are used in the synthesis of FA [23-26]. Most chemical methods require very precise control of parameters of the synthesis process, product composition control and control of its characteristics, which is not so easy to achieve. Therefore, those methods are not suitable for the synthesis of FHA / FA on an industrial scale [27].

On the other hand, mechanochemical process is simple method that takes place in the solid state, allows synthesis of materials through the extremely efficient process of mixing different ion types due to shear forces, which using reduction of particle size and their alternating layers positioning improve thermodynamics and kinetic reactions between different solid substance precursors. In addition, compared to other above-mentioned processes, this method is more suitable regarding economic and technical sides because it enables mass production of nanocrystalline powders and high flexibility of process parameters [13].

The aim of this study was to present the method of synthesis of Nano powders fluorhydroxyapatite / fluorapatite using the method of mechanical alloying. Milling parameters such as speed of rotation, diameter, number of spheres, and weight ratio of the dust-spheres were constant, while the influence of milling time on phase composition was carefully defined. The kinetics and mechanism to obtain FHA / FA and other transitional phases were examined using XRD and FTIR spectroscopy.

MECHANISMS OF FLUORAPATITE SYNTHESIS

The mechanisms of fluorapatite synthesis are shown in the case of the most commonly used precursors such as calcium hydroxide $Ca(OH)_2$, phosphorus pentoxide P_2O_5, and calcium fluoride CaF_2 (synthesis 1) and calcium hydroxide $Ca(OH)_2$, phosphorus pentoxide P_2O_5 and ammonium fluoride NH_4F with the addition of surface-active substance vinyl acetate / versatate (EVA / AVV) (synthesis 2). Both mechanisms are carried out through the series of processing steps that can be analyzed using IR spectroscopy and X-ray diffraction [24]. It was noted that each phase is followed by certain degree of transformation of starting reactants in fluorhydroxyapatite with smaller segments of OH^- groups and larger segments of F^- ions instead of OH^- groups, until complete transformation of fluorapatite is finished. Generally, the reaction is carried out with an incomplete stoichiometry where x is thevalue that defines deviation from complete symmetry and can be found in the interval $x_1<x<x_k$

$$Ca_{10}(PO_4)_{1-y}(CO_3)_y(PO_4)_5\,(OH)_{2-2x1}\,(F)_{2x1} + (2-2x_1)HF \rightarrow$$
$$Ca_{10}(PO_4)_{1-y}(CO_3)_y\,(PO_4)_5(OH)_{2-xk}F_{xk} + (2-x_k)\,HF + H_2O;$$

where x_k maximum value is 1 and x_1 minimum value is 0.

So, the summary reaction in the reaction with total stoichiometry would be:

$$9\,Ca(OH)_{2-2y}(CO_3)_y + 3P_2O_5 + CaF_2 \rightarrow$$
$$Ca_{10}(PO_4)_{1-y}(CO_3)_y\,(PO_4)_5F_2 + 9H_2O\,;\ x=1$$

Based on X-ray diffraction, it was found that after only 1h of mechanochemical treatment, amorphization had occurred.

Due to extremely high concentration of mechanical strain on a very small contact surface (the contact that is realized in mutual globe collision or in globe collision with the surface of the inner lining) conditions are generated for the emergence of high shear stresses in a relatively small contact surface. Thus, the size of tension strain depends primarily on the diameter of spheres used in mechanochemical treatment (the size of the contact portion of a sphere indentation deformation in a crash) and the speed collision. Simultaneously, strain transfer leads to mechanical activation of the system and highly resilient flow that follows intense chemical and phase changes in the material (reaction shift, mixing ionic types, creating new phase, etc.). These changes can be such that material during the relaxation time partly suffers reversible deformation (highly resilient flow) or can be entirely irreversible when creeping material mechanism dominates.

The tension of critical deformation depends on the system exposure time to deformation (the number of sphere blows), in other words the number of pressure cycles, so that with time, the tension which provokes critical deformation demolition/formation of fissures and new areas, has less and less value, leading to larger amorphousness of the system. The process of mechanical activation in which water appears as a reaction product, is additionally accelerated by facilitating the transport of adequate ion types to places that correspond to the minimum of free energy of the system.

In this case, because of the exceptional hydrophilicity of P_2O_5, immediately upon its adding to other reactive substances, the process creates phosphorous acid (P_2O_5+ $3H_2O \rightarrow 2H_3PO_4$), which then reacts with $Ca(OH)_{2-2y}(CO_3)_y$ and creates $Ca(HPO_4)_{1-y}(CO_3)_y$(carbonate calcium hydrogen phosphate).

After 4h of milling, the distinctive HPO_4^{2-} start to vanish intensively and 5h after completely disappears, while the band on 963 cm^{-1}, appears (carbonate calcium efficienthydroxide fluorapatite) (Figure 1). Simultaneously, during the whole process, CaF_2 dissociates and F ions that enter into reactionare created with calcium deficient hydroxide fluorapatite until the formation of its final chemical form. Finally, on previously mechanically treated samples during the period of 6 and 9 hours, and their afterwards thermic treatment on 1100°C, the bands belonging to CO_3^{2-}disappear on 1420 and 1455 cm^{-1}. In samples mechanically treated for 6 hours one band ap-

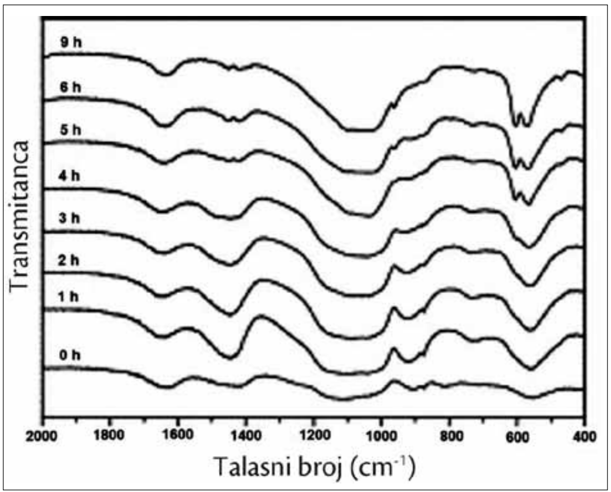

Figure 1. FTIR spectrum of untreated and mechanochemically treated mixture of $Ca(OH)_2–P_2O_5–CaF_2$
Slika 1. FTIR spektar netretirane i mehanohemijski tretirane smeše $Ca(OH)_2–P_2O_5–CaF_2$

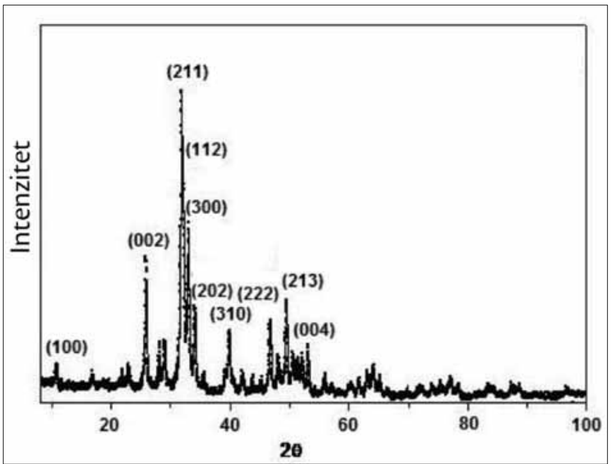

Figure 2. Refined XRD fluorapatite obtained after 9 hours of mechanochemical treatment
Slika 2. Utačnjeni XRD fluorapatita dobijenog mehanohemijskim tretmanom tokom 9 h

pears on 630 cm^{-1} indicating that exchange between fluor and hydroxide group was not completed. The band on 726 cm^{-1} that belong to F ions, is constantly present during the whole process demonstrating that F coordination is not significantly changed through the process of F transfer from CaF_2 into calcium hydroxide fluorapatite.

The rate of deprotonation of HPO_4^{2-} and ion exchange of OH^- and F^- regulate the rate of formation of fluorapatite in all stages of the process. The process of dissociation of calcium fluorite occurs through the process of chemical etching of its particles, within the defects of the system (open surfaces, corrosion pits, dislocations, dislocation loops, vacancies), and tears Ca^{2+} ions away from these places, leaving exposed F^- ions which are carried by water molecules andthen transported to places that correspond to a given fluctuating concentration gradient / concentration gradient of the local surface.

This indicates that it is realistic to assume that reactions in larger and smaller initial particles of the system take place in different ways and at different time intervals reach equilibrium conditions for the final reaction of fluorapatite formation. The reaction on the surface of large particles probably runs immediately after the begining of the mechanochemical treatment, while the cores that are still associated to given initial reactants remained in the depth of the particles. The morphology of the particles, which even in the remote stages of the treatment (4-6 hours) remains the same, testifies that the reactions in every particle / particle group advance individually. The reaction in smaller particles proceeds quickly, and in medium and large particles it progresses with full intensity along the newly created paths (new surface areas, the border of the crystallites / block mosaics, etc.), until the reaction of conversion of the calcium hydroxyfluorapatite into the fluorapatite is fully implemented, as it is shown on the refined XRD spectrum (Figure 2).

Figure 3 shows the nearest neighbours of F^- ion in the structure of carbonate fluorapatite. It is noted that there are three $CaII^{2+}$ located near F^- ion at a distance of 2.3 Å.

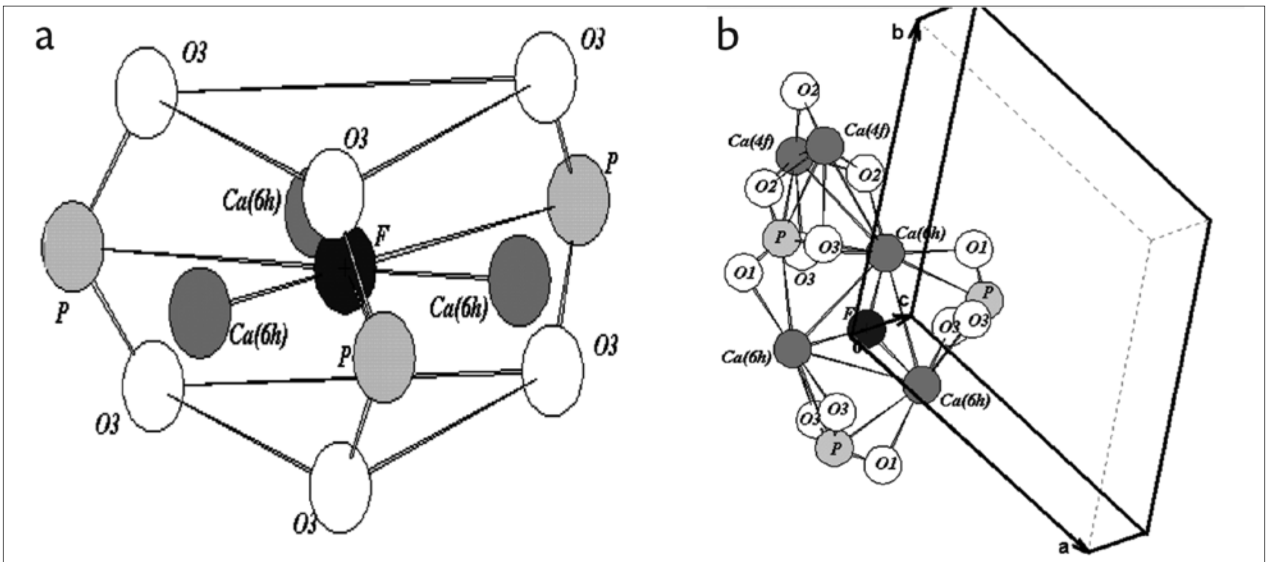

Figure 3. a) The closest neighbours of F- ion in the carbonate fluoroapatite structure; b) Fragment of crystal lattice compared with a unit cell
Slika 3. a) Najbliži susedi F- jona u karbonatnoj fluoroapatitnoj strukturi; b) Fragment kristalne ćelije upoređen sa jediničnom ćelijom

