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Contents / Sadržaj

Serbian Dental Journal, vol. 63, N° 4, 2016

Ukoliko ne znate gde idete,
svaki put će vas tamo odvesti.
Indijska poslovica

U vremenu „svekolikog napretka" u svim segmentima života, pa i u nauci, i ideje za urednički komentar obično ne manjkaju. Ovog puta za to je poslužio komentar jedne koleginice o važnosti „zvanja" u naučnim okvirima.

Ovaj komentar najbolje oslikava izreka na početku ovog teksta „jer daje najbolju sliku stanja u naučnoj zajednici, ali i sliku onih koji tu zajednicu čine.

Često su upravo osobe koje mnogo toga ne razumeju „najslobodnije" za demonstraciju bahatosti i beskrupuloznosti, jer osim nesposobnosti i naučne inferiornosti imaju problem i pravilnog poimanja „naučnog zvanja".

Naime, oni svoje naučno zvanje doživljavaju kao sredstvo za ostvarivanje ličnih (prevelikih ambicija), ali i kao sredstvo uticaja na druge sa tako „visokih" pozicija. U takvim situacijama trpe svi, i ustanova i sredina i široko okruženje, ali i cela društvena zajednica. A poznato je da osobe bez ikakvih vrednosnih potvrda imaju i najčvršća uverenja. Ova uverenja su upravo najčvršća u poimanju i razumevanju sopstvenih vrednosti.

Naučna znanja su najčešće posledica ličnog u određenoj profesiji, odnosno kvaliteta koji uglavnom „odstoji" od normalnih vrednosnih lestvica propisanih odgovarajućim propisima.

Ali problem nastaje onog momenta kada se u spoznaji sopstvenog lika pojavi ponor između realnosti i opijenosti sobom. Kada se lična inferiornost u naučnom i stručnom pogledu pokuša „nadomestiti" naučnim zvanjem stečenim zahvaljujući „sumnjivim" kriterijumima za napredovanje i uz malo političke potpore, onda je problem još veći jer, nažalost, „čovek nije ono što misli da jeste, niti ono što bi voleo da bude, već samo ono što svojim radom potvrđuje". Upravo ta diskretanca između realnog stanja i ličnog doživljaja sebe, odnosno nerazumevanje činjenice da jedino rad donosi rezultat jeste problem bitnih „krivo stečenih zvanja". Neki su isuviše rano sebe proglasili genijalcima, bez obzira na kvalitet koji poseduju. Oni se često ni u ogledalu ne vide realno. Vide se imaginarno i uveličano. Ali to nije njihov problem. To je problem svih onih koji su u njihovom okruženju i dele sa njima iste vrednosne koncepte, koje oni čak i ne prepoznaju. Oni obično ne razumeju osnovni životni postulat da su stručnost i znanje temeljni kriterijumi za uspeh, a naporan rad osnov svakog uspešnog ishoda. Možda je to najbolje opisao Aleksandar Grajam Bel: „Čovek vrlo malo duguje onom s čim je rođen, čovek je ono što radom napravi od sebe".

Ali sumornost sadašnjeg trenutka se može rešiti jedino posvećenošću i odgovornim radom u svakom segmentu života i rada. Uspeh je posledica upravo napornog i odgovornog rada ali i pokušaja da sopstveni rezultati budu pouka, putokaz drugima.

Ovaj urednički komentar ću završiti citatom Beverly Sills: „Nema prečice do mesta na koje je vredno otići", jer najbolje objašnjava životnu filozofiju uspeha, odnosno značaja upornog rada kao najvažnijeg recepta za uspeh.

Prof. dr Slavoljub Živković

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The effect of zinc oxide based sealer on bone defects healing

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SUMMARY

Introduction Obturation as the final phase of endodontic treatment aims to provide complete hermetic filling along the entire length of the canal system from the coronal opening to the apical end. The aim of this study was to evaluate histological response of bone tissue on the implantaton of zinc oxide based material in artificially prepared defect in the mandible of rats.

Material and method For the experiment, sixteen male Wistar rats were used. Using sterile steel burs a defect was made in mandible, between the midline and mental foramen. Zinc oxide based sealer was implanted in the defects of experimental group while the defects of control group healed spontaneously. One half of animals in both groups were sacrificed after thirty days, and the second half after ninety days. Microscopic preparations consisted of the defect with surrounding bone and after processing were analysed using light microscopy.

Results The thirtieth day after implantation of the material, fibrovascular connective tissue was noted, with scant chronic inflammatory cell infiltrate. Away from the experimentally made defect, in the depth of the bone, lamellar bone with well-formed larger osteons was noted as well as enlarged Volkmann and Haversian canals. Ninety days after implantation of the material, there was no *restitutio ad integrum*, but intense focal remodelling of bone tissue was noted.

Conclusion Endomethasone N slowed down bone tissue healing process by showing the signs of prolonged inflammation in bone tissue in which it has been implanted. Extension of the healing process is reflected in the slow replacement of fibrovascular connective tissue with newly formed bone tissue.

Keywords: sealers; obturation; bone healing

INTRODUCTION

The aim of obturation as the final phase of endodontic treatment is to provide complete hermetic filling along the entire length of the canal system from the coronal opening to the apical end. It should break communication between endodontic and periodontal tissues and thus prevent reinfection of periapical region [1]. Materials used for obturation should have a number of physical, chemical and biological properties and should not extend beyond the end of the canal system [1]. When a biomaterial comes in contact with the tissues and fluids of the human body there is always some form of interaction and therefore it is necessary for material to be biocompatible and harmless in the biological surrounding [2].

Overinstrumentation and consequently overfilling of the root canal significantly increase the risk of adverse impacts of filling material [3]. Overfilling causes inflammation and slows down wound healing [4, 5]. The negative effect is usually more pronounced until the material fully sets and it is attributed to the components of the material [6]. Slow release of harmful agents over long periods of time depends on the solubility of materials and the degree of material exposure to tissue fluids [2].

Zinc oxide based sealers are most commonly used in the clinical practice as well as comparative sealers in studies that examine the effect of biomaterials [4, 7]. The results of these studies, however, are not encouraging. According to some authors, certain materials from this group are highly toxic, and should not be used as sealers [7]. The toxicity is attributed mainly to the presence of eugenol or formaldehyde in some formulations of these materials [8, 9].

The aim of this study was to evaluate histological response of bone tissue on the implantation of zinc oxide based material in artificially prepared defects in the mandible of rats.

MATERIALS AND METHOD

For the experiment, 16 Wistar male rats were used, weight 160-180 g (approved by the Ethics Committeeof the Faculty of Medicine in Niš No. 01 3797). During the experimental procedure, animals were anaesthetized by intraperitoneal injection of ketamine hydrochloride (0.1 ml per 100 g of weight). After preparation procedure, between midline and mental foramen of the left mandible of all animals, a defect (diameter of 1.4mm and 1.6mm depth) was made using sterile stainless steel fissure burs.

Animals of the experimental group (n = 12) were divided into the two subgroups:

- The first subgroup (n = 6) was sacrificed after 30 days;
- The second subgroup (n = 6) was sacrificed after 90 days.

In bone defects of experimental group animals, „Endomethasone N" (Septodont, France) prepared by the manu-

Address for correspondence: Address for correspondence: Marija NIKOLIĆ, Clinic of Dentistry, Bulevar dr Zorana Đinđića 52, 18000 Niš, Serbia; *makinis80@yahoo.com*

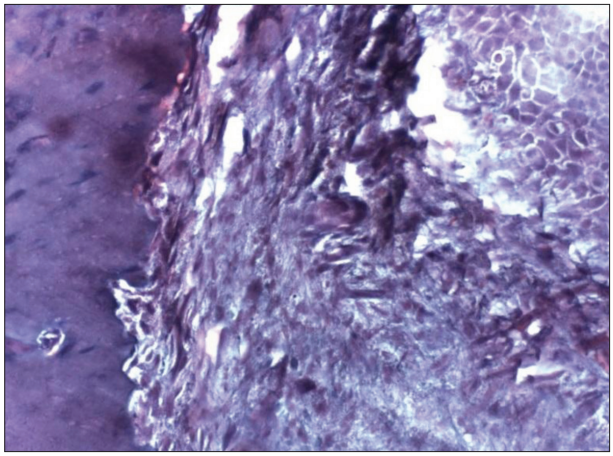

Figure 1. Histological finding of the defect 30 days after implantation of Endomethasone N. Fibrovascular connective tissue with scarce inflammatory cell infiltrate is visible. Surrounding bone is without osteon-type structure (HE, 800×).
Slika 1. Histološki nalaz defekta u kosti 30 dana nakon implantacije endometazona N. Fibrovaskularno vezivno tkivo sa oskudnim inflamatornim ćelijskim infiltratom. Okolna kost bez građe po tipu osteona (HE, 800×).