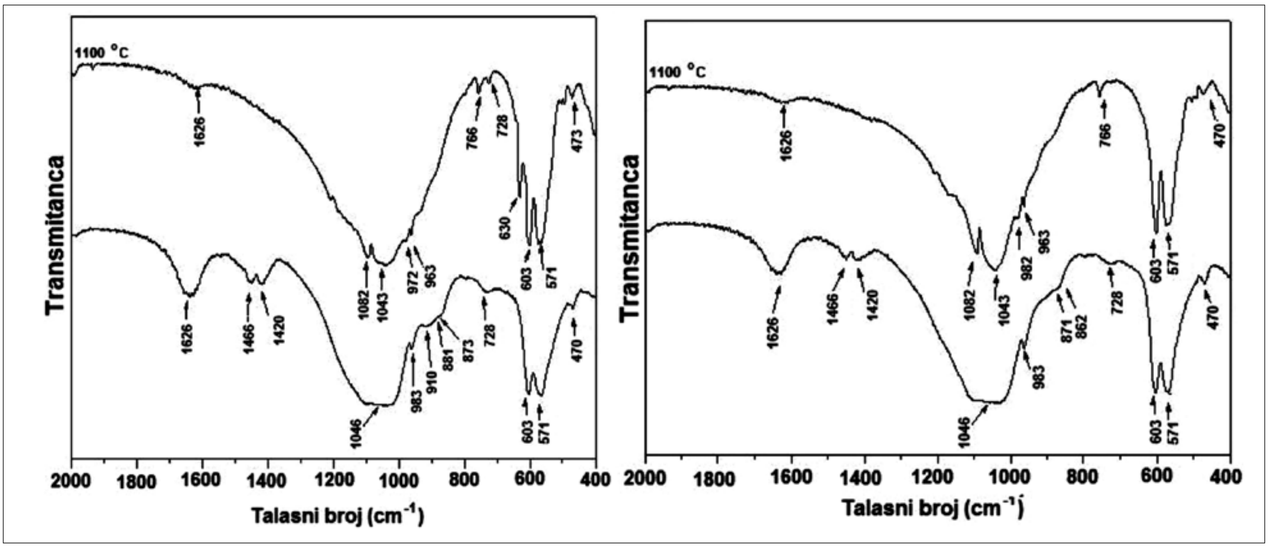

Figure 4. FTIR spectrum of mechanochemically treated mixture of Ca(OH)2–P2O5–CaF2 for various milling times: a) 6 hours, and b) 9 hours after and before thermal treatment

Slika 4. FTIR spektar mehanohemijski tretirane smeše Ca(OH)2-P2O5-CaF2 za različita vremena mlevenja: a) 6 časova i b) 9 časova posle i pre termičkog tretmana

These CaII^{2+} ions form the vertices of an equilateral triangle with F$^-$ ion in the center. There are three CaII^{2+}, P^{5+}, and O^{2-} ions in the second coordination sphere, which mutually form the vertices of triangles. The distance between F$^-$ and P^{5+} is 3.6 Å, and between P^{5+} and O^{2-}(1) is 3.9 Å. There are O^{2-}(3) ions above and below the plane containing F$^-$ and CaII^{2+}. The oxygen ions occupy the vertices of a dodecahedron. The distance between F$^-$ and O^{2-}(3) ions is 3.1 Å, whereas between P^{5+} and O^{2-}(3) ions it is 1.5 Å. Figure 2 shows a fragment of the crystal lattice compared with the unit cell. According to the research of Pandaet al.CaII^{2+} has larger atomic size compared toCaI^{2+}. When OH$^-$ ions are substituted with F$^-$, there is greater distortion in the structure due to the larger size of the ionic radius of F$^-$. At the end of the mechanochemical synthesis process, F$^-$ ion occupies large space in the center of the latticeforming a stable fluorapatite structure.

According to the research of Jokanovicet al. [24] (OH$^-$, F$^-$), in addition to the three types of [OH$^-$], the chain of apatite also contains the fourth type with different vibrational energies. It is observed in this study that if the criteria for displacement of free vibration OH$^-$ is taken as the criteria for quantifying the changes of OH$^-$ with F$^-$, then it is indicated that about 50% of OH$^-$ groups are modified with F$^-$, while the system, with almost completely changed OH$^-$ groups with F$^-$ (for pure fluorapatite), provides the value of the wave number of 758 cm^{-1} (Figure 4 and Table 1).

THE METHOD OF SYNTHESIZING WITH ADDITIONAL LOW-TEMPERATURE THERMAL TREATMENT

Another method of mechanochemical synthesis using the precursorsCa(OH)$_2$, P_2O_5, NH_4F and surfactant of vinyl acetate / versatate, shows that mechanochemical process only can not form fluorapatite. That is why it is necessary to carry out an additional low-temperature treatment.

Table 1. Assignment of IR absorption bands

Tabela 1. Asignacija IR apsorpcionih traka

Mod	Sample Uzorak			
	FA (t = 6 h)	FA (t = 9 h)	FA (t = 6 h, 1100 C)	FA (t = 9 h, 1100 C)
ν_3(O-H)	3430	3430	3430	3430
	2926	2926	2926	2926
δ(O-H)	1626	1626	1626	1626
$\nu_3(CO_3^{2-})$	1455	1455	-	-
$\nu_3(CO_3^{2-})$	1420	1420	-	-
$\nu_3(PO_4^{3-})$	1092	1092	1093	1093
$\nu_3(PO_4^{3-})$	1046	1042	1043	1043
$\nu_1(PO_4^{3-})$	963	962	963	963
$\nu(F^-)$	726	726	-	726
ν_L(O-H)	-	-	630	-
$\nu_4(PO_4^{3-})$	603	603	603	603
$\nu_4(PO_4^{3-})$	571	571	571	571
$\nu_2(PO_4^{3-})$	473	473	473	473

FTIR method (Figure 5) proved to be the most suitable method for monitoring the synthesis. In order to obtain complete picture of phase transitions that occur in materials during mechanochemical and low-temperature treatment, the method of X-ray diffraction [23] can be used in addition to FTIR method.

XRD spectra of samples (Figure 6) show very intense peaks of portlandite (P), while β-$Ca_2P_2O_7$(CP) peaks are also visible. In addition, $CaCO_3$ (C) and CaF_2 (CF) peaks are strongly emphasized. All the characteristic diffraction peaks for FA are almost negligible, and some of them absent [29].

After 5 minutes of milling, the most intense peaks belong to Ca(OH)$_2$, and typical peaks for β-$Ca_2P_2O_7$, CaF_2 and $CaCO_3$ are clearly visible. The peak corresponding to Ca(OH)$_2$ is clearly visible and shows low rate of reaction for forming fluorapatite. Consequently, the amount of synthesized FA in the mixture is negligible.

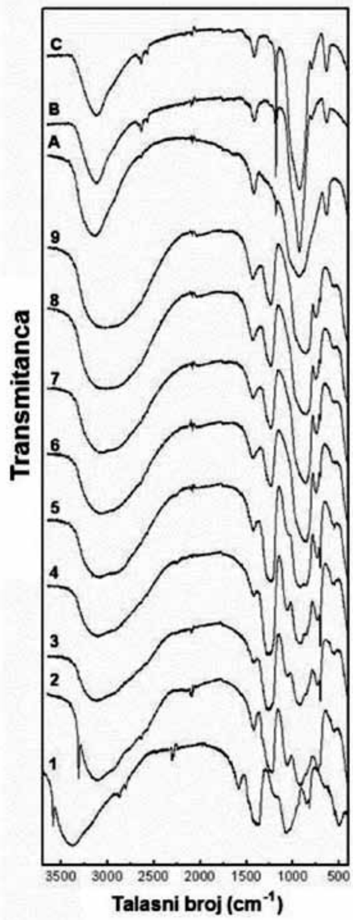

Figure 5. FTIR spectrum of $Ca(OH)_2$-P_2O_5-NH_4F mechanically treated mixture: 1 – 5 minutes, 2 – 20 minutes, 3 – 35 minutes, 4 – 1 hours, 5 – 2 hours, 6 – 3 hours, 7 – 4 hours, 8 – 6 hours, 9 – 8 hours, A-C – thermally treated
Slika 5. FTIR spektar $Ca(OH)_2$-P_2O_5-NH_4F smeše mehanički tretirane: 1 – 5 min., 2 – 20 min., 3 – 35 min., 4 – 1 h, 5 – 2 h, 6 – 3 h, 7 – 4 h, 8 – 6 h, 9 – 8 h, A-C – termički tretirane

There were identified changes in the structure from amorphous to crystalline, for all thermally treated samples. The typical peaks of FA confirmed transformation that took place in almost all samples (Figure 7). The amount of residual $CaCO_3$ and CaF_2 was still significant only in the sample milled for 5 minutes. The emphasized peaks of these phases indicate that parts of the samples remained unchanged, despite the high energy involved in the mechanochemical treatment during the preparation of the precursors mixture (Figure 7a).

As shown in Figure 7 a-c, clearly emphasized and sharp peaks typical for FA are present as a result of an adequate mixing of samples for 2 hours and particularly milling for 8 hours. The sample milled for 5 minutes shows that the transformation of the reaction mixture in flourapatite was only partial, despite the thermal treatment at 550°C for 3 hours. In addition to FA peaks, there are also $CaCO_3$ and CaF_2 peaks. This proves that even though the inside of the micelle of a surfactant contains the components of the building blocks of precursors phase that can be easily transformed into the pure fluorapatite, they still remain unchanged.

On the contrary, the mixture that was milled for at least 2 hours and additionally thermally treated for 3 hours is

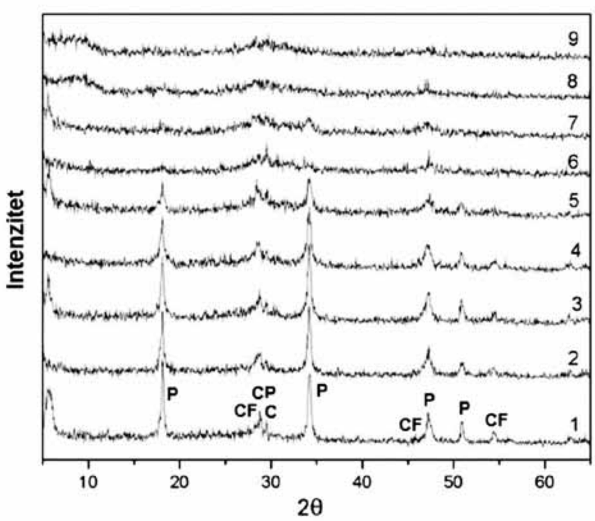

Figure 6. XRD spectrum of $Ca(OH)_2$-P_2O_5-NH_4F mechanically treated mixture: 1 – 5 minutes, 2 – 20 minutes, 3 – 35 minutes, 4 – 1 hours, 5 – 2 hours, 6 – 3 hours, 7 – 4 hours, 8 – 6 hours, 9 – 8 hours
Slika 6. XRD spektar $Ca(OH)_2$-P_2O_5-NH_4F smeše mehanički tretirane: 1 – 5 min., 2 – 20 min., 3 – 35 min., 4 – 1 h, 5 – 2 h, 6 – 3 h, 7 – 4 h, 8 – 6 h, 9 – 8 h

completely transformed into the pure fluorapatite. Similar was for a sample that was milled for 8 hours. The peak corresponding to FA only moves towards the greater angles of diffraction. This means that the content of OH^- groups was reduced during the milling of the sample and that FA finally became predominant phase (possibly mixed with a small amount of hydroxyapatite).

REACTION MECHANISM

During 5 minutes and 2 hours of milling, the reactions in which β- $Ca_2P_2O_7$ and CaF_2 are formed are dominant, while $Ca(OH)_2$ part remains unchanged. This has a strong influence on the synthesis rate of FA, which is very slow and cannot be completed only by milling (even after 8 hours of milling). This low rate comes from a very slow diffusion and rearrangement of certain ions that are necessary for FA formation. The exchange and incorporation of Ca^{2+} ions in β- $Ca_{2(1-x)}P_{2(1-x)}O_{7(1-x)}$ and $Ca_{(1-x)}F_{2(1-x)}$ pre-formed cells is strongly inhibited by the presence of EVA/EVV. Therefore, in order to provide bigger reaction rate of formation of FA, crystal structure of β- $Ca_{2(1-x)}P_{2(1-x)}O_{7(1-x)}$ and $Ca_{(1-x)}F_{2(1-x)}$ must firstly be transformed to amorphous by additional milling (2 – 8 hours). This procedure provides good mixing, which reduces the diffusion paths of different ions.

The second stage of the formation of FA started after low temperature treatment of the samples at 550°C for 3 hours. In this step, according to certain researches, the reactions can be initiated on the surface of the dominant phase β- $Ca_2P_2O_7$ through the surface diffusion of additional Ca^{2+} and F^- ions in its volume. According to the diffraction peaks, it is evident that the process of formation of FA during these thermal treatments is very intense. The only exception was a sample milled for 5 minutes. Despite conducted thermal treatment, short milling time was not

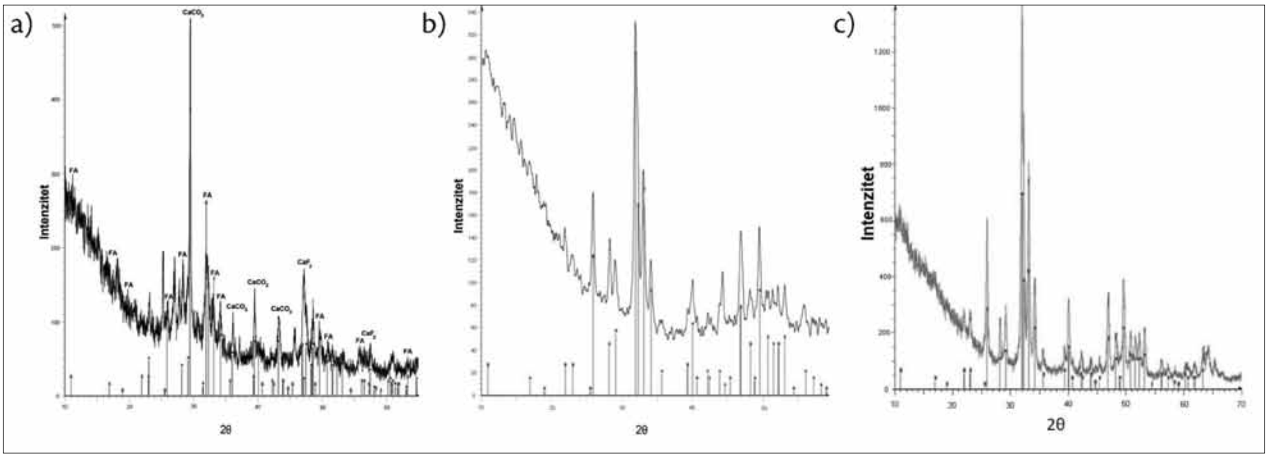

Figure 7. XRD spectra of $Ca(OH)_2$-P_2O_5-NH_4F mixture after different milling times: a) 5 minutes, b) 2 hours, c) 8 hours
Slika 7. XRD spektri $Ca(OH)_2$-P_2O_5-NH_4F smeše nakon različitih vremena mlevenja: a) 5 min., b) 2 h, c) 8 h

enough to obtain a single-phase system (FA). However, the milling of at least 2 hours led to the formation of pure FA phase. The presence of almost negligible peaktypical for β- $Ca_2P_2O_7$ proves that this milling time is the border time for obtainingthe single-phase FA.

It is important to emphasize that, no matter how long the samples were milled; this reaction wouldn't be possible without the thermal treatment. With the extended milling time (up to 3 hours or more), the system became amorphous. The reaction did not progress even during the longest milling time (8 hours), which was confirmed by XRD. The progress was observed only in the amorphous samples (milled for at least 2 hours), which were subjected to subsequent thermal treatment. The mechanism of the process, which took place during milling, was possibly significantly activated by the water present in $Ca(OH)_2$. The explanation provided by some researchers shows that smaller $Ca(OH)_2$ particles, during milling under the influence of shearing forces, tend to grow, causing further disintegration [29-31], so that the exchange of different ion species becomes more efficient.

During milling, the distortion of Ca^{2+}-O polyhedra is much more prominent in comparison with P^{5+}-O tetrahedra. The distortion led to displacement of cations from the center of its coordination sphere. It has a strong impact on the diffusion rate of the remaining amount of Ca^{2+} and F^- ions and consequent destruction of β-$Ca_2P_2O_7$ during milling. Thus, the "empty" space in β-$Ca_2P_2O_7$is increasinglyfilled with these ions, untilβ-$Ca_2P_2O_7$ cell is completely destroyed. It should be noted that the capacity of dissolution of Ca^{2+} and F^- ions within β-$Ca_2P_2O_7$ is very high. Accordingly, the smaller size of CaF_2, and especially $Ca(OH)_2$ crystallites is suitable for further propagation reaction of the formation of FA. Although crystallization of amorphous phase cannot be achieved through milling, the distribution of ions provides very rapid crystallization of the samples in FA in the next step of a very low thermal treatment (at 550 °C for 3 hours).

Therefore, it seems that there is enough space within β-$Ca_2P_2O_7$ structure for F- anions to be placed in large gaps, and within the network of calcium and phosphate ions. In addition to F^- ions, very small CaF_2 nanoparticles are placed randomly in the gaps within β-$Ca_2P_2O_7$ lattices, which produce significant changes in its symmetry, causing the corresponding chemical changes responsible for the transformation of mixture into fluorapatite during the next thermal treatment. This treatment can significantly accelerate the processes of diffusion, causing degradation of EVA/EVV micellar cages and supporting small ion redistribution by distortion in β-$Ca_2P_2O_7$ structure induced by shear forces, until the final transformation of the mixture into fluorapatite.

CONCLUSION

Mechanochemical process of fluorapatite synthesis is based on the use of two kinds of precursors: calcium hydroxide, phosphorus pentoxide and calcium fluoride, or calcium hydroxide, phosphorus pentoxide and ammonium fluoride with the addition of surfactant vinyl acetate/verstat. On the basis of XRD and FTIR analysis it was observed that fluorapatite has significant advantages in comparison with hydroxyapatite. These benefits are related to its greater stability, lower solubility and especially better protection against cavities.

APPRECIATION

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Strukturne karakteristike i mehanizmi mehanohemijske sinteze fluorapatita

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KRATAK SADRŽAJ

U radu su analizirani mehanizmi mehanohemijske sinteze fluorapatita i njegove strukturne karakteristike. Suštinu istraživanja čine istraživanja V. Jokanovića i saradnika objavljena u odgovarajućim časopisima i knjizi *Nanomedicina, najveći izazov 21. veka*. Karakteristike dobijenih materijala pokazuju njihove brojne biološke prednosti, koje su povezane sa specifičnostima strukturnog dizajna materijala tokom procesa sinteze.

Kao osnove za proučavanje procesa sinteze i mehanizma nastajanja fluorapatita korišćene su metode rendgenske difrakcije (XRD) i infracrvene spektroskopije sa Furijeovom transformacijom (FTIR).

Ključne reči: fluoroapatit; mehanohemijska sinteza; difrakcija X zraka; infracrvena spektroskopija; tretman niskom temperaturom

UVOD

Fluorapatit (FA), hemijske formule $Ca_5(PO_4)_3F$, ili $Ca_{10}(PO_4)_6F_2$, najstabilniji je, najmanje rastvorljiv i najtvrđi kalcijum-ortofosfatni mineral (tvrdoća po Mosu 5). Takve osobine fluorapatita povezane su sa specifičnom pozicijom F^- jona u centru Ca_2 trougla, njegove kristalne strukture. Tehnike sinteze su slične kao kod hidroksiapatita, s tim što sinteza podrazumeva prisustvo F^- jona, koji se unose u sintezu preko CaF, NaF ili NH_4F. Za razliku od hidroksiapatita (HA) deficijentnog kalcijumom, nema podataka o fluorapatitima deficijentnim kalcijumom. Hemijska formula fluorapatita je $Ca_{10}(PO_4)_6(F,OH)_2$, zbog toga što najčešće izmena OH^- jona F^- jonima nije potpuna. Među svim kalcifikovanim tkivima čoveka, najveća koncentracija fluorapatita nađena je u kostima, a najmanja u zubnoj gleđi. Ipak, čak i tamo gde je najveća koncentracija fluorapatita, količina fluorida je najčešće smanjena u odnosu na stehiometrijsku količinu. Zbog svoje slabe rastvorljivosti (brzine degradacije) retko se koristi kao zamenik kostiju.

Poslednjih godina fluorohidroksiapatit/fluorapatit (FHA/FA) koristi se u kliničkoj restauraciji, jer mu je zahvaljujući mehaničkoj stabilnosti smanjena rastvorljivost i unapređena proliferacija ćelija koštanog tkiva [1, 2]. Pored toga, HA i FHA/FA se koriste u biomedicini kao nosači lekova ikatalizatora i adsorbensi [3, 4].

Takođe, FHA/FA ima bolju termičku i hemijsku stabilnost od HA [1]. Ovaj fenomen je opisao Elliott sa saradnicima [5, 6]. Kada se određeni broj OH^- grupa u HA matriksu zameni jonima F, termička i hemijska stabilnost FHA/FA keramika značajno raste. Teorijski, odnos F:OH ≥ 1 unutar OH lanaca (u FHA strukturi) bio bi dovoljan da uredi HA kristale, stabilišući njihovu strukturu zahvaljujući naizmeničnom rasporedu F^- jona između OH^- jona.

U praksi se materijali koji sadrže jone F^- široko koriste za stomatološke restauracije, jer sprečavaju razvoj karijesa i smanjuju bakterijsku aktivnost u kiseloj sredini. Pored toga, sami F^- joni favorizuju mineralizaciju i kristalizaciju kalcijum-fosfata tokom formiranja kosti [7]. Osim toga, *in vitro* istraživanja FHA/FA ukazala su na njegovo sporo rastvaranje, bolje taloženje sloja sličnog hidroksiapatitu, bolju adsorbciju proteina [6-8] i uporedivo ili bolje vezivanje ćelija u poređenju sa čistim HA [7, 9], odnosno poboljšanu aktivnost alkalne fosfataze [6].

Takođe je pokazano da prisustvo fluorida utiče na povećanje količine i kvaliteta kostiju u organizmu [5]. Fluoridni jon se koristi za lečenje osteoporoze, jer koštana masa raste sa primenom F^- jona [9], a F^- joni stimulišu aktivnost osteoblasta, i u *in vitro* i u *in vivo* uslovima. Osim toga, mineralna faza zubne gleđi sastoji se od HA (95–97%) sa 0,04–0,07 tež. % fluorida. Doza od oko 1.5–4 mg fluorida dnevno značajno smanjuje rizik od karijesa [5]. Pored FHA i FHA faza, materijali slični CaF_2 takođe su značajni u stomatologiji, jer mogu da se koriste kao rezervoari labilnog fluorida u prevenciji karijesa [10-14].

Neke studije su pokazale da je dvostruki sistem isporuke F^- i Ca^{2+} jona neophodan da se omogući homogena nukleacija i formiranje vrlo malih CaF_2 kristala u ustima. Ove količine su veoma efikasne u povećanju taloženja i zadržavanju labilnih F^- jona u ustima, uz istovremeno povećanje efekata remineralizacije, bez konsekventnog povećanja F^- sadržaja [15-23]. Zbog toga je u istraživanjima V. Jokanovića i saradnika [24] prvi put opisan ne samo specifičan način sinteze fluorapatita već i sinteze kombinovanih sistema inkapsuliranih u površinski aktivnu supstancu polietilen vinil acetat/versatat, koja je potencijalni izvor labilne CaF_2 faze. Ovo je veoma važno za održavanje ravnoteže sadržaja jona F, ali i za unapređenje hemijske i mehaničke stabilnosti zuba.

Za sintezu FHA/FA, kao što je precipitacija, koriste se različite metode, kao što susol-gel, hidroliza, hidrotermalna metoda, kao i čvrsto fazne reakcije, koje podrazumevaju odgovarajuću izmenu jona između reaktanata, koji se koriste u sintezi FA [23-26]. Većina hemijskih metoda zahteva vrlo preciznu kontrolu parametara procesa sinteze, sastav proizvoda i njegove osobine, što nije tako lako postići, pa zato navedene metode nisu pogodne za sintezu FHA/FA na industrijskoj skali [27].

S druge strane, mehanohemijska obrada, kao jednostavna metoda koja se odvija u čvrstom stanju, omogućava sintezu materijala kroz izuzetno efikasan proces mešanja različitih jonskih vrsta, zahvaljujući silama smicanja, koje redukcijom veličine čestica i njihovim naizmeničnim slojevitim pozicioniranjem poboljšavaju termodinamiku i kinetiku reakcija između različitih prekursora čvrste supstance. Osim toga, u poređenju sa drugim gorenavedenim procesima, ovo je pogodnija metoda i sa ekonomske i sa tehničke tačke gledišta, jer omogućava masovnu proizvodnju nanokristalnih prahova i veliku fleksibilnost procesnih parametara [13].

Zbog toga, cilj ovog rada je bio da se predstavi suština metode sinteze nanopraha fluorhidroksiapatita/fluorapatita metodom mehaničkog legiranja.

Parametri mlevenja, kao što su brzina rotacije, prečnik i broj kugli, maseni odnos kugle–prah, bili su konstantni, dok je uticaj vremena mlevenja na sastav faze veoma pažljivo određen. Kinetika i mehanizam reakcije za dobijanje FHA/FA i druge prelazne faze su ispitivani koristeći XRD i FTIR spektroskopiju.

MEHANIZMI SINTEZE FLUORAPATITA

Tipični mehanizmi sinteze fluorapatita prikazani su na primeru najčešće primenjivanih postupaka mehanohemijske sinteze iz najčešće korišćenih prekursora kao što su kalcijum-hidroksid – $Ca(OH)_2$, fosfor-pentoksid – P_2O_5 i kalcijum-fluorid – CaF_2 (sinteza 1) i kalcijum-hidroksid – $Ca(OH)_2$, fosfor-pentoksid – P_2O_5, i amonijum-fluorid – NH_4F, uz dodatak površinski aktivne supstance vinil acetat/versatata (EVA/AVV) (sinteza 2). Oba mehanizma odvijaju se kroz niz procesnih faza, koje je moguće pratiti na adekvatan način prvenstveno primenom IR spektroskopije i rendgenske difrakcije [24]. Na osnovu rezultata izvedenih analiza uočeno je da svaku fazu prati određeni stepen transformacije polaznih reaktanata u fluorhidroksiapatit, sa sve manjim i manjim udelima OH^- grupe i sve većim i većim udelima jona F^- umesto OH^- grupe, sve do potpune transformacije sistema u fluorpatit.