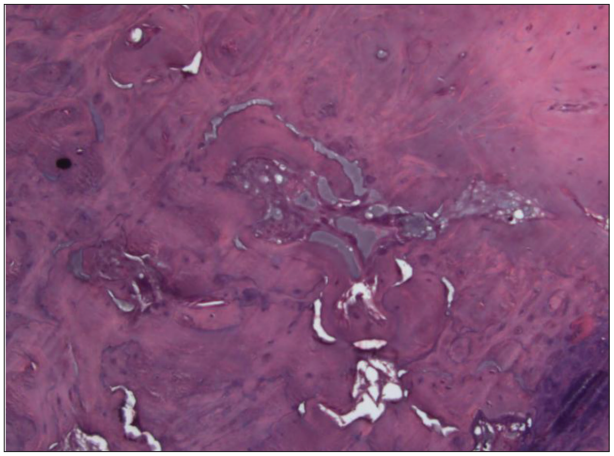

Figure 2. Histological finding of the defect 30 days after implantation of Endomethasone N. Thickening of cement lines and expansion of Volkmann and Haversian canals in the bone tissue away from the edges of the defect are visible (HE, 400×).
Slika 2. Histološki nalaz defekta u kosti 30 dana nakon implantacije endometazona N. Zadebljanje cementnih linija i proširenje Folkmanovih i Haversovih kanala u koštanom tkivu udaljenom od ivice defekta (HE, 400×).

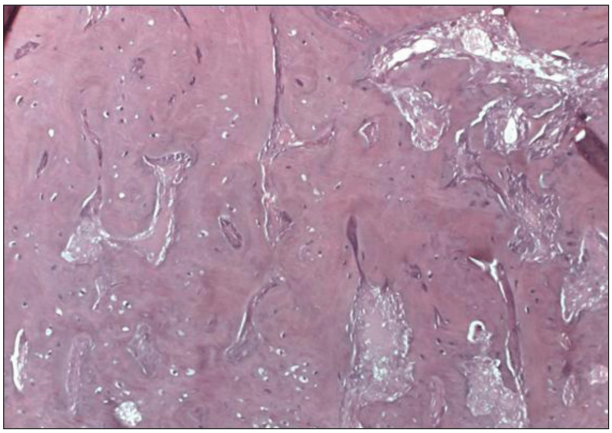

Figure 3. Histological finding of the defect 90 days after implantation of Endomethasone N. Newly formed bone with diffuse distribution of mature connective tissue is noted (HE, 400×).
Slika 3. Histološki nalaz defekta u kosti 90 dana nakon implantacije endometazona N. Novoformirana kost sa difuzno raspoređenim zrelim vezivnim tkivom (HE, 400×).

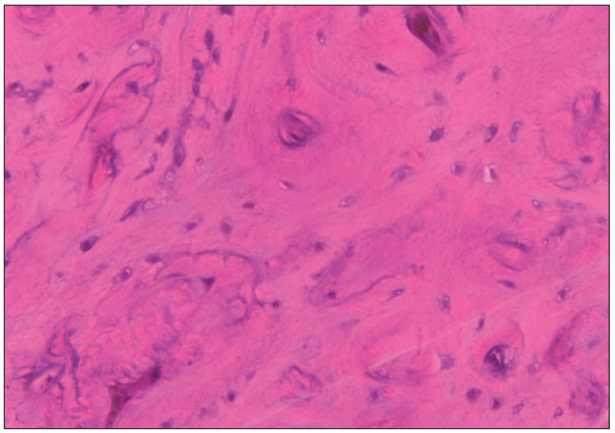

Figure 4. Histological finding of the defect 30 days after implantation of Endomethasone N. Bone shows normal micromorphology away from the defect (HE, 800×).
Slika 4. Histološki nalaz defekta u kosti 90 dana nakon implantacije endometazona N. Kost udaljena od defekta je normalne mikromorfologije (HE, 800×).

facturer's instructions was implanted. Bone defects in the control group (n = 4) were left to heal spontaneously without any implants. Two animals of the control group were sacrificed after thirty days and two animals after ninety days. After the estimated time the animals were sacrificed by an overdose of anaesthetic (ketamine hydrochloride). The tissue samples were made by resection of the mandible consisting of the defect and surrounding bone.

Tissue samples were fixed in 10% buffered formalin, demineralized in 10% formic acid, dehydrated in a series of graded alcohols and embedded in paraffin wax. Cutting was performed in the buccolingual direction on the microtome with 2μm thick glass knives. The slides were stained with hematoxylin and eosin. Microscopic analysis was performed using light microscopy with digital camera Leica DFC 295.

RESULTS

Experimental group

The thirtieth day after the implantation of material, fibrovascular connective tissue was noted, with scant focal chronic inflammatory cell infiltrate, while surrounding bone was hypocellular without the presence of Haversian system (in 5 out of 6 samples) (Figure 1). Small osteocytes with hyperhromatic nuclei were stored in slightly enlarged lacunae. Osteoblasts were few in number, while the presence of osteoclasts was inconspicuous. In areas of non-resorbed material, which was lost during processing of histopathological preparations, empty spaces were noticed. Away from experimentally made defect, in the depth of the bone, lamellar bone with well-formed larger osteons was noted as well as enlarged Volkmann and Haversian canals (Figure 2). Expanded interstitial lamellae were observed in osteons, osteocytes and lacunae were

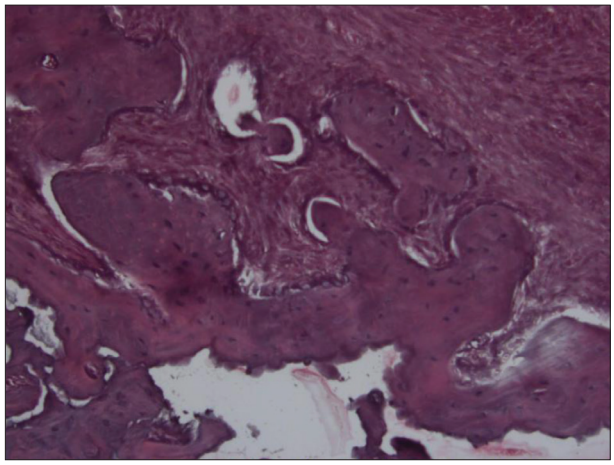

Figure 5. Histological finding of the defect after 30 days (no implantation of material- control group). Fibrovascular connective tissue surrounding newly formed bone can be seen (HE, 400×).
Slika 5. Histološki nalaz defekta kosti nakon 30 dana (kontrolna grupa). Fibrovaskularno vezivno tkivo okružuje ostrvca novoizgrađene kosti (HE, 400×).

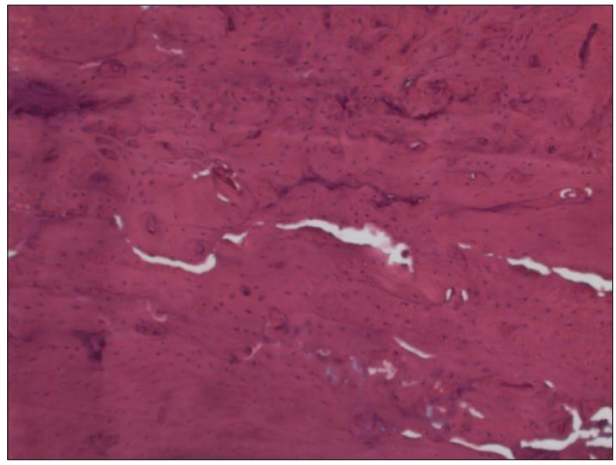

Figure 6. Histological finding of the defect after 90 days (no implantation of material- control group). Newly formed bone with osteon- type organization and borders to the lamellar bone are visible (HE, 200×).
Slika 6. Histološki nalaz defekta kosti nakon 90 dana (kontrolna grupa). Novostvorena kost sa organizacijom po tipu osteona i granica prema lamelarnoj kosti (HE, 200×).

placed in oval contours. Border cement lines were basophilic, enlarged, with amorphous to fine-grained appearance. In one case (1 of 6) giant cell reaction to a foreign body was observed.

Ninety days after material implantation, osteoplasia and trabeculae of different widths surrounded by osteoblasts were observed. Newly formed bone was young and morphologically immature. There was no *restitutio ad integrum*, but focal remodeling of bone tissue was intense (Figure 3). Fibrous connective tissue was focally distributed and reduced (in all samples). Bone tissue, away from experimental area showed normal morphology (Figure 4).

Control group

Thirtieth day after preparation of experimental defect in all control samples, osteosynthesis activity and fibrovascular connective tissue were observed (Figure 5). Young bone tissue with osteocytes in enlarged oval lacunae was noted. Thickened edges of osteons with increased basophil responses were present away from the experimental defect. No signs of inflammation in tissue were noted.

Ninety days after preparation of artificial defect, an *ad integrum* bone healing was noted in all samples of the control group. Osteons of newly formed bone tissue had smaller diameter and smaller number of concentric lamellae (Figure 6).

DISCUSSION

Before introducing any material in the clinical practice it is necessary to conduct appropriate tests. Cytotoxicity testing of endodontic materials by *in vitro* tests provides control of the experiment conditions but does not correspond completely to clinical situation. For this reason, implantation of materials (subcutaneous, intramuscular, intaoseal) is considered to be superior test. Tested materials are commonly implanted into the tissues of rats [5, 8, 10], rabbits [11], guinea pigs [12]. Intraoseal models provide an adequate environment for *in vivo* testing of endodontic materials. In this experiment, the method of mandible bone implantation was chosen because of its specificity [13].

Eugenol (4-allyl-2-methoxyphenol) is clove oil extract and a component of zinc oxide eugenol (ZnOE). It causes periapical toxicity and inhibits growth and proliferation of human osteoblastic U2OS cell line. As cell growth, attachment, proliferation and synthesis of matrix play an important role in wound healing and tissue regeneration, it can be assumed that eugenol in tissue may disturb the processes of wound healing [14, 15].

Thirty days after the material (Endomethasone N) implantation, its presence was macroscopically observed inside the experimental defects. During the process of making histological preparations, the material was in most cases partially or completely rinsed from the implantation site and experimental defects were represented as empty spaces. After the experimental period of 90 days, microscopic analysis did not show tested material (Endomethasone N) present.

Thirtieth day after implantation of Endomethasone N, fibrovascular connective tissue by type of callus was observed as well as new bone formation. Inflammatory response, in all samples, was slightly pronounced except in one case where the response was more type of reaction to the foreign body with granulation tissue. On the ninetieth day the absence of inflammatory reactions was recorded and slower degree of fibrovascular proliferation in relation to the period of 30 days. Connective tissue was mostly replaced with young bone tissue with osteon type structure.

In the control group after the period of 30 days an inflammatory reaction was observed which disappeared until the ninetieth day. Also, in the control group, the presence of connective tissue in the second period (90

days) was not observed, and the experimental defect was completely replaced by newly formed bone tissue. The presence of connective tissue in the experimental group after 30 days and its reduction after 90 days suggest that after longer time it may be completely replaced by newly formed bone.

Results of the current study were similar to other studies [13, 14]. Tassery et al. describe mild to moderate infiltration of inflammatory cells after 4 weeks when material Super EBA (based on zinc oxide) was implanted in the mandible of guinea pigs. This reaction decreased over time and it was not observed after 12 weeks. This study did not find healing disruption due to increased inflammatory reaction [13]. The initial inflammatory effect was attributed to eugenol and etoxybenzoic acid (Super EBA ingredient) that were released [16], but the setting of the material reduced their amount [17]. High degree of binding of these substances to serum and extracellular proteins may contribute to reduced toxicity and mild inflammation [18].

Triches et al. examined inflammatory reaction of subcutaneous tissue in rats using histomorphological parameters such as - quantity of inflammatory cells, presence of blood vessels, area of necrosis and the thickness of fibrous capsule. A mild to moderate tissue reaction to Endomethasone N was described after 7 days with predominance of chronic inflammatory cells, while after 42 days tissue remained inflamed with the presence of lymphocytes, fibroblasts and well-organized collagen fibres [14]. The authors came to the conclusion that all tested sealers (Endomethasone N, Endofil (based on ZnO), Sealer 26 (based on $CaOH_2$)) were irritants; which toxicity was reduced over time.

Our study showed that after longer periods of time Endomethasone N slowed down the process of reparation and caused late inflammatory reaction which is consistent with the results of other authors [14, 19, 20]. In the study conducted by Suzuki & Souza, Endomethasone did not provide complete healing even after 90 days. The experiment was carried out on dogs whose root canals were overfilled with this material, and then periradicular tissue was analyzed histologically. Occasionally inflammatory cell infiltrate of different degree was found in all samples, while giant cells were present in eight out of ten samples [20]. Our results also showed chronic weak inflammatory response of bone tissue to Endomethasone in the period after 30 days. That is consistent with another study that found mild inflammation in bone with signs of recovery after 42 days of subcutaneous implantation of Endomethasone in rats [14].

Ninetieth day after the implantation no signs of inflammation were noted and regenerative processes had prevalence in our study. These results do not agree with the findings of chronic inflammatory infiltrate even after the period longer than 90 days (six months) for overfilled root canals with Endomethasone in monkeys [19]. The results were explained by irritation that material caused when it was extruded beyond the apex. Discrepancies can be attributed to the variety of animal models and experimental design.

Other authors reported different results from ours. Zafalon et al. found moderate to severe inflammatory reaction after 15 days of implantation, while after 30, 60, 90 days, there was no reaction [10]. The time period was the same so the difference can be attributed to the experimental procedure and the difference in material composition of Endomethasone and Endomethasone-N.

The most ingredients of Endomethasone are released during setting reaction due to its high solubility [21]. Sealer components diffuse easier in the freshly mixed state, although they could be released by hydrolysis even after material is set [15]. Continuous release of toxic components such as paraformaldehyde and eugenol may explain persistent inflammation over longer period of time.

CONCLUSION

Endomethasone N slows down bone tissue healing process by showing the signs of prolonged inflammation in bone tissue in which it was implanted. Prolonged healing is reflected in slow replacement of fibrovascular connective tissue with newly formed bone tissue. However, permanent disturbance of morphofunctional relations in the bone tissue was not found.

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Uticaj materijala za opturaciju na bazi cink-oksida na zarastanje koštanih defekata

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KRATAK SADRŽAJ

Uvod Cilj opturacije kao završne faze endodontskog tretmana je da se obezbedi kompletno hermetično zatvaranje duž cele dužine kanala od koronarnog otvora da apeksne granice.

Cilj studije bio je da se proveri histološki odgovor koštanog tkiva na implantaciju materijala na bazi cink-oksida u arteficijalno preparisan defekt u mandibuli pacova.

Metode rada U eksperimentu je upotrebljeno 16 pacova Wistar soja, muškog pola. Upotrebom sterilnih, čeličnih fisurnih borera formiran je defekt između medijalne linije i mentalnog otvora u mandibuli pacova. Siler baziran na cink-oksidu je implantiran u defekte eksperimentalne grupe, dok su defekti kod kontrolne grupe ostavljeni da spontano zarastaju. Polovina životinja iz obe grupe je žrtvovana posle trideset, a druga polovina posle devedeset dana. Mikroskopski preparati su se sastojali od defekta i okolne kosti i posle pripreme su analizirani svetlosnom mikroskopijom.