Reakcija se u osnovi odvija sa nepotpunom stehiometrijom, pri čemu se vrednost x, koja definiše odstupanje od potpune simetrije, nalazi u intervalu $x_1<x<x_k$,

$$Ca_{10}(PO_4)_{1-y}(CO_3)_y(PO_4)_5 (OH)_{2-2x1} (F)_{2x1} +(2-2x_1)HF \rightarrow Ca_{10}(PO_4)_{1-y}(CO_3)_y (PO_4)_5(OH)_{2-xk}F_{xk} + (2-x_k) HF + H_2O;$$

pri čemu x_k može imati maksimalnu vrednost 1, a x_1 minimalnu vrednost 0.

Dakle, sumarna reakcija, kod reakcije sa potpunom stehiometrijom, imala bi oblik:

$$9\ Ca(OH)_{2-2y}(CO_3)_y + 3P_2O_5 + CaF_2 \rightarrow Ca_{10}(PO_4)_{1-y}(CO_3)_y (PO_4)_5F_2 + 9H_2O;\ x=1$$

Na osnovu rendgenske difrakcije utvrđeno je da je već posle 1 h mehanohemijskog tretmana došlo do amorfizacije sistema/mešavine.

Naime, zbog izuzetno visoke koncentracije mehaničkih naprezanja na veoma maloj površini kontakta (kontakt koji se ostvaruje pri međusobnom sudaru kugli ili sudaru kugli sa površinom unutrašnje obloge), ostvaruju se uslovi za nastajanje visokih smicajnih naprezanja na relativno maloj površini kontakta. Pri tome veličina napona deformacije zavisi prvenstveno od prečnika kugli koje se koriste kod mehanohemijskog tretmana (veličina kontaktne kalote utiskivanja deformacije pri sudaru) i brzine sudara. Istovremeno sa procesom prenosa deformacije dolazi do mehaničke aktivacije sistema i visokoelastičnog toka, koji prati intenzivne hemijske i fazne promene unutar materijala (reakcije izmene, mešanja jonskih vrsta, nastajanja novih faza itd.). Ove promene mogu biti takve da materijal tokom relaksacije delimično trpi povratnu deformaciju (viskoelastični tok), ili su pak u celosti nepovratne, kada dominira mehanizam puzanja materijala.

Napon kritične deformacije zavisi od vremena izlaganja sistema deformaciji (broja udara kugli), odnosno broja ciklusa opterećenja, tako da vremenom napon koji izaziva kritičnu deformaciju razaranja/formiranja pukotina i novih površina ima sve manju i manju vrednost, što vodi sve većoj amorfizaciji sistema. Proces mehaničke aktivacije u sistemima u kojima kao reakcioni produkt nastaje voda dodatno je ubrzan time što je olakšan transport odgovarajućih jonskih vrsta na mesta koja odgovaraju minimumu slobodne energije sistema.

U ovom slučaju, zbog izuzetne hidrofilnosti P_2O_5 odmah po njegovom dodavanju ostatku reakcione smeše dolazi do nastanka fosforne kiseline (P_2O_5+ $3H_2O \rightarrow 2H_3PO_4$), koja potom reaguje sa $Ca(OH)_{2-2y}(CO_3)_y$ i nastaje $Ca(HPO_4)_{1-y}(CO_3)_y$ (karbonatni kalcijum-hidrogenfosfat).

Nakon 4 h mlevenja karakteristične trake za HPO_4^{2-} počinju intenzivno da iščezavaju, a nakon 5 h potpuno iščezavaju, a pojavljuje se traka na 963 cm^{-1} (karbonatni kalcijum-deficijentni hidroksifluorapatit) (Slika 1). Istovremeno tokom celog procesa dolazi i do disocijacije CaF_2, pri čemu nastaju joni F^- koji tokom celog procesa ulaze u reakciju sa deficijentnim kalcijum-hidroksifluorapatitom sve do formiranja njegove konačne hemijske forme. Napokon, na uzorcima koji su termički tretirani na 1100°C, a prethodno mehanički tretirani 6 i 9 h iščezavaju trake na 1420 i 1455 cm^{-1}, koje pripadaju CO_3^{2-}, a kod uzorka koji je mehanički tretiran 6 h pojavljuje se traka na 630 cm^{-1}, koja govori da nije došlo do kraja reakcije izmene fluora i hidroksilne grupe. Traka na 726 cm^{-1} pripada jonima F i konstantno je prisutna tokom celokupnog procesa, što govori o tome da se kordinacija F^- ne menja bitno kroz sam proces transfera F^- iz CaF_2 u kalcijum-hidroksifluorapatit.

Brzina deprotonizacije HPO_4^{2-} i jonske izmene OH^- i F^- regulišu brzinu nastajanja fluorapatita u svim fazama procesa. Proces disocijacije kalcijum-fluorita odvija se kroz proces hemijskog nagrizanja njegovih čestica, u okviru defekata sistema (otvorene površine, jamice nagrizanja, dislokacije, dislokacijske petlje, vakancije), čime se otkidaju joni Ca^{2+} sa tih mesta ostavljajući ogoljene jone F^- koji se tek u tom trenutku nošeni molekulima vode transportuju na mesta koja odgovaraju datom fluktuacionom koncentracionom gradijentu / koncentracionom gradijentu lokalne površine.

To ukazuje da je realno pretpostaviti da se reakcija u većim i manjim polaznim česticama sistema odvija na različit način i da u različitim vremenskim intervalima doseže ravnotežne uslove za krajnju reakciju nastajanja fluorapatita. U velikim česticama verovatno reakcija na površini teče odmah po početku mehanohemijskog tretmana, dok u dubini čestica ostaju jezgra koja su još uvek pripadajuća datim polaznim reaktantima. Sama morfologija čestica, koja i u udaljenim fazama tretmana (4–6 h) ostaje ista, svedoči o tome da reakcija u svakoj čestici / grupi čestica napreduje na individualan način. U manjim česticama reakcija se odvija brzo, u srednjim česticama i posebno u velikim ona napreduje punim intenzitetom duž novootvorenih puteva (novih površina, granica kristalita / blok mozaika itd.), sve dok se u celosti ne realizuje reakcija transformacije kalcijum-hidroksifluorapatita u fluorapatit, kao što je pokazano na utačnjenom XRD spektru (Slika 2).

Slika 3. pokazuje najbliže susede F^- jona u strukturi karbonatnog fluorapatita. Uočava se da se tri $CaII^{2+}$ nalaze u neposrednoj blizini F^- jona na rastojanju od 2,3 Å. Ovi $CaII^{2+}$ joni formiraju temena jednakostraničnog trougla sa F^- jonom u centru. U drugoj koordinacionoj sferi nalaze se po tri $CaII^{2+}$, P^{5+} i O^{2-} jona koja međusobno formiraju temena trouglova. Rastojanje između F^- i P^{5+} je 3,6 Å, a između P^{5+} i O^{2-}(1) je 3,9 Å. O^{2-}(3) jona nalaze se ispod i iznad ravni koja sadrži F^- i $CaII^{2+}$. Kiseonični joni zauzimaju temena dodekaedrona. Rastojanje između F^- i O^{2-}(3) jona je 3,1 Å, dok je između P^{5+} i O^{2-}(3) jona 1,5 Å. Slika 2b pokazuje fragment kristalne rešetke upoređen sa jediničnom ćelijom. Saglasno istraživanjima Pande i njegovih saradnika, $CaII^{2+}$ ima veću atomsku veličinu nego CaI^{2+}. Kada su OH^- joni supstituisani sa F^- dolazi do veće distorzije u strukturi zbog veće veličine jonskog radijusa F^-. Na kraju mehanohemijskog procesa sinteze F^- jon zauzima veliki prostor u centru rešetke, formirajući na taj način stabilnu fluorapatitnu strukturu.

Saglasno istraživanjima V. Jokanovića i saradnika [24] (OH^-, F^-), lanac apatita sadrži pored tri tipa [OH^-] i četvrti tip sa različitim vibracionim energijama. U ovim istraživanjima uočeno je da, ako se kao kriterijum kvantifikacije izmene OH^- sa F^- uzme kriterijum pomeranja slobodne vibracije OH^-, sledi da je oko 50% OH^- grupa izmenjeno sa F^-, dok je kod sistema sa skoro potpuno izmenjenim OH^- grupama sa F^- (kod čistog fluorapatita) vrednost talasnog broja od 758 cm^{-1} (Slika 4 i tabela 1).

POSTUPAK SINTEZE SA DODATNIM NISKOTEMPERATURNIM TERMIČKIM TRETMANOM

Drugi postupak mehanohemijske sinteze, koji je izveden primenom prekursora $Ca(OH)_2$, P_2O_5, NH_4F i površinski aktivne supstance vinil acetata/versatata, pokazuje da samo u mehanohemijskom postupku nije moguće dobiti fluorapatit, nego da je za tu svrhu potrebno izvesti dodatni niskotemperaturni tretman.

Kao najadekvatnija metoda za praćenje same sinteze pokazala se kao i u prethodnom primeru FTIR metoda (Slika 5).Radi dobijanja kompletne slike o faznim prelazima koji se događaju u materijalu tokom mehanohemijskog i niskotemperaturnog tretmana, pored FTIR metode korišćena je i metoda rendgenske difrakcije, koje su detaljno opisane u referenci 23.

XRD spektri uzoraka (Slika 6) pokazuju vrlo intenzivne pikove portlandita (P), dok su pikovi β-$Ca_2P_2O_7$(CP) takođe vidljivi. Osim toga, veoma su naglašeni i pikovi $CaCO_3$(C) i CaF_2(CF). Svi karakteristični difrakcioni pikovi za FA su gotovo zanemarljivi, a neki od njih su odsutni [29].

Posle pet minuta mlevenja, najintenzivniji vrhovi pripadaju $Ca(OH)_2$, a pikovi karakteristični za β-$Ca_2P_2O_7$, CaF_2 i $CaCO_3$ jasno su vidljivi. Pik koji odgovara $Ca(OH)_2$ je jasno vidljiv i pokazuje nisku brzinu reakcije formiranja fluorapatita. Usled toga, količina sintetisanog FA u smeši je zanemarljiva.

Za sve termički tretirane uzorke identifikovane su promene u strukturi od amorfne do kristalinične. Karakteristični pikovi FA potvrđuju transformacije koji su se desile u gotovo svim uzorcima (Slika 7). Samo u uzorku mlevenom pet minuta količina rezidualnog $CaCO_3$ i CaF_2 je bila još značajna. Izraženi pikovi ovih faza ukazuju da su delovi uzoraka ostali nepromenjeni, pored visoke energije uključene u mehanohemijski tretman prilikom pripreme smeše prekursora (Slika 7a).

Kao što je prikazano na slici 7a–c, vrlo izraženi i oštri vrhovi karakteristični za FA prisutni su kao posledica adekvatnog mešanja uzorka u toku 2 h i posebno 8 h mlevenja. Uzorak mleven pet minuta pokazuje da je transformacija reakcione smeše u fluorapatit samo delimična, uprkos termičkom tretmanu na 550°C u toku 3 h. Osim pikova FA, javljaju se i pikovi $CaCO_3$ i CaF_2. Ovo je dokaz da iako se unutar micela površinski aktivne supstance delovi gradivnih blokova faza prekursora mogu lako transformisati u čisti fluorapatit, oni ipak ostaju nepromenjeni.

Naprotiv, smeša mlevena najmanje 2 h i dodatno termički tretirana 3 h potpuno je transformisana u čist fluorapatit. Slično je primećeno za uzorak mleven 8 h. Pik koji odgovara FA samo se pomera ka većim uglovima difrakcije. To znači da je sadržaj OH-grupa smanjen tokom mlevenja uzorka i da je FA postao konačno prevladavajuća faza (pomešan verovatno sa malom količinom hidroksiapatita).

MEHANIZAM REKACIJE

Tokom mlevenja od pet minuta i 2 h, reakcije u kojima nastaju β- $Ca_2P_2O_7$ i CaF_2 su dominante, a deo $Ca(OH)_2$ ostaje nepromenjen. Ovo ima snažan uticaj na brzinu sinteze FA, koja se odvija veoma sporo i ne može biti završena samo mlevenjem (čak i posle 8 h mlevenja). Ova niska brzina potiče od jako spore difuzije i preuređenja određenih jonskih vrsta, koje su neophodne za formiranje FA. Razmena i inkorporacija Ca^{2+} jona u prethodno formirane ćelije β- $Ca_{2(1-x)}P_{2(1-x)}O_{7(1-x)}$ i$Ca_{(1-x)}F_{2(1-x)}$ snažno je inhibirana prisustvom EVA/EVV. Zbog toga, za veću brzinu reakcije formiranja FA, kristalna struktura β- $Ca_{2(1-x)}P_{2(1-x)}O_{7(1-x)}$ i$Ca_{(1-x)}F_{2(1-x)}$ mora biti prvo transformisana u amorfnu dodatnim mlevenjem (2–8 h). Ova procedura omogućava dobro mešanje, čime se umanjuju difuzioni putevi različitih jonskih vrsta.