Rezultati Trideset dana posle implantacije je uočeno fibrovaskularno vezivno tkivo sa oskudnim hroničnim inflamatornim infiltratom. Dalje od eksperimentalnog defekta, u dubini kosti, zapažena je lamelarna kost sa dobro formiranim većim osteonima i proširenim Haversovim i Folkmanovim kanalima. Devedeset dana posle implantacije materijala nije došlo do *restitutio ad integrum,* ali je fokalno remodelovanje kosti bilo intenzivno.

Zaključak Endometazon N usporava proces zarastanja koštanog tkiva jer pokazuje znake produženog zapaljenja u koštanom tkivu u koje je implantiran. Produženje procesa zarastanja ogleda se i u usporenoj zameni fibrovaskularnog vezivnog tkiva novonastalim koštanim tkivom.

Ključne reči: sileri; opturacija; zarastanje kosti

UVOD

Opturacija kao završna faza endodontskog tretmana ima za cilj obezbeđivanje kompletnog hermetičnog punjenja duž celokupne dužine kanalskog sistema od koronarnog otvora do apeksne granice. Uloga opturacije je da se spreči komunikacija između endodonta i parodonta i na taj način onemogući reinfekcija periapeksne regije [1]. Materijali koji se upotrebljavaju bi trebalo da poseduju niz fizičkih, hemijskih i bioloških osobina i po pravilu ne bi trebalo da se prostiru izvan granica kanalskog sistema [1]. Kada biomaterijal dođe u kontakt sa tkivima i tečnostima ljudskog organizma, uvek postoji neka forma interakcije i zato je potrebno da materijal bude biokompatibilan, neškodljiv u biološkoj sredini, a da je pacijent zaštićen od bilo kakvih negativnih uticaja [2].

Preterana instrumentacija i sledstveno prepunjavanje kanala korena značajno povećavaju rizik od nepovoljnog uticaja materijala za punjenje [3]. Prepunjavanje utiče na povećanu inflamaciju i usporeno zarastanje [4, 5]. Negativno dejstvo je obično izraženije dok materijal još nije potpuno vezan i uglavnom se pripisuje sastavnim komponentama materijala [6]. Sporo oslobađanje negativnih agenasa tokom dužih perioda vremena zavisi od rastvorljivosti materijala, odnosno stepena izloženosti materijala tkivnim tečnostima [2].

Materijali na bazi cink-oksida sa eugenolom su najčešće upotrebljavani i obično se koriste kao komparativni sileri u studijama koje ispituju biološke efekte materijala [4, 7]. Rezultati ovih studija, međutim, nisu ohrabrujući. S obzirom na to da su prema nekim autorima pojedini materijali iz ove grupe veoma toksični, oni ih ne preporučuju za upotrebu [7]. Toksičnost se uglavnom pripisuje prisustvu eugenola ili formaldehida, koji neki od ovih materijala sadrže [8, 9].

CILJ RADA

Cilj istraživanja bio je da se proveri histološki odgovor koštanog tkiva na implantaciju materijala na bazi cink-oksida u arteficijalno preparisan defekt u mandibuli pacova.

METOD RADA

U eksperimentu je korišćeno 16 Wistar pacova muškog pola, telesne mase 160–180 g (odobreno od strane Etičkog komiteta Medicinskog fakulteta u Nišu, broj 01 3797). Tokom eksperimentne procedure životinje su anestezirane intraperitonealnom injekcijom ketamin-hidrohlorida (0,1 ml na 100 ml telesne mase). Nakon postupka pripreme, između središnje linije i mentalnog otvora sa leve strane donje vilice svih životinja, napravljen je defekt (prečnika 1,4 mm i dubine 1,6 mm) korišćenjem sterilnog fisurnog čeličnog borera. Životinje eksperimentalne grupe podeljene su u dve podgrupe:

- Prva podgrupa (n = 6) žrtvovana nakon 30 dana
- Druga podgrupa (n = 6) žrtvovana nakon 90 dana

U formirane defekte kod eksperimentalne grupe životinja implantiran je endometazon N (*Septodont, France*) pripremljen po uputstvu proizvođača. Preparisan defekt kod životinja kontrolne grupe (n = 4) ostavljen je da spontano zarasta bez ikakvog implantata. Dve životinje iz kontrolne grupe su žrtvovane posle 30, a dve posle 90 dana. Nakon predviđenog vremena životinje su žrtvovane predoziranjem anestetikom (ketamin-hidrohlorid). Uzorci tkiva su napravljeni resekcijom mandibule, a sastojali su se od defekta i okolne kosti. Uzorci tkiva su fiksirani u 10% puferisanom formalinu, demineralizovani u 10% mravljoj kiselini, dehidrirani u seriji alkohola, a zatim kalupljeni u parafinskom vosku. Sečenje je izvedeno u bukolingvalnom

pravcu na mikrotomu staklenim noževima 2 μm debljine. Preparati su bojeni hematoksilin-eozin metodom i mikroskopski analizirani svetlosnim mikroskopom *VH50* (*Olimpys*, Japan) opremljenim digitalnom kamerom *Leica DFC* 295.

REZULTATI

Eksperimentalna grupa

Tridesetog dana posle implantacije materijala uočava se fibrovaskularno vezivno tkivo, sa oskudnim hroničnim inflamativnim ćelijskim infiltratom u vidu fokusa, dok je okolna kost hipocelularna i bez prisustva Haversovih sistema (kod pet od šest uzoraka) (Slika 1). Osteociti malih hiperhromatičnih jedara su smešteni u lako proširene lakune. Osteoblasti su malobrojni, dok je prisustvo osteoklasta neupadljivo. Na mestima neresorbovanog materijala, ispalog tokom obrade histopatoloških preparata, zapažaju se prazni prostori. Dalje od eksperimentalno načinjenog defekta, prema dubini kosti, uočava se lamelarna kost sa dobro formiranim većim osteonima, proširenih Haversovih i Folkmanovih kanala (Slika 2). U osteonima se zapažaju proširene intersticijalne lamele, a osteociti su smešteni u lakune ovalnih kontura. Granične cementne linije su bazofilne, proširene, sitnozrnastog do amorfnog izgleda. U jednom slučaju (jedan od šest) uočena je džinovsko-ćelijska reakcija na strano telo.

Devedeset dana nakon implantacije materijala u preparisan defekt u koštanom tkivu se zapaža osteoplazija i formiranje trabekula različite širine, koje su okružene osteoblastima. Novoformirana kost je mlada, morfološki nezrela. Nije došlo do *restitutio ad integrum,* ali je fokalno remodelovanje koštanog tkiva intenzivno (Slika 3). Fibrozno vezivno tkivo je fokalno raspoređeno i redukovano (kod svih uzoraka). Koštano tkivo dalje od eksperimentalnog područja pokazuje normalnu morfologiju (Slika 4).

Kontrolna grupa

Tridesetog dana od preparacije eksperimentalnog defekta kod svih uzoraka kontrolne grupe zapaža se osteosintetska aktivnost i fibrovaskularno vezivno tkivo (Slika 5). Prisutno je mlado koštano tkivo čiji osteociti imaju proširene ovalne lakune. Dalje od eksperimentalnog defekta se uočavaju zadebljali rubovi osteona pojačano bazofilne reakcije (Slika 6). U tkivu nema znakova zapaljenja.

Devedeset dana od preparacije arteficijalnog defekta kod kontrolne grupe (svi uzorci) uočava se potpuno zarastanje *ad integrum*. Osteoni novonastalog koštanog tkiva pokazuju manji dijametar i manji broj koncentričnih lamela (Slika 6).

DISKUSIJA

Pre preporuke za kliničku upotrebu nekog materijala neophodno je sprovesti odgovarajuće testove. Testiranje citotoksičnosti endodontskih materijala *in vitro* testovima omogućava kontrolisanje uslova eksperimenta, ali ne odgovara u dovoljnoj meri kliničkoj situaciji. Iz tog razloga se tehnike implantacije (subkutane, intramuskularne, intraosealne) smatraju superiornijim. Materijali koji se testiraju implantiraju se u tkiva pacova [5, 8, 10], zečeva [11], zamoraca [12]. Intraosealni modeli obezbeđuju adekvatno okruženje za *in vivo* testiranje endodontskih materijala. U ovom eksperimentu je korišćena metoda koštane implantacije, a odabrana je mandibula zbog specifičnosti svoje građe [13].

Eugenol (4 alil-2-metoksifenol) jeste ekstrakt ulja karanfilića i predstavlja sastojak cinkoksid-eugenola (*ZnOE*). Izaziva periapeksnu toksičnost i inhibiše rast i proliferaciju *U2OS* humane osteoblasne ćelijske linije. S obzirom na to da ćelijski rast, vezivanje, proliferacija i sinteza matriksa igraju važnu ulogu u zarastanju rana i tkivnoj regeneraciji, može se pretpostaviti da oslobođeni eugenol može uzrokovati poremećaje u zarastanju [14, 15].

U eksperimentalnom periodu od 30 dana unutar eksperimentalnih defekata makroskopski je zapaženo prisustvo ili manji ostaci materijala (endometazon N). Tokom tehnike obrade uzorkovane kosti i izrade histoloških preparata opturacioni materijal je u najvećem broju slučajeva potpuno ili delimično ispao sa mesta implantacije, a eksperimentalni defekti svetlosno-mikroskopski su bili predstavljeni kao prazni prostori. U eksperimentalnom periodu od 90 dana makroskopski i svetlosno-mikroskopski primenjeni opturacioni materijal (endometazon N) nije uočen.

Tridesetog dana od implantacije materijala endometazona N uočeni su fibrovaskularno vezivno tkivo po tipu kalusa i stvaranje nove kosti. Inflamativni odgovor je kod svih uzoraka bio blago izražen osim u jednom slučaju gde je uočen odgovor po tipu reakcije na strano telo sa granulacionim tkivom i okolnom reaktivno izmenjenom kosti. Devedesetog dana zabeleženi su odsustvo inflamativne reakcije u tkivu i manji stepen fibrovaskularne proliferacije u odnosu na period od 30 dana. Vezivno tkivo je najvećim delom zamenjeno mladim koštanim tkivom sa građom po tipu osteona.

U eksperimentalnoj grupi u ovom istraživanju je u ranijem periodu (30 dana) uočena zapaljenska reakcija, koja se do devedesetog dana potpuno povukla. Takođe, kod kontrolne grupe, za razliku od eksperimentalne, nije uočeno prisustvo vezivnog tkiva u drugom vremenskom periodu (90 dana), već je eksperimentalni defekt potpuno zamenjen novonastalim koštanim tkivom. Prisustvo vezivnog tkiva kod eksperimentalne grupe nije bilo izraženo i primećeni su znaci njegovog smanjivanja u odnosu na raniji period, pa bi se moglo pretpostaviti da će s vremenom potpuno biti zamenjeno novostvorenom kosti.

Reakcija tkiva slična dobijenoj u ovoj studiji sreće se i u istraživanjima drugih autora [13, 14]. Kod implantacije materijala *Super EBA* (na bazi cink-oksida) u mandibule zamoraca, *Tassery* i sar. opisuju blagu do umerenu infiltraciju inflamativnim ćelijama posle četiri nedelje. Ova reakcija se smanjivala vremenom i nije zapažena posle 12. nedelje. Pomenuto istraživanje ne ukazuje na ometanje zarastanja niti na pojačanu reakciju inflamacije [13]. Inicijalni inflamativni efekat se pripisuje eugenolu i etoksibenzoevoj kiselini (sastojak *Super EBA*), koji se oslobađaju [16] i čije se otpuštanje smanjuje sa vezivanjem materijala [17]. Visok stepen vezivanja ovih supstanci za serum i ekstracelularne proteine može doprineti redukovanoj toksičnosti i pratećoj blagoj inflamaciji [18].

Inflamativna reakcija subkutanog tkiva pacova je dobijena i u studiji *Triches* i sar., gde su ispitivani histomorfološki parametri kao što su kvantitet inflamativnih ćelija, prisustvo krvnih sudova, zona nekroze i debljina fibrozne kapsule. Opisana je blaga do umerena tkivna reakcija na endometazon N posle

sedam dana sa predominacijom hroničnih inflamativnih ćelija, dok je 42. dana tkivo ostalo blago inflamirano, sa prisustvom limfocita, fibroblasta i dobro organizovanih kolagenih vlakana [14]. Autori su došli do zaključka da su svi testirani sileri (endometazon N, endofil (na bazi ZnO), *Sealer 26* (na bazi $CaOH_2$)) iritansi, čija se toksičnost vremenom smanjuje.

Kada su u pitanju dugi vremenski periodi, rezultati dobijeni u ovoj studiji su saglasni sa rezultatima drugih autora koji potvrđuju da endometazon N usporava procese reparacije i izaziva kasnu zapaljensku reakciju [14, 19, 20].

U istraživanju koje su sproveli *Suzuki* & *Souza,* kod endometazona nije zapaženo idealno zarastanje ni posle 90 dana. Eksperiment je sproveden na psima čiji su kanali korena zuba prepunjavani ovim materijalom, a zatim patohistološki analizirana reakcija periradiksnog tkiva. Hronični zapaljenski ćelijski infiltrat različitog stepena javio se kod svih uzoraka, dok su gigantske ćelije bile prisutne kod osam od deset uzoraka [20].

Dobijeni rezultati u ovoj studiji takođe ukazuju na hroničnu inflamativnu reakciju koštanog tkiva na endometazon u periodu posle 30 dana, koja je slabog intenziteta, što je u saglasnosti sa autorima koji ukazuju na blagu inflamaciju sa znacima regeneracije posle 42. dana od subkutane implantacije endometazona kod pacova [14].

Devedesetog dana od implantacije nisu uočeni znaci zapaljenja, već preovladavanje regenerativnih procesa. Ovi se rezultati ne slažu sa nalazima autora koji hronični zapaljenski infiltrat registruju i posle perioda dužih od 90 dana (šest meseci) kod prepunjenih kanala endometazonom kod majmuna [19]. Dobijene rezultate objašnjavaju iritacijom koju materijal izaziva kada se prebaci preko apeksa korena zuba. Neslaganje rezultata može se pripisati različitim životinjskim modelima i eksperimentalnom dizajnu.

Podaci iz literature, kod ispitivanja endometazona *in vivo* subkutanim testom ukazuju na rezultate različite od dobijenih u ovoj studiji. *Zafalon* i sar. su ustanovili umerenu do jaku inflamativnu reakciju nakon 15 dana od implantacije, a posle 30, 60, 90 dana reakcije nije bilo [10]. S obzirom na to da se radi o istom vremenskom periodu, razlike se mogu pripisati eksperimentalnoj proceduri i razlici u sastavu materijala endometazona i endometazona N.

Najviše sastojaka endometazon oslobađa tokom reakcije vezivanja, što se može objasniti velikom rastvorljivošću materijala [21]. Komponente silera lakše difunduju u sveže zamešanom stanju, iako se eugenol može osloboditi hidrolizom i iz vezanog materijala [15]. Kontinuirano ispuštanje toksičnih komponenata poput eugenola i paraformaldehida može objasniti perzistiranje inflamacije tokom dužeg vremenskog perioda.

ZAKLJUČAK

Na osnovu dobijenih rezultata može se zaključiti da endometazon N usporava proces zarastanja koštanog tkiva jer pokazuje znake produženog zapaljenja u koštanom tkivu u koje je implantiran. Produženje procesa zarastanja ogleda se i u usporenoj zameni fibrovaskularnog vezivnog tkiva novonastalim koštanim tkivom, ali materijal ne dovodi do trajnog narušavanja morfofunkcionalnih odnosa u tkivu.

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En-face parameters change after orthodontic treatment of Class II malocclusion

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SUMMARY

Introduction The aim was to evaluate the difference in en-face anthropometric facial parameters and proportions of patients with Class II malocclusion, before and after orthodontic treatment as well as changes in linear parameters and facial proportions and their deviation from ideal values.