Druga faza formiranja FA počela je nakon niskotemperaturnog tretmana uzoraka na 550°C tokom 3 h. Tokom ovog koraka, prema nekim istraživanjima, reakcije mogu biti pokrenute na površini dominantne faze β- $Ca_2P_2O_7$ kroz površinsku difuziju dodatnih Ca^{2+} i F^- jona u njenu zapreminu. Prema difrakcionim pikovima, evidentno je da je proces formiranja FA tokom tih termičkih tretmana veoma intenzivan. Jedini izuzetak bio je uzorak mleven pet minuta. Uprkos primenjenom termičkom tretmanu, tako kratko vreme mlevenja nije bilo dovoljno da se dobije monofazni sistem (FA). Međutim, mlevenje od najmanje 2 h dovelo je do formiranja čiste FA faze. Prisustvo gotovo zanemarljivog pika karakteristnog za β- $Ca_2P_2O_7$ dokazuje da je ovo vreme mlevenja granično vreme za dobijanje mono faze FA.

Veoma je važno naglasiti da bez obzira na to koliko su uzorci mleveni, bez termičkog tretmana ne bi došlo do ove reakcije. Sa produženim vremenom mlevenja (do 3 h ili duže), sistem je postao amorfniji. Reakcija nije napredovala čak i tokom najdužeg vremena mlevenja (8 h), što je potvrđeno pomoću XRD. Napredak je primećen samo u amorfnijim uzorcima (mlevenim najmanje 2 h) izloženim naknadnom termičkom tretmanu. Mehanizam procesa koji se dogodio tokom mlevenja verovatno je značajno aktiviran vodom prisutnom u $Ca(OH)_2$. Objašnjenje Hedina i nekih drugih istraživača jeste to da manje čestice $Ca(OH)_2$ tokom mlevenja pod dejstvom sila smicanja pokazuju tendenciju da rastu, izazivajući dalju dezintegraciju [29-31], pa razmena različitih jonskih vrsta postaje efikasnija.

Tokom mlevenja distorzija Ca^{2+}-O poliedara je mnogo izraženija nego P^{5+}-O tetraedara. Distorzija je dovela do pomeranja katjona od centra njegove koordinacione sfere. Ona ima jak uticaj na brzinu difuzije preostalih količina Ca^{2+}i F^- jona i konsekventno razaranje β-$Ca_2P_2O_7$ tokom mlevenja. Dakle, „prazan“ prostor u β-$Ca_2P_2O_7$ ispunjen je sve više ovim jonskim vrstama, dok ćelija β-$Ca_2P_2O_7$ ne bude potpuno razorena.

Treba napomenuti to da je kapacitet rastvaranja Ca^{2+}i F^- jona unutar β-$Ca_2P_2O_7$ veoma visok. Shodno tome, manje veličine CaF_2, a naročito $Ca(OH)_2$ kristaliti, prikladni su za dalju propagaciju reakcije formiranja FA. Iako se kristalizacija amorfnih faza ne može postići kroz mlevenje, raspodela jona omogućava vrlo brzu kristalizaciju uzoraka u FA u sledećem koraku veoma slabog termičkog tretmana (na 550°C tokom 3 h).

Stoga, čini se da unutar β-$Ca_2P_2O_7$ strukture ima dovoljno prostora za F^- anjone da se postave u velike praznine, unutar mreže kalcijumovih i fosfatnih jona. Pored F^- jona, veoma male nanočestice CaF_2 smeštaju se nasumice u praznine u β-$Ca_2P_2O_7$ rešetki, koje proizvode značajne promene u njenoj simetriji, izazivajući odgovarajuće hemijske promene koje su odgovorne za transformaciju smeše u fluorapatit tokom naredne termičke obrade. Ovaj tretman, kako je dokazano u ovim istraživanjima, može značajno ubrzati procese difuzije, uzrokujući degradaciju EVA/EVV micelarnih kaveza i podrške malim jonskim preraspodelama distorzijom u strukturi β-$Ca_2P_2O_7$ izazvanoj silama smicanja, do konačne transformacije mešavine u fluorapatit.

ZAKLJUČAK

Mehanohemijski postupak sinteze fluorapatita bazira se na primeni dve vrste prekursora: kalcijum-hidroksida, fosfor-pentoksida i kalcijum-fluorida, odnosno kalcijum-hidroksida, fosfor-pentoksida i amonijum-fluorida uz dodatak površinski aktivne supstance vinil acetat/verstata. Na osnovu XRD i FTIR analize, kao mehanizma formiranja FA, uočeno je da fluorapatit ima značajne prednosti u poređenju sa hidroksiapatitom. Te prednosti su vezane za njegovu bolju stabilnost, nižu rastvorljivost i pre svega bolju zaštitu od karijesa.

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Diabetes Mellitus And Reparative Response Of Dental Pulp

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SUMMARY

Anatomically, dental pulp is connective tissue and specific microcirculatory system with significant reparatory abilities intending to preserve pulp vitality. Various therapeutic approaches in the treatment of affected pulp may be compromised by various factors leading to treatment failure. Due to microcirculatory system disorders, treatment of affected dental pulp in patients with diabetes mellitus (DM) is additional challenge. The function and levels of growth factors could be altered in various diabetic tissues including dental pulp. Among them are growth factors important for reparative response of the pulp. There are experimental evidences that DM impede dental pulp reparation. Therefore, clinical procedures aiming to preserve vitality of diabetic dental pulp should be applied with caution.

The aim of this paper is to present basic factors and parameters that affect reparative response of dental pulp in patients with DM.

Keywords: dental pulp; diabetes mellitus; growth factors

INTRODUCTION

Dental pulp is a connective tissue that has some unique characteristics. Namely, it has specific microcirculatory system with no lateral blood vessel branches. Also, dental pulp is located within solid dentinal walls that cannot accept any significant change of its volume. These features make it very prone to irreversible inflammatory changes shortly after the impact of noxious stimuli. This can be significantly intensified in persons with diabetes mellitus (DM) known for their tissue vulnerability caused by macro- and micro-circulatory disorders.

Dental pulp has significant reparatory abilities that are important for therapeutic approaches aiming to preserve pulp vitality. These treatment modalities may be compromised by various factors leading to treatment failure. Therefore, these factors should be well known and described in order to establish appropriate indication for maintaining pulp vitality. Diabetic dental pulp, with possible various pathological changes, may present additional challenge for these treatment modalities.

The aim of this paper is to present basic factors and parameters with influence on reparative response of dental pulp in patients with diabetes mellitus.

REPARATIVE RESPONSE OF DENTAL PULP

The primary role of pulp is to produce dentin, but it is well known that this tissue has several functions: nutritive, sensory, defensive and reparative. Ability to repair is of special clinical interest because it has fundamental influence on all therapeutic procedures aiming to maintain pulp vitality. Reparative response of dental pulp is modified by morphological and functional pulp status (younger or older subjects, specific location, previous history of reparation process, etc). Reparative response depends on the type and intensity of harmful stimuli [1]. Specificity of pulp tissue compared to other connective tissues is dentin production up regulated during the repair process known as tertiary dentinogenesis. The layers of dentin are deposited on the pulp-dentinal interface, particularly towards noxious stimuli. The aim of this process is to protect pulp tissue by blocking harmful effects. The prerequisite for this process is localized, controlled and mild inflammation that will allow spreading blood vessels and providing adequate nutrient supply for up-regulated pulp secretory activity [2].

Tertiary dentinogenesis may progresses in two completely different ways depending on the intensity of noxious stimuli. In mild to moderate stimuli (for example shallow caries lesion) odontoblasts, specific secretory cells of dental pulp, may survive and increase their activity forming layers of tertiary dentin [3]. This process is called reactionary dentinogenesis. In case of strong stimuli, odontoblasts will not survive. Progenitor pulp cells will then activate, migrate and differentiate into odontoblast-like cells. These cells will form layers of new dentin in complex process known as reparative dentinogenesis [4]. Various signaling molecules regulate both reactionary and reparative dentinogenesis.

Although the process of tertiary dentinogenesis is well recognized and described, cellular and molecular mechanisms of its regulation are still not fully identified. It is known that specific cells conduct tertiary dentinogenesis. The cell lines involved in up-regulated dentin production

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are odontoblasts or odontoblast- like cells. The origin of the later ones is still unclear, but they are most probably derived from progenitor pulp cells (stem cells, Rougett pericytes, etc) [5]. Tziafas et al. have found that dentinogenetic activity of odontoblast- like cells may occur only in pulpal environment indicating that there are specific stimulators in pulp intercellular substance that initiate and conduct this process[6]. It is now well known that these "stimulators" are growth factors (GF).

THE ROLE OF GROWTH FACTORS IN PULP REPARATION

GF are signaling molecules that have the structure of polypeptides or small proteins. Cells produce GF and their regulatory function is achieved through binding to specific trans-membrane receptors. GF may activate receptors on cells they have been excreted by (autocrine mode of regulation), or they may react with receptors of adjacent cells (paracrine mode of regulation). When GF binds to extracellular domain of receptor, the intracellular enzymatic part will activate cascade of specific cellular reactions. In most cases this will lead to expression of specific genes and consequent synthesis of proteins included in regulation of particular cellular event [7]. Non-transcriptional responses may also occur as a result of GF activity, leading to activation of already existing proteins.

Numerous experimental evidences indicate that the key GF's with regulatory role in processes of pulp reparation are members of transforming growth factor β (TGF-β) superfamily[8]. Bone morphogenetic proteins (BMP), members of TGF-β superfamily, are also identified within pulp tissue[9]. Angiogenic GF's are of special importance, having in mind the need for adequate vascular supply in the pulp with up-regulated secretory activity. Vascular endothelial growth factor (VEGF) is the most prominent angiogenic GF, identified in dental pulp tissue[10]. Besides pulp tissue, all of this GF was identified in dentin matrix, too [11, 12]. During the progression of caries lesion, or as a result of pulp capping therapy, they will be released from dentin matrix and take role in regulation of dentinogenetic events [13].

DIABETES MELLITUS

DM is systemic metabolic disorder with hyperglycemia as main characteristic. It may be the result of pancreatic β cells dysfunction (DM type 1), or increased tolerance of cells and tissues to secreted insulin (DM type 2). DM, specifically type 2, is one of the most prevalent systemic diseases in human population [14]. DM is specific for its numerous complications that may seriously damage patients' life quality. All DM complications are in fact pathologic result of either macrovascular or microvascular changes in different tissues and organs. Therefore, these complications may be classified as macrovascular (atherosclerosis of cardiac blood vessels, coronal disease, etc.) or microvascular (diabetic retinopathy, diabetic nephropathy, etc.) [15].

There are significant physiological changes in DM. The main pathophysiological mechanism of changes during DM is mitochondrial overproduction of superoxides due to hyperglycemia which results in increase of tissue oxidative stress [15]. This leads to activation of specific pathophysiological mechanisms, signed to be direct promoter of histological changes. Activation of protein kinase C and formation of glycozilated end products are the main mechanisms to provoke changes in levels and function of different GF, contributing profoundly to the specific diabetic pathology [15].

Vascular endothelial growth factor (VEGF) is the most prominent GF responsible for diabetic complications. In physiological conditions it is responsible for vasculogenesis, angiogenesis and processes associated with them [16]. The levels and function of VEGF is altered in tissues and organs during DM. There are tissues with up-regulation of this GF leading to complications with extensive pathological hyper-angiogenesis such as diabetic retinopathy. On the other hand there are tissues where VEGF is down-regulated causing complications based on insufficient blood supply such as wound healing difficulties [17].

DM also provokes changes in TGF-β superfamily members [15]. Bone morphogenetic protein 2 (BMP-2), one of the members of this GF group, has significant influence on a specific pathological change in DM. Namely, BMP-2 is up-regulated in walls of diabetic blood vessels causing differentiation of osteoblasts and ectopic vascular calcifications. This is the main mechanism for atherosclerosis and similar changes in vascular beds to occur in DM [18, 19].

THE EFFECTS OF DM ON DENTAL PULP

DM may cause various pathological changes in oral tissues. Most of the studies on oral status in diabetic patients reported changes in oral mucosa and marginal periodontal tissues [14]. Endodontic research about the effect of DM on dental pulp is very scarce. Epidemiological studies reported higher prevalence of pulp and periapical diseases in diabetic patients[20], while Wang et al. [21] found higher frequency of tooth extraction after endodontic therapy in patients with DM, suggesting lower success rate of root canal treatment. Amatyakul et al. [22] found lowered blood flow in diabetic pulp, while Inagaki et al. reported higher prevalence of intrapulpal calcifications [23]. Histological studies showed increased thickness of blood vessel basement membrane, reduction in collagen level of intercellular substance and signs of chronic inflammation and angiopathy [24].It was concluded in the study of Garber et al. that pulp in diabetic rats had compromised reparatory response resulting in chronic pulp inflammation and reduced dentin bridge formation in comparison to pulp in non-diabetic rats [25]. Histological studies revealed higher concentrations of inflammatory mediators and enzymes in diabetic pulp, as well as oxidative stress parameters indicating higher level of reactive oxygen species in diabetic pulp comparing to non-diabetic one [26, 27]. Ilić et al. analyzed human diabetic dental pulp and found significant changes in levels of VEGF and BMP-2,GF's important for pulp reparative response [28].

VEGF is of great importance for microcirculatory system of dental pulp. The expression of VEGF and its receptors has been identified in pulp using polymerase chain reaction (PCR) method[29, 30] as well as immunohistochemical identification [31]. Increased expression of VEGF has been noticed during some pathological conditions of dental pulp such as inflammation, injury and hypoxia [32-34].

BMP-2 is GF with significant role in dental pulp functions. Expression of BMP-2 in dental pulp was demonstrated immunohistochemicaly [35] and by PCR method [9, 36]. This GF is responsible for odontoblast differentiation of pulp stem cells and for up-regulation of odontoblast secretion in primary, secondary and tertiary dentinogenesis [37, 38].

Changes in these GF levels could be of special interest when analyzing the effect of DM on pulp reparative response. Their altered levels in DM could provoke inadequate reaction of dental pulp on noxious stimuli. This is in concordance with the investigation of Garber et al. [25] that provided evidences that DM impede dental pulp reparation on rat model and with empirically known fact from practice that reactions of diabetic dental pulp on capping procedures may be very unpredictable.

CONCLUSION

Clinical dilemma whether to conduct pulp capping or to extend indications for pulpectomy in medically compromised patients with DM still exists. Fundamental physiological and clinical data on this problem are scarce. Extensive investigation should be conducted to reveal and describe cellular and molecular mechanisms of recognized changes in pulp reparative response during DM. Although these mechanisms are still insufficiently known, therapeutic procedures in order to preserve the pulp vitality in DM patients should be realized with caution.

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Dijabetes melitus i reparativni odgovor zubne pulpe

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KRATAK SADRŽAJ

Zubna pulpa je vezivno tkivo i poseban mikrocirkulatorni sistem sa značajnim reparatornim sposobnostima u cilju očuvanja njenog vitaliteta. Terapijski postupci u lečenju obolele pulpe mogu da budu kompromitovani delovanjem brojnih faktora koji mogu uticati na ishod lečenja. Zubna pulpa kod pacijenata sa dijabetes melitusom (DM), zbog mikrocirkulatornih poremećaja često predstavlja poseban terapijski izazov. Poznato je da funkcije i nivoi faktora rasta mogu biti promenjeni u dijabetičnim tkivima, pa samim tim i u zubnoj pulpi. Ovde su od najveće važnosti faktori rasta značajni za regulaciju reparatornog odgovora pulpe. Postoje eksperimentalni dokazi da DM umanjuje reparativnu aktivnost zubne pulpe, pa zbog toga kliničke procedure vezane za očuvanje vitaliteta dijabetične zubne pulpe treba realizovati uz značajne mere opreza.

Cilj ovog rada je da se predstave osnovni faktori i parametri koji utiču na reparatorni odgovor pulpe zuba kod obolelih od DM.

Ključne reči: zubna pulpa; dijabetes mellitus; faktori rasta

UVOD

Zubna pulpa je vezivno tkivo koje poseduje posebne karakteristike jer predstavlja jedinstven mikrocirkulatorni sistem bez kolateralnih grana. Takođe, pulpa je smeštena unutar čvrstih dentinskih zidova, što onemogućava bilo kakvu promenu njenog volumena. Ove osobine uslovljavaju ireverzibilne inflamatorne promene u kratkom vremenskom periodu nakon delovanja štetnih nadražaja. Ova sklonost je posebno potencirana kod osoba obolelih od dijabetesa melitusa (DM) usled pojačane sklonosti ka tkivnim oštećenjima uzrokovanu prisutnim promenama na velikim i malim krvnim sudovima.

Zubna pulpa ima značajne reparatorne sposobnosti, što je značajno za terapijske pristupe koji imaju za cilj očuvanje njenog vitaliteta. Međutim, terapijski modaliteti mogu biti kompromitovani brojnim uticajima koji vode neuspehu tretmana. Zbog toga je veoma važno poznavati ove faktore radi postavljanja pravilne indikacije, ali i realizacije terapijskih postupaka u cilju očuvanja vitaliteta ovog tkiva. Zato dijabetična zubna pulpa, sa brojnim patološkim promenama, predstavlja poseban izazov u terapiji.

Cilj ovog rada je da se predstave osnovni faktori i parametri koji utiču na reparatorni odgovor pulpe zuba kod obolelih od DM.

REPARATORNI ODGOVOR ZUBNE PULPE

Primarna uloga zubne pulpe je da stvara dentin. Reparatorna sposobnost pulpe je predmet posebnog kliničkog interesovanja jer leži u osnovi svih terapijskih procedura koje imaju za cilj očuvanje vitaliteta. Ovaj odgovor zubne pulpe može biti modifikovan njenim morfološkim i funkcionalnim stanjem (pulpa zuba starijih ili mlađih osoba, lokalizacija pulpe, prethodni reparatorni procesi itd.), a značajno zavisi od vrste i intenziteta štetnog nadražaja [1]. Specifičnost pulpe u odnosu na druga vezivna tkiva je produkcija dentina, koja je povećana tokom reparacije (tercijarna dentinogeneza). Slojevi novog dentina nastaju na pulpo-dentinskom spoju, prvenstveno u smeru delovanja štetnog nadražaja sa ciljem zaštite pulpnog tkiva mogućim blokiranjem štetnih uticaja. Preduslov za odvijanje ovog procesa je lokalizovana, kontrolisana i blaga zapaljenska reakcija koja dovodi do širenja mreže malih krvnih sudova, što sledstveno obezbeđuje adekvatno snabdevanje nutrijentima neophodnim za povećanu sekretornu aktivnost pulpe [2].

Tercijarna dentinogeneza može da se odvija u dva potpuno različita pravca i zavisi od intenziteta štetnih nadražaja. Kod blagih i umerenih nadražaja (na primer plitka karijesna lezija) odontoblasti mogu da prežive i pojačaju svoju sekretornu aktivnost formirajući nove slojeve tercijarnog dentina [3] (reaktivna dentinogeneza). U slučaju jakih stimulusa, odontoblasti bivaju oštećeni, pa se tada progenitorne ćelije pulpe aktiviraju, migriraju i diferenciraju u ćelije slične odontoblastima. Ove ćelije potom formiraju slojeve novog dentina u procesu reparativne dentinogeneze [4]. Procesi kako reaktivne tako i reparatorne dentinogeneze su regulisani brojnim signalnim molekulama.

Iako je tercijarna dentinogeneza proces koji je jasno prepoznat i dobro opisan, ćelijski i molekularni mehanizmi njegove regulacije nisu još uvek potpuno jasni. Poznato je da ove procese sprovode specifične ćelije. Ćelijske vrste neposredno odgovorne za povećanu pulpnu sekreciju su odontoblasti i ćelije slične odontoblastima. Poreklo ovih drugih još uvek je nedovoljno jasno, ali je najverovatnije da nastaju iz progenitornih pulpnih ćelija (stem ćelija, Rudžetovih (*Rougett*) pericita i sl.) [5]. Cafas (*Tziafas*) i sar. su utvrdili da odontoblastima slične ćelije mogu da stvaraju dentin samo u okuženju pulpnog tkiva, što ukazuje da u međućelijskoj supstanci pulpe postoje specifični stimulatori koji iniciraju i vode ovaj proces [6]. Danas se zna da su ovi tkivni stimulatori u stvari faktori rasta (FR).

ULOGA FAKTORA RASTA U REPARACIJI ZUBNE PULPE

Faktori rasta su signalne molekule koje su po svojoj hemijskoj strukturi polipeptidi ili mali proteini. Oslobađaju ih ćelije, a njihova regulatorna uloga se ostvaruje vezivanjem i aktivacijom specifičnih transmembranskih receptora. FR se mogu vezati za receptore na istoj ćeliji koja ih je proizvela (autokrina vrsta dejstva) ili za receptore na susednim ćelijama (parakrina vrsta dejstva). Kada se FR veže za vanćelijski deo receptornog molekula (tzv. ekstraćelijski domen), unutarćelijski enzimski deo receptora će započeti kaskadu specifičnih ćelijskih reakcija. U najvećem broju slučajeva ova kaskada reakcija dovodi do ekspresije specifičnog gena i sledstvene sinteze proteina odgovornih za regulaciju određenog ćelijskog procesa [7]. Kao rezultat aktivnosti FR mogu se desiti i netranskripcioni odgovori koji dovode do aktivacije već postojećih proteina u ćeliji.

Veliki broj eksperimentalnih studija ukazuje da su ključni FR za regulaciju procesa reparacije pulpe oni iz familije faktora rasta transformacije β (TGF-β) [8]. Kostni morfogenetski proteini, koji su inače članovi TGF-β familije, takođe su identifikovani u tkivu pulpe [9]. Angiogeni FR imaju poseban značaj, imajući u vidu potrebu za adekvatnim vaskularnim snabdevanjem pulpe koja ima povećanu sekretornu aktivnost. Faktor rasta vaskularnog endotela (VEGF) najznačajniji je angiogeni FR identifikovan u pulpi [10]. Osim tkiva zubne pulpe, svi pomenuti FR identifikovani su i u dentinskom matriksu [11, 12]. Tokom razvoja karijesne lezije, ali i kao posledica terapijskih postupaka prekrivanja pulpe, ovi faktori se oslobađaju iz dentinskog matriksa i ulaze u sledstvene procese regulacije [13].

DIJABETES MELITUS

Dijabetes melitus (DM) je sistemsko metaboličko oboljenje sa hiperglikemijom kao glavnom karakteristikom. Ono može nastati kao rezultat disfunkcije β ćelija pankreasa (DM tipa 1), ili kao rezultat povećane tolerancije ćelija i tkiva na insulin (DM tipa 2). DM, prvenstveno tipa 2, jedno je od najzastupljenijih sistemskih oboljenja u ljudskoj populaciji [14]. DM je posebno značajno oboljenje zbog brojnih komplikacija koje mogu ozbiljno da ugroze kvalitet života pacijenata. Sve komplikacije DM su u suštini posledica makrovaskularnih ili mikrovaskularnih promena u različitim tkivima i organima i zato se mogu klasifikovati kao makrovaskularne (ateroskleroza velikih krvnih sudova, koronarna bolest i sl.) i mikovaskularne (dijabetična retinopatija, dijabetična nefropatija i sl.) [15].

Međutim, postoje i suštinske fiziološke promene kod obolelih od DM. Osnovni patofiziološki mehanizam promena tokom DM je hiperprodukcija superoksida u mitohondrijama, koja nastaje kao posledica hiperglikemije i dovodi do povećanja oksidativnog stresa u tkivima [15]. Ovo vodi u aktivaciju specifičnih patofizioloških mehanizama, koji se smatraju direktnim pokretačima histoloških promena. Aktivacija proteinske kinaze C i stvaranje glikozilisanih završnih produkata metabolizma glavni su mehanizmi koji izazivaju promene u funkcijama različitih FR i značajno doprinose specifičnoj dijabetičnoj patologiji [15].

VEGF je najznačajniji FR odgovoran za razvoj dijabetičnih komplikacija. U fiziološkim uslovima, on je odgovoran za vaskulogenezu, angiogenezu i njima povezane procese [16]. Nivoi i funkcije VEGF su izmenjeni u tkivima i organima tokom DM. Postoje tkiva sa povišenom aktivnošću ovog FR, što dovodi do komplikacija usled ekstenzivne patološke angiogeneze kao kod dijabetične retinopatije. Sa druge strane, ima tkiva u kojima je aktivnost VEGF snižena izazivajući komplikacije usled neadekvatnog snabdevanja krvlju, kakvo je otežano zarastanje rana [17].

DM izaziva promene i u aktivnosti TGF-β familije [15]. Kostni morfogenetski protein 2 (BMP-2) ima značajan uticaj na razvoj specifičnih patoloških promena tokom DM. Naime, BMP-2 ima pojačanu aktivnost u zidovima dijabetičnih krvnih sudova i utiče na diferencijaciju osteoblasta i ektopične kalcifikacije. Ovo je glavni mehanizam ateroskleroze i sličnih promena koje nastaju na krvnim sudovima tokom DM [18, 19].

EFEKTI DM NA ZUBNU PULPU

DM dovodi do brojnih patoloških promena na oralnim tkivima. Većina studija vezanih za oralno stanje dijabetičara ukazala je na promene na oralnoj mukozi i marginalnom parodoncijumu [14]. Endodontske studije o efektima DM na zubnoj pulpi veoma su retke. Epidemiološke studije su pokazale veću prevalenciju pulpnih oboljenja i oboljenja apeksnog parodoncijuma [20], dok suVang (*Wang*) i sar. [21] uočili veću učestalost ekstrakcija zuba nakon sprovedenog endodontskog tretmana kod pacijenata sa DM. Amatjakul (*Amatyakul*) i sar. [22] našli su smanjen protok krvi u dijabetičnoj pulpi, dok su Inagaki i sar. utvrdili veću učestalost intrapulpnih kalcifikacija [23]. Studije na tkivnom nivou pokazale su zadebljalost bazalne membrane krvnih sudova, smanjenje količine kolagena u međućelijskoj supstanci i znake hronične inflamacije i angiopatije [24]. U istaživanju Garbera i sar. utvrđeno je da je reparatorni odgovor u pulpi dijabetičnih pacova bio kompromitovan, što je dovelo do hronične inflamacije i redukovanog stvaranja dentinskog mosta [25]. Studije na ćelijskom nivou su pokazale da su u dijabetičnoj pulpi povećane koncentracije medijatora i enzima inflamacije, kao i parametri oksidativnog stresa, što ukazuje na povećani nivo slobodnih radikala u dijabetičnoj pulpi u poređenju sa zdravom [26, 27]. Ilić i sar. su analizirali ljudsku dijabetičnu pulpu i uočili značajne promene u nivoima VEGF i BMP-2, FR značajnih za reparatorni odgovor pulpe [28].