Material and method In this study, en-face photographs before and after the treatment of 50 Class II malocclusion patients were used. Patients were divided in two groups; first group comprised 25 patients treated with multibracket appliance with extractions, and second group included 25 patients treated without extractions, using fixed functional Herbst and multibracket appliance. On each and every photo before and after the treatment facial points and lines were drawn, and linear parameters were determined, based on those markers.

Results showed change in anthropometric parameters in both groups of patients. Statistically significant difference was found for parameters in the middle and lower facial third. Facial proportions changed after the treatment in both groups and they approached ideal values and golden proportion 1:1.618 in the lower facial third.

Conclusion Patients with Class II, division 1 malocclusion, deviate from an ideal set of proportions, particularly in the lower facial third. After the orthodontic treatment, anthropometric parameters in the lower facial third were approaching ideal values.

Keywords: Class II Malocclusion; anthropometry; facial proportions

INTRODUCTION

Facial esthetics evaluation has long history of development. In the last century, one of the first ways to measure facial parameters was actually the measurement of soft tissue profile characteristics. Soft tissue profile parameters were measured on cephalograms, while en-face parameters were not taken into consideration due to poor visibility on anterior and posterior x-rays of head. These x-rays are indicated for skeletal, rather than dental structures analysis, in case of facial asymmetry. However, they were not the choice for soft tissue en-face parameter measurements. Although soft tissue analyses, known and described by authors in the last century, found their use in orthodontic diagnostics, they cannot be used for determining facial beauty in broader concept [1]. In the past, several soft tissue analyses were developed for measuring facial parameters [2, 3]. These out-of-date analyses have not been combined with clinical assessment and none of them was used for important facial elements evaluation. Just recently, facial balance, beauty diagnosis and treatment planning are improved with combined clinical analysis and soft tissue cephalometric analysis [4].

At this moment, cephalometric three-dimensional soft tissue analysis, that would be useful for facial beauty guidelines determination, do not exist. Orthodontic treatment that corrects dentofacial anomaly (dental, skeletal, soft tissue anomaly) does not change facial appearance, therefore the answer lies in appearance comparison (before and after the treatment) [5, 6]. For that reason, it is necessary to measure en-face parameters on facial photographs before and after the orthodontic treatment, in terms of precise and highly justifiable facial perception change [7, 8].

Facial harmony is associated with golden proportions. However, one cannot be assured that achieving golden proportions would change facial beauty perception. Several studies confirmed proportions influence in facial attractiveness; these results need to be taken with caution in terms of orthodontic treatment only [9, 10, 11].

The aim of this study was to determine the difference in en-face anthropometric facial parameters and proportions in patients with Class II malocclusion, before and after orthodontic treatment. Using en-face facial photographs, deviation from ideal proportions, especially in the lower facial third was determined.

MATERIALS AND METHODS

In this study en-face photographs of 50 Class II patients before and after the treatment were used. Patients were divided in two groups; first group of 25 patients was treated with extractions using multibracket appliance - Figure 1a, and second group, 25 patients were treated with the cast splint Herbst appliance followed by multibracket appliances treatment - Figure 1b. On each and every photo before and after the treatment facial points and lines were

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Figure 1a. Before and after treatment photographs of the patient treated with extractions
Slika 1a. Fotografije pacijenta pre i posle terapije (ekstrakciona metoda)

Figure 1b. Before and after treatment photographs of the patient treated without extractions
Slika 1b. Fotografije pacijenta pre i posle terapije (neekstrakciona metoda)

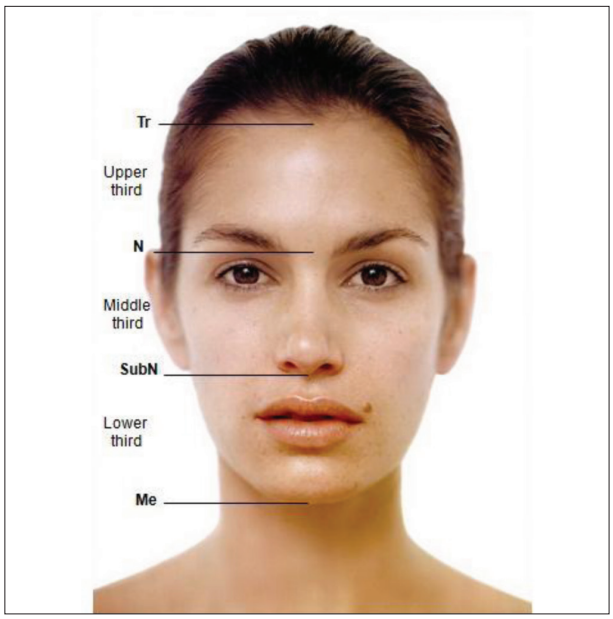

Figure 2. Division of the face into horizontal thirds
Slika 2. Podela lica na horizontalne trećine

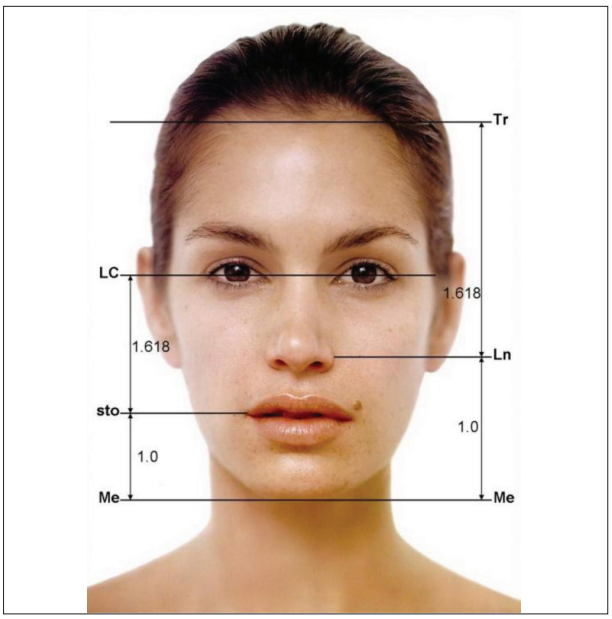

Figure 3. Lengths of the face and set of ideal proportions
Slika 3. Proporcije linearnih parametara

drawn, and linear parameters were determined, based on those markers.

Facial markers, measured on photographs are as follows:

Ch (cheilion) – the point on the corner of the mouth

LC (lateral canthus) – the point on the lateral corner of the eye

Ln (lateral nose) – the outer edge of the nostril

Lchk (lateral cheek) – the most lateral point on the cheek

Ts (temporal soft tissue) – the most lateral point of eyebrows

Tr (trichion) – highest point of the forehead

N (nasion) – the point between upper and middle third of the face

Sn (subnasale) – the point where lowest edge of the nose and upper lip merge

sto (stomion) – the point where the upper and lower lip merge

Me (menton) – the lowest point on the chin.

On the photographs, the following parameters were measured:

I. The horizontal thirds of the face (Figure 2):
   1) Upper third: Tr-N
   2) Middle third: N-subN
   3) Lower third: subN-Me.

II. Lengths – proportions (Figure 3):
   1) Me-sto
   2) sto –LC
   3) Me-Ln
   4) Ln- Tr.

III. Dimensions of the face (Figure 4):
   1) Length (height) of the face (Tr-Me)
   2) Width of the face (lchk r-lchk l).