VEGF je od velikog značaja za mikrocirkulatorni sistem zubne pulpe. Ekspresija VEGF i njegovih receptora je identifikovana u tkivu pulpe metodom lančane reakcije polimeraze (PCR) [29, 30] kao i imunohistohemijski [31]. Povećana ekspresija VEGF utvrđena je u nekim patološkim stanjima zubne pulpe kao što su zapaljenje povreda i hipoksija [32-34].

BMP-2 je FR sa značajnom ulogom u funkciji zubne pulpe. Ekspresija BMP-2 u zubnoj pulpi je dokazana imunohistohemijski [35] i PCR metodom [9, 36]. Ovaj FR je odgovoran za diferencijaciju odontoblasta iz pulpnih stem ćelija i za povećanu sekretornu aktivnost odontoblasta u primarnoj, sekundarnoj i tercijarnoj dentinogenezi [37, 38].

Promene u nivoima ovih faktora rasta mogle bi biti od posebnog interesa kada se analiziraju efekti DM na reparatorni odgovor pulpe, imajući u vidu njihov značaj za regulaciju ovog procesa. Njihovi promenjeni nivoi tokom DM mogu izazvati neadekvatnu reakciju zubne pulpe na štetne nadražaje. Ovo je u saglasnosti sa istraživanjem Garbera i sar. [25], koji su utvrdili da DM ugrožava reparaciju zubne pulpe na animalnom modelu pacova, ali i sa empirijski poznatom činjenicom da su reakcije dijabetične pulpe na proceduru prekrivanja pulpe vrlo nepredvidljive.

ZAKLJUČAK

Klinička dilema da li sprovesti postupak prekrivanja pulpe ili proširiti indikacije za pulpektomiju kod pacijenata sa kompromitovanin opštim stanjem usled DM i dalje postoji. Bazični fiziološki pokazatelji i na dokazima zasnovani klinički podaci o ovom problemu su oskudni. Trebalo bi sprovesti opsežna istraživanja o ćelijskim i molekularnim mehanizmima reparatornog odgovora pulpe tokom DM. Zato je neophodno uvek sa oprezom pristupati terapijskim procedurama u cilju očuvanja vitaliteta zubne pulpe kod osoba obolelih od DM.

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Traumatic neuroma of mental nerve following lower lip mucocele excision

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SUMMARY

Traumatic neuroma represents reactive hyperplasia of irregularly positioned Schwann neurofibril cells and connective tissue - stroma that develop as a result of unsuccessful attempts to regenerate peripheral nerve after injury.
This paper presents the case of a patient with painless traumatic neuroma of the lower lip formed in the same place where he previously had surgically removed salivary cyst of minor salivary gland – mucocele. Histopathological examination confirmed the diagnosis of traumatic neuroma.
Keywords: traumatic neuroma; lower lip; mucocele

INTRODUCTION

Nerve severance or damage can inevitably occur during minor oral surgery interventions. That can result in temporary or permanent sensory changes related to the region of nerve distribution. Furthermore, the development of a traumatic neuroma at the injury site can occur as a consequence of nerve damage.

Traumatic neuroma is reactive nonneoplastic process rather than true neoplasm. It's pseudo-tumorous lesion that forms when proximal segment of peripheral nerve undergoes proliferative response while distal segment undergoes Walerian degeneration [1].

Traumatic neuromas, or amputation neuromas, represent a reactive hyperplasia of irregularly arranged Schwann cells and neurofibrils in a connective tissue stroma resulting from unsuccessful attempts of regeneration [2]. These lesions are generally not considered to be frequent but the most common intraoral sites are mental area, lower lip and tongue [3]. However, only few cases of traumatic neuroma have been described on the lower lip [4, 5].

The current case report presents traumatic neuroma of mental nerve on the lower lip after mucocele excision was done.

CASE REPORT

A 41-year old male was referred to the Clinic of Oral Surgery, Faculty of Dentistry, University of Belgrade, Serbia, for extraction of the right maxillary third molar. During clinical examination, the presence of localized submucosal swelling in his lower right fornix was noticed. The patient reported that he had surgical excision of a painless, soft tissue mass of approximately 10 mm in diameter, localized in his right lower fornix 26 years ago. The histopathological finding has confirmed the clinical diagnosis of mucocele. Postoperatively, the patient experienced numbness on the right side of the lower lip and chin. His symptoms lasted about twelve months and spontaneously disappeared. About thirteen years following the surgery, the patient noticed a soft tissue mass localized in the same place as previous lesion. New lesion has been growing slowly without numbness or any other symptoms therefore the patient did not visit an oral surgeon until the third molar extraction was indicated. Intraoral examination showed a half spherical, movable, firm, painless, soft tissue mass covered by a normal mucosa, measuring 10 mm in diameter, located in the right lower fornix.

The patient underwent surgical excision of the lesion. During surgical procedure the lesion was easily separated from surrounding tissues. It was connected to mental nerve with a pedicle of 10 mm in length and 1 mm in diameter (Figure 1). The pedicle was carefully separated from mental nerve and surgically removed together with the lesion. Both, spherical soft tissue mass and pedicle were sent for histological examination. The day after the surgery the patient had mild burning sensations in the area of the mental foramen. He also complained of a mild skin paresthesia of the chin but not the lip. The third postoperative day all symptoms disappeared.

Microscopically, the lesion was composed of irregularly arranged and interlacing nerve fibres in a fibrous stroma. The lesion comprised spindle cells with scant cytoplasm, elongated oval nuclei arranged in interlacing bundles, and variably sized fascicles with abundant fibrous stroma. Occasionally, a single axon surrounded by Schwann cells was observed in the peripheral areas of the mass (Figure 2).

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Figure 1. Intraoperative view showing lesion connected to the mental nerve
Slika 1. Intraoperativni nalaz nakon odvajanja od okolnog tkiva. Neurom je peteljkom bio vezan za n. mentalis

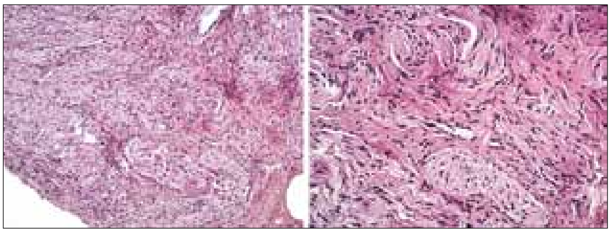

Figure 2. Irregularly arranged and interlacing nerve fibers in a fibrous stroma, HE; 40×, 100×
Slika 2. Iregularno raspoređeni i isprepletani snopovi proliferisanih nervnih vlakana u fibroznoj stromi, HE; 40×, 100×

DISCUSSION

Traumatic neuroma is not a true neoplasm but a reactive proliferation of neural tissue after interruption or other damage of a nerve bundle [6] representing an ineffective attempt of nerve repair [7]. The nerve elements can be injured by different factors, including pressure, ischemia, crushing, cuts, and lacerations, stretching or bleeding into the surrounding area [8].

The most common intraoral sites are mental area, lower lip, and tongue [3]. From surgical trauma point of view, neuromas of mental nerve have been reported to occur following sagittal split osteotomy, chin augmentation, and tooth extraction [3, 7, 9-12]. Clinical findings may vary. The most common symptoms include pain, tenderness and paresthesia [8, 11, 13, 14]. The pressure on a local area may aggravate the pain. Infiltration of local anesthesia into the painful region provides relief [11, 13, 14]. However, 25% cases of traumatic neuromas could be asymptomatic lesions which confuses diagnosis [15].

In our paper the case of painless traumatic neuroma is presented. Considering that painless lesion occurred at the same site as previous lesion we firstly assumed that it was newly formed mucocele. We came to that conclusion as mucocele is one of the most common oral lesions on the lower lip according to numerous authors [16]. Mucocele is developed after an injury of a minor salivary duct, usually after biting trauma and a spillage of mucus into the surrounding submucosal connective tissue as a consequence. The most common complaint is painless swelling. Depending on location the clinical appearance is different. The superficial lesions are blue and fluctuant while deeper ones have normal color of mucosa and they are firm. The clinical findings in our patient corresponded to the clinical presentation of deeper mucocele lesions. The treatment of choice was surgical excision of the mucocele and surrounding minor salivary glands. But, improper surgical treatment as well as continued lip biting habits could result in recurrence as described in 27.78% of cases [17]. However, as the new lesion appeared thirteen years later it was less likely to be mucocele recurrence. On the other hand there was a great possibility that new mucocele or some other benign connective tissue lesion have been developed. Beside mucocele the differential diagnosis included fibrolipoma, schwannoma, neurofibroma, fibroephithelial polyp and small salivary gland tumors.

Traumatic neuroma can be differentiated from fibrolipoma by the lack of lobular pattern, fibrosis and absence of lipoblasts [18]. Traumatic neuroma differs from schwanoma by a more superficial position, absence of Antoni type tissues architecture, and additional number of axons and myelin sheaths within the lesion. Neurofibromas have expansive growth, usually are located deeply, and have no specific site of occurrence. They exhibit pattern in which cells are arranged in short fascicles. They are commonly found in patients with Von Recklinghausen's disease that was not present in our case. Traumatic neuroma differs from neurofibroma in the presence of broader fascicles with more axons and myelin sheath and also the lack of significant mucopolysaccaride ground substance and mast cells [18].

Mucocele is one of the most common lesions of lower lip and preliminary diagnosis was based on that fact. During the surgical treatment it was found that the lesion was connected to mental nerve suggesting neural origin of the lesion. Also the spherical mass neither matched in color nor in consistency with deep mucocele. The histological features of the mass were consistent with the diagnosis of traumatic neuroma. Treatment of choice for traumatic neuroma is surgical excision. Apart classical surgical excision another techniques like cryosurgery and laser therapy could be a method of choice for the treatment of traumatic neuroma.

In this case we assumed that traumatic neuroma had occurred as a result of tissue damage by blade during the previous surgical excision of mucocele or by needle during local anaesthetic administration. This is supported by the fact that after the first surgical intervention patient complained about lower lip paraesthesia that lasted about a year.

In conclusion, nerve injury and consequently traumatic neuroma are possible by-effects of mucocele excision even though it is not a major facial surgical procedure.

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Traumatski neurom mentalnog nerva nakon ekscizije mukokele donje usne

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KRATAK SADRŽAJ

Traumatski neurom predstavlja reaktivnu hiperplaziju iregularno postavljenih Švanovih ćelija i neurofibrila u vezivno-tkivnoj stromi koja nastaje kao posledica neuspešnih pokušaja regeneracije nakon povrede perifernog nerva.

U ovom radu je prikazan slučaj pacijenta sa bezbolnim traumatskim neuromom donje usne koji se pojavio na istom mestu gde je prethodno hirurški uklonjena salivarna cista male pljuvačne žlezde donje usne – mukokela. Histopatološki nalaz je pokazao da je uklonjena promena bila traumatski neurom.

Ključne reči: traumatski neurom; donja usna; mukokela

UVOD

Opšte je poznato da u toku oralno-hirurških intervencija može doći do povrede nervnih struktura. Kao posledica, dolazi do subjektivnih senzornih ili motornih promena u inervacionoj zoni povređenog nerva. Osim toga, ponekad postoje i objektivni znaci koji se mogu manifestovati pojavom traumatskog neuroma na mestu povrede.

Traumatski neurom predstavlja reaktivnu ne-neoplastičnu leziju više nego pravu neoplazmu. Ovaj patološki entitet je poznat kao pseudotumor i nastaje usled proliferatibnog odgovora proksimalnog segmenta perifernog nervnog vlakna, dok distalni segment podleže Valerovoj degeneraciji (*Wallerian degeneration*) [1].

Traumatski neurom, odnosno amputacijski neurom, predstavlja reaktivnu hiperplaziju iregularno postavljenih Švanovih ćelija i neurofibrila u vezivno-tkivnoj stromi koja nastaje kao posledica neuspešnih pokušaja regeneracije [2]. Ove lezije nisu česte, a uobičajena mesta pojave u usnoj duplji su područja bradnog otvora, donje usne i jezika [3]. Kada je u pitanju područje donje usne, u literaturi postoji samo nekoliko opisanih slučajeva traumatskog neuroma [4, 5].

U ovom radu opisan je traumatski neurom bradnog nerva u području donje usne, nastao nakon ekscizije mukokele.