After determining facial markers and connecting them into appropriate lengths, proportions were measured. There are ideal proportions for each of parameters, so

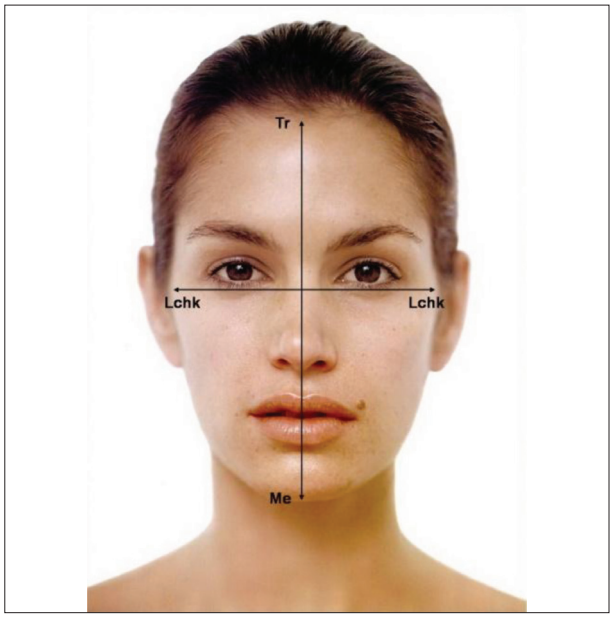

Figure 4. Dimensions of the face – height and width
Slika 4. Dimenzije lica – dužina i širina

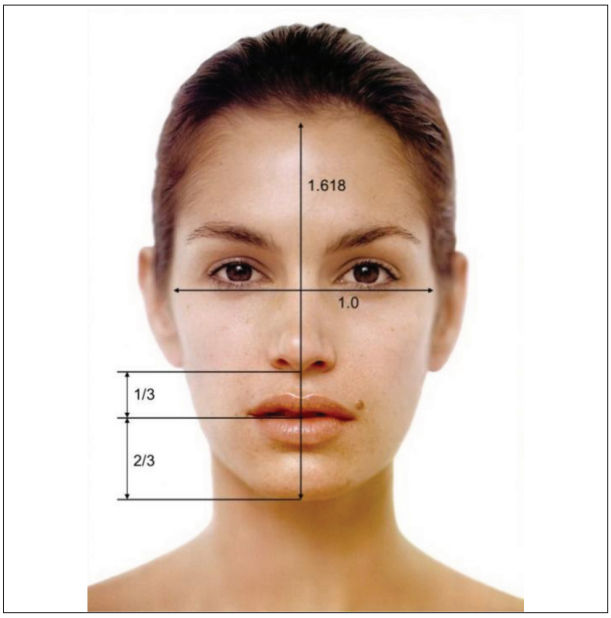

Figure 5. Set of ideal proportions – guidelines
Slika 5. Idealne proporcije – smernice

that the actual length of various parameters does not play role and errors are minimized.

Horizontal thirds of the face should be equal, forehead, nose and jaw thirds should be the same length on each photo individually, their actual length is not important, but only their relationship (Figure 2).

Upper third of the jaw third, the distance between Subnasale and stomion should be 1/3 of the total length of the jaw third, ie. distance between Subnasale - Menton (Figure 5).

The distance Menton - stomion and stomion - Lateral Canthus should be 1 to 1.618 (Figure 3).

The distance Menton - lateral nose and Lateral nose - Trichion should be 1 to 1.618 (Figure 3).

If the width of the face (distance between two points lateral cheek (lchk)) is 1, the length of the face (distance between points Trichion and Menton) should be 1.618 (Figure 5).

RESULTS

The results are presented in Tables 1 and 2. Parameter change after the orthodontic treatment in both groups is presented in Table 1. Statistically significant difference was found for parameters of the middle and lower third (N-subN and subN-Me), in both groups. Their ratio changed and in the first group it was 0.9 while in the second group it was 0.92.

The length parameters and their ratio are presented in Table 2. Parameters Me-Ln and Tr-Me showed significant change after the treatment (Sig < 0.01). When we compare the ratio of middle and lower third of the face, it can be noted that before the therapy it was 1.1 (length N- subN was 46.77mm, length of subN-Me was 52.20 mm), while after the treatment this ratio became 0.9 (length N- subN was 43.72 mm and the length of subN-Me was 48.44 mm). In the first group of patients, the ratio of Me-Ln and Ln-Tr length before the treatment was 1:1.36 (length Me-Ln was 61.93mm and length Ln-Tr was 84.38mm) while after the treatment this ratio was 1:1.5 (length Me-Ln was 55.71mm and length Ln-Tr was 83.76mm). In the second group of patients, the ratio of these two parameters before the treatment was 1.45 (length Me-Ln was 48.72mm and length Ln-Tr was 70.72 mm). After the treatment this ratio changed at 1.62 (length Me-Ln was 48.57mm and length Ln-Tr was 78.77 mm).

DISCUSSION

Analysis of facial parameters and their comparison with standard-average values are necessary in different fields of medicine and dentistry that are able to change facial characteristics in different ways [12, 13, 14]. Some of the specialties like plastic surgery, maxillofacial surgery, orthodontics and prosthodontics are able to perform these kinds of changes. Assumingly, it is important to balance the outcome of the treatment with patient`s expectations, as well their family and friends. Individual beauty assessments and variations in broader opinion about beauty concept, which is highly dependent of modern trends and fashion, are subject of investigations of various authors. [15] Therefore, orthodontists and surgeons have to be united when it comes to objective clinical goals about patient's appearance improvement [16, 17].

In our research en-face parameters were measured. They indicate facial symmetry and division of the face into various proportions and regions, and compatibility with the set of ideal proportions. As it was already mentioned, beauty is very individual; it lies in the eye of observer, and it is difficult to measure and compare it. Therefore, quantitative and numeric comparison between the two groups of patients was not done [18].

Table 1. Horizontal thirds of the face
Tabela 1. Horizontalne trećine lica i njihov odnos

Statistical Parameters Statistički parametri	EXTRACTION VAĐENJE ZUBA							
	Tr–N (mm)		N–subN (mm)		subN–Me (mm)		subN–sto (mm)	
	Before Pre	After Posle	Before Pre	After Posle	Before Pre	After Posle	Before Pre	After Posle
Mean Srednja vrednost	47.54	47.31	46.77	43.72	52.20	48.44	18.08	16.79
St. Dev. Standardna devijacija	4.01	4.09	3.74	1.67	3.23	3.24	2.58	2.46
Significance Značajnost	0.86		0.00		0.00		0.13	
Ratio/Odnos N–subN / subN–Me					1.1	0.9		
Statistical Parameters Statistički parametri	**NON EXTRACTION BEZ VAĐENJA ZUBA**							
	Before Pre	After Posle	Before Pre	After Posle	Before Pre	**After Posle**	**Before Pre**	**After Posle**
Mean Srednja vrednost	40.01	46.01	36.33	39.23	43.39	42.07	13.78	14.57
St. Dev. Standardna devijacija	3.41	3.62	4.79	5.87	4.47	4.77	1.58	1.99
Significance Značajnost	0.60		0.00		0.00		0.20	
Ratio/Odnos N–subN / subN–Me					1.1	0.92		

Table 2. Linear parameters and their relation to ideal set of proportions
Tabela 2. Linearni parametri i njihov odnos prema idealnim proporcijama

Statistical Parameters Statistički parametri	EXTRACTION VAĐENJE ZUBA											
	Me–sto (mm)		Sto–LC (mm)		Me–LN (mm)		LN–Tr (mm)		Tr–Me (mm)		Lchk–Ichk (mm)	
	Before Pre	After Posle	Before Pre	After Posle	Before Pre	After Posle	Before Pre	After Posle	Before Pre	After Posle	Before Pre	After Posle
Mean Srednja vrednost	34.23	31.69	52.74	52.04	61.93	55.71	84.38	83.76	146.51	139.46	99.08	96.03
St. Dev. Standardna devijacija	4.79	4.87	3.51	2.91	6.80	3.93	5.23	4.87	2.62	8.89	13.81	11.52
Significance Značajnost	0.12		0.52		0.00		0.00		0.71		0.48	
Ratio/Odnos Me–Ln / Ln–Tr = 1 : 1.618									1 : 1.36	1 : 1.5		
Statistical Parameters Statistički parametri	**NON EXTRACTION BEZ VAĐENJA ZUBA**											
	Before Pre	After Posle	Before Pre	After Posle	Before Pre	After Posle	Before Pre	After Posle	Before Pre	After Posle	Before Pre	After Posle
Mean Srednja vrednost	29.67	27.53	43.50	47.40	48.72	48.57	70.72	78.77	119.72	127.30	87.33	91.93
St. Dev. Standardna devijacija	5.83	5.02	4.84	9.49	7.28	7.39	6.59	3.44	7.18	9.21	7.03	5.42
Significance Značajnost	0.25		0.13		0.00		0.00		0.95		0.09	
Ratio/Odnos Me–Ln / Ln–Tr = 1 : 1.618									1 : 1.45	1 : 1.62		

Upper, middle and lower facial third should be, in case of harmonically balanced face, approximately equal (ratio 1:1). Lower facial third is divided with line that goes through the point stomion, in upper and lower part, and their ratio should be 1:2 [19].