PRIKAZ PACIJENTA

Četrdesetjednogodišnji muškarac primljen je na Kliniku za oralnu hirurgiju Stomatološkog fakulteta Univerziteta u Beogradu radi ekstrakcije gornjeg desnog umnjaka. U toku kliničkog pregleda primećeno je prisustvo submukoznog, loptastog, jasno ograničenog otoka u predelu donje usne sa desne strane. Pacijent je naveo da je pre 26 godina bio podvrgnut hirurškoj eksciziji bezbolne mekotkivne strukture prečnika 1 cm koja je bila lokalizovana na istom mestu. Promena je klinički odgovarala mukokeli, što je potvrđeno histopatološkom analizom. Nakon operacije pacijent se žalio na utrnulost u regiji desne polovine donje usne i brade. Prema rečima pacijenta, simptomi su trajali oko 12 meseci, nakon čega su spontano iščezli. Trinaest godina posle operacije pacijent je primetio novu mekotkivnu promenu na mestu prethodne lezije. Tokom tog perioda lezija se uvećavala veoma sporo (bez utrnulosti, kao i bilo kakvih drugih simptoma), te pacijent nije došao na pregled sve dok ekstrakcija gornjeg trećeg molara nije bila indikovana. Intraoralnim pregledom je ustanovljeno postojanje polukružne, pokretne, čvrste i bezbolne mekotkivne strukture koja je prekrivena normalnom sluzokožom, čija je veličina bila 10 mm u prečniku. Pacijentu je sugerisano hirurško uklanjanje lezije. U toku operacije lezija je lako odvojena od okolnog tkiva i primećeno je da je peteljkom dužine oko 10 mm povezana sa mentalnim nervom (Slika 1). U skladu sa tim, peteljka je pažljivo odvojena od mentalnog nerva i uklonjena zajedno sa lezijom. Odstranjeno tkivo zajedno sa peteljkom poslato je na histopatološku analizu. Prvog postoperativnog dana pacijent je prijavio osećaj pečenja u zoni projekcije mentalnog otvora. Takođe, naveo je i blagi osećaj parestezije kože brade, ali ne i usne. Tri dana nakon operacije svi simptomi su nestali.

Mikroskopski, lezija se sastojala od iregularno raspoređenih i isprepletanih nervnih vlakana u fibroznoj stromi. Pored toga, tkivo je sadržalo i vretenaste ćelije sa oskudnom citoplazmom, izduženim ovalnim jedrom, raspoređene u međusobno isprepletanim snopovima različitih veličina uz obilje fibrozne strome. Mestimično, pojedinačni akson okružen Švanovim ćelijama nalazio se u perifernim delovima tumorskog tkiva (Slika 2).

DISKUSIJA

Traumatski neurom nije neoplazma u pravom smislu te reči, već reaktivna proliferacija nervnog tkiva koja nastaje nakon prekida ili neke druge povrede nervnog snopa [6] i predstavlja neuspešan pokušaj nervne regeneracije [7]. Nervni elementi mogu biti povređeni delovanjem različitih faktora uključujući pritisak, ishemiju, nagnječenje, presecanje, kao i laceracije, rastezanje i krvarenje u okolnom tkivu [8]. Što se tiče intraoralne lokalizacije, traumatski neurom se najčešće javlja u području bradnog otvora, donje usne i jezika [3]. Sa aspekta hirurške traume, pojave neuroma mentalnog nerva su opisane nakon sagitalne osteotomije, koštane augmentacije bradnog predela, ali i vađenja zuba [3, 7, 9-12].

Klinički simptomi mogu varirati. Uobičajena klinička slika uključuje bol, žarenje i paresteziju [8, 11, 13, 14]. Bol se može pojačati pritiskom na zahvaćenu regiju. Infiltracija lokalnog anestetičkog rastvora u bolno područje donosi olakšanje [11, 13, 14]. Sa druge strane, u 25% pacijenata traumatski neuromi mogu biti asimptomatske lezije, što često otežava postavljanje dijagnoze [15].

U ovom radu je prikazan slučaj bezbolnog traumatskog neuroma. Budući da se bezbolna mekotkivna formacija javila na istom mestu kao i prethodna lezija, postojala je pretpostavka da se radi o recidivu mukokele. Mukokele su jedan od najčešćih patoloških entiteta usne duplje i predominantno se javljaju u regiji donje usne [16]. Nastaju kao posledica povrede malih pljuvačnih kanala i žlezda, najčešće ugrizanjem. Usled oštećenja salivarnog kanala dolazi do izlivanja mukusa u okolno submukozno vezivno tkivo. Najčešći simptom na koji se pacijenti žale je bezbolni otok. Treba istaći da klinička slika zavisi od lokalizacije. Površno lokalizovane lezije su plavičaste boje i pokazuju fenomen fluktuacije, dok su dublje lezije tvrđe i imaju boju normalne sluzokože. Terapija ovih lezija je hirurška i podrazumeva eksciziju mukokele i uklanjanje okolnih malih pljuvačnih žlezda. Neadekvatna hirurška tehnika kao i dalje prisustvo navike grickanja usne mogu rezultovati recidivom u 27,78% pacijenata [17].

Klinički nalaz kod pacijenta iz ovog rada je odgovarao kliničkoj slici dublje lokalizovane mukokele. Kako je do pojave nove lezije došlo 13 godina nakon prve intervencije, manje je verovatno da je u pitanju recidiv. Sa druge strane, postojala je velika mogućnost da je u pitanju nova mukokela ili neka druga benigna mekotkivna struktura.

Diferencijalno dijagnostički, pored mukokele, u vidu treba imati fibrolipom, švanom, neurofibrom, fibroepitelijalni polip i tumor malih pljuvačnih žlezda. Traumatski neurom se može razlikovati od fibrolipoma po odsustvu lubalarne građe, fibroze kao i masnih ćelija [18]. Sa druge strane, od švanoma se razlikuje po tome što je obično postavljen površnije. Takođe, odusustvo Antoni tipa tkivne arhitekture i veći broj aksona i mijelinskih struktura ukazuju na to da je u pitanju traumatski neurom pre nego švanom. Neurofibromi imaju ekspanzivan rast, obično su lokalizovani dublje u tkivu i nemaju specifično mesto nastajanja. Odlikuje ih građa u kojoj su ćelije raspoređene u kratkim snopovima. Po pravilu se javljaju kod pacijenata sa von Recklinghausenovom bolešću, što nije bio slučaj kod prikazanog pacijenta u ovom radu. Traumatski neurom se od neurofibroma razlikuje po prisustvu širih snopova sa više aksona i mijelinskih obloga, kao i po odsustvu značajne količine mukopolisaharida i mast ćelija [18].

Mukokela je jedna od najčešćih lezija donje usne, te je tako i privremena dijagnoza postavljena u ovom slučaju. U toku operacije ustanovljeno je da je lezija povezana sa mentalnim nervom, što je ukazalo na to da lezija može biti porekla nervnog tkiva. Pored toga, ni boja ni konzistencija lezije nisu odgovarale mukokeli.

Ono što je važno istaći je to da hirurška ekscizija predstavlja tretman izbora za traumatski neurom. Osim klasične hirurške ekscizije postoje i druge tehnike lečenja kao što su kriohirurgija i terapija laserom. Zahvaljujući manjoj invazivnosti, ove tehnike se nameću kao dostojna alternativa klasičnoj hirurgiji.

Histopatološka analiza je nesumnjivo potvrdila da je u pitanju traumatski neurom. Na osnovu preoperativnih anamnestičkih podataka i podataka dobijenih nakon operacije, kao i histopatološkog nalaza, traumatski neurom je najverovatnije nastao kao posledica povrede skalpelom tokom prethodne hirurške intervencije, ili povrede iglom u toku aplikacije lokalnog anestetičkog rastvora ili šivenja.Takođe, ovo potkrepljuje i podatak da se nakon prve operacije pacijent žalio na paresteziju donje usne.

Može se zaključiti da je povreda nerva i posledična pojava traumatskog neuroma jedna od mogućih komplikacija ekscizije mukokele, iako ova intervencija ne predstavlja ekstenzivnu oralnohiruršku proceduru. Imajući u vidu da je usna izgrađena od vezivnog i masnog tkiva, krvnih sudova, nerava i pljuvačnih žlezda, jasno je da svaki tumor usne može imati širok spektar diferencijalno dijagnostičkih patoloških entiteta. Kako je klinička prezentacija benignih i malignih tumora u regiji usne veoma slična, ekscizionа biopsija je neophodna kako bi se mogla postaviti sigurna dijagnoza.

Da li ste pažljivo čitali radove?

1. Zubna pulpa kod osoba sa dijabetesom ima nekompromitovan reparatorni odgovor na nadražaje?
   a) Da
   b) Ne
   c) Zavisi od nadražaja

2. Traumatski neurom je:
   a) reaktivna hiperplazija Švanovih ćelija
   b) karcinom donje vilice
   c) melanom

3. Fluorapatit je:
   a) najstabilniji kalcijum-ortofosforni mineral
   b) najrastvorljiviji mineral
   c) najmekši mineral

4. Automatsko određivanje linearnih veličina lica na osnovu fotografija je proveravano na uzorku od:
   a) 20 fotografija
   b) 30 fotografija
   c) 35 fotografija

5. Kod osoba sa dijabetesom nivoi faktora rasta su:
   a) promenjeni
   b) nepromenjeni
   c) zavisi od nadražaja

6. Traumatski neurom je:
   a) česta pojava
   b) retka pojava
   c) izuzetno retka pojava

7. Pozicije definisanih tačaka na digitalnoj fotografiji su određivane:
   a) sa pauzom od 3 dana
   b) sa pauzom od 7 dana
   c) sa pauzom od 14 dana

8. Eksperimentalni dokazi o umanjenoj restaurativnoj aktivnosti pulpe kod osoba sa dijabetesom:
   a) postoje
   b) ne postoje
   c) nema ni kliničkih ni eksperimentalnih potvrda

9. Traumatski neurom se češće javlja u:
   a) donjoj vilici
   b) gornjoj vilici
   c) u obe vilice podjednako

10. Najveća koncentracija fluoroapatita se kod čoveka nalazi:
    a) u kostima
    b) u gleđi
    c) u vezivnim tkivima

11. Odgovor pulpe može biti modifikovan njenim morfološkim i funkcionalnim stanjem?
    a) Da
    b) Ne
    c) U zavisnosti od oboljenja

12. Klinički nalaz neuroma odgovara:
    a) ekspanzivnom karcinomu
    b) mukokeli
    c) melanomu

13. Traumatski neurom se u usnoj duplji najčešće javlja:
    a) u predelu bradnog otvora donje usne
    b) u predelu sekutića gornje usne
    c) u predelu molara gornje usne

14. Faktori rasta su po hemijskoj strukturi:
    a) protein
    b) vitamin
    c) neutralne materije

15. Fluorotit se u biomedicini koristi kao:
    a) aktivno terapijsko sredstvo
    b) nosač lekova
    c) zaštita mekih tkiva

16. Traumatski neurom je obično:
    a) sa jako izraženim simptomima
    b) sa simptomima u vidu bola, žarenja i parestezije
    c) sa probadajućim bolovima

17. Fluorat je:
    a) manje stabilan od hidroksiapatita
    b) rastvorljiviji od hidroksiapatita
    c) efikasniji u zaštiti od karijesa od hidroksiapatita

18. Ključni faktor rasta za regulaciju procesa reparacije pulpe je:
    a) BMP-2
    b) TGF B
    c) VEG F

19. Za sintezu fluorapatita najčešće se koristi:
    a) kalcijum-hidroksid
    b) kalcijum-karbonat
    c) kalcijum-silikat

20. Odstupanja od merenih veličina dobijenih vrednosti kretala su se od:
    a) 0,1 do 0,5%
    b) 0,1 do 0,6%
    c) 0,03 do 0,6%

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Instructions for Authors

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General instructions The manuscript should be typed in MS Word, with double line spacing, only in Times New Roman font and letters size 12 pt. Page margins should be 25 mm, page size set to A4 format, and text typed aligned left with paragraph indentations of 10 mm. Words should not be hyphenated. If special symbols are used in the text, preferred font is Symbol. References should be marked with Arabic numbers in brackets, e.g. [1, 2], in the order of appearance in the text. Page numbers should be inserted at the bottom of the page, starting from the title page.

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Summary and keywords The second page should contain a structured summary of the paper with Introduction (with the aim), Material and Methods, Results and Conclusion with up to 250 words. Each of these segments should be written as a new paragraph with bold subtitles. Only the most important results should be indicated with the statistical level of significance. Following summary it is recommended to list 3 to 6 keywords related to the paper. Keywords should be chosen according to the Medical Subject Headings – MeSH (*http://www.nlm.nih.gov/mesh*).

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Text of the manuscript Text should be written in short and clear sentences, avoiding foreign language words and inadequate terms and interpretation from the literature. Medications should be indicated by their generic names. For each abbreviation, full term should be indicated when first mentioned in the text, except for standard measuring units. Decimals should be separated with a comma in Serbian, and with a dot in English. Numbers should be approximated to one decimal place. All results of hematological, clinical and biochemical measurements should be quoted in the metrical system according to the International Unit System (SI).

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