In the group of patients who had some teeth extracted, statistically significant difference for parameters of middle and lower third was found. As mentioned earlier ratio is more important than the actual numbers. Not taking into consideration values in millimeters but change in relation between parameters we can see slight increase in vertical dimension of lower facial third after finished orthodontic treatment. The reason and explanation can be found in changing the parameters of vertical growth, and increasing vertical dimension due to the application of Class II intermaxillary elastics. However, observing these values and comparing them with the set of ideal proportions, it is noticeable that the ratio between these proportions and their closeness to ideal values did not significantly change, so that difference cannot be described as facial attractiveness change. Nevertheless, the change is visible to clinicians and in the eye of observer. Shell et al. [20] came to the same conclusion when they compared ideal proportions in Class II patients treated with activator during growth, and after the period of active growth, with multibracket appliance with or without orthognatic surgery. They compared re-

lation between ideal proportions and facial attractiveness perception and found that although orthodontic treatment changed facial appearance, it is debatable to what extent that change means achievement of ideal proportions [21].

In the group of patients treated without extractions comparing the parameters of the horizontal division of the face into equal thirds, statistically significant difference was found for the parameter Tr-N. As the upper third of the face significantly change, it is important to identify and interpret newly established relationship of face division into thirds and whether it affects patient's perception in accordance with possible approach to golden proportions. In this group of patients, similar findings about changes in relationship of the lower and middle thirds of face on one side and upper facial third on the other side were found. The original ratio was reduced from 1.1 to 0.92, which was explained as slight change in vertical dimension of the lower third of the face [22].

Ratio between linear parameters in ideal proportions should be, in numerical terms, 1:1.618. Linear parameter of Me-Ln and Ln-Tr before the treatment was 1:1.36, while after the treatment that relation was 1:1.5. The ideal ratio is 1:1.618, and after the treatment the ratio came closer to this value because the parameter Me-Ln in the lower facial third changed.

Even though the parameter Tr-Me (the length of the face) significantly changed after the orthodontic treatment, the ratio between facial length and width that should be close to golden ratio of 1 to 1.618 did not change much. This ratio between the facial length (Tr-Me) and width (lchk-lchk) before treatment was 1.47 and after the treatment it was 1.45. As these values talk more about the broader aspect of enface look and can be associated with the shape of the face, it is expected that this segment will not be much changed after the treatment.

In the group of facial length parameters defined by ideal proportions, in patients treated with fixed functional appliance without extraction, statistically significant difference was observed for the parameter Ln-Tr, which was in golden proportion with the parameter Me-Ln. These two parameters had ratio 1.45 before the treatment, while after the treatment this ratio changed to 1.62 that is ideal relationship between these two parameters. Therefore, in this group of patients ideal ratio was achieved between these two parameters, one of which is directly related to the outcome of orthodontic treatment, new position of chin and Menton (the lowest point on the chin). Our results are consistent with the research of Scolozzi et al. [23] who also reached ideal ratio after completing treatment for the same parameter in the lower third of the face.

Baker et al. [24] performed study about relation between ideal facial proportions and attractiveness perception after orthodontic and surgical treatment taking into consideration facial entities that influence facial beauty concept in general. They used a questionnaire and in results reported significant improvement of facial attractiveness after the treatment, although without strong connection with ideal values. They suggested this analysis to be used only as addition to cephalometric and anthropometric analysis.

Expectedly, Class II malocclusion treatment had limited influence on en-face facial parameters, and consequently achieving ideal values. However, significant changes occurred in the soft tissue profile, so that the measurement of these parameters on the profile photographs, as shown in many studies, is an indispensable supplement to complete analysis of facial soft tissue parameters [25].

CONCLUSION

After anthropometric measurement of linear parameters and proportions, it can be concluded that patients with Class II, division 1 malocclusion, deviate from an ideal set of proportions, particularly in the lower facial third. After the orthodontic treatment, these parameters were approaching the set of ideal values, in both groups of treated patients (with and without extractions).

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Promena parametara anfasa nakon ortodontske terapije malokluzije II klase

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KRATAK SADRŽAJ

Uvod Cilj ovog rada bio je da se antropometrijskim merenjem fotografija anfasa pacijenata pre i posle ortodontske terapije malokluzije II klase, 1. odeljenja, utvrde promene linearnih parametara i proporcija, kao i njihovo odstupanje od idealnih vrednosti.

Materijal i metod U istraživanju su korišćene fotografije anfasa 50 pacijenata pre i posle ortodontske terapije. Pacijenti su podeljeni u dve grupe, od kojih je jedna lečena ekstrakcionom (fiksni aparati sa intermaksilarnim gumicama II klase), a druga neekstrakcionom (fiksni aparati sa fiksnim funkcionalnim Herbst aparatom) metodom terapije malokluzije II klase. Na fotografijama su, nakon iscrtavanja mekotkivnih tačaka, mereni linearni parametri: podela lica na horizontalne trećine, dužina i širina lica, kao i set linearnih proporcija koji su u odnosu idealnih proporcija.

Rezultati Dobijeni rezultati pokazali su da je kod obe grupe pacijenata došlo do promene u antropometrijskim parametrima srednje i donje trećine lica. Dobijena razlika bila je statistički značajna (Sig < 0,01). Kod obe grupe pacijenata dobijena je razlika u odnosu linearnih parametara prema setu idealnih proporcija, naročito u donjoj trećini lica, u kojoj se taj odnos približio idealnim vrednostima i proporciji 1 : 1,618.

Zaključak Pacijenti sa malokluzijama II klase, 1. odeljenja, odstupaju od idealnih vrednosti facijalnih proporcija, pogotovo u donjoj trećini lica. Nakon završene ortodontske terapije vrednosti antropometrijskih parametara u donjoj trećini lica se približavaju idealnim vrednostima, u obe grupe pacijenata.

Ključne reči: malokluzije II klase, antropometrija, facijalne proporcije

UVOD

Merenja lica i facijalne estetike su imala svoj razvojni put. U prošlom veku prvi od načina merenja facijalnog izgleda odnosio se pre svega na izgled mekotkivnog profila pacijenta.

Na telerendgenskim snimcima mereni su parametri mekotkivnog profila, dok je merenje parametara anfasa zapostavljeno zbog nemogućnosti dobijanja dobre vidljivosti mekotkivnih struktura na posteroanteriornim snimcima glave. Ovi snimci su indikovani za analize skeletnih struktura, manje dentalnih, ali nisu izbor za analizu mekih tkiva. Iako je poznato da su mekotkivne analize opisane od autora još u prošlom veku našle svoju primenu u ortodontskoj dijagnostici i pokazale da su jako korisne, ne mogu nam pomoći u određivanju šireg pojma facijalne lepote [1]. U prošlosti, nekoliko analiza mekih tkiva lica su razvijene da bi se merile facijalne pozicije [2, 3]. Ove rane mekotkivne analize nisu bile kombinovane sa kliničkom procenom i nijedna od njih nije istraživala sve važne elemente lica. Nedavno, facijalni balans, dijagnoza lepote i planiranje tretmana su unapređeni kombinacijom kliničke analize lica i mekotkivne kefalometrije [4].

Može se reći da trenutno ne postoji nijedna kefalometrijski bazirana trodimenzionalna analiza mekotkivnih parametara koja bi nam tačnije predstavila smernice za facijalnu lepotu i atraktivnost. Sama metoda ortodontske terapije u cilju ispravljanja anomalije (koja je kako dentoalveolarna, tako i skeletna, i kao takva obuhvata i meka tkiva koja određuju izgled lica) ne menja bitno facijalni izgled, tako da se odgovor mora potražiti u komparaciji izgleda pre i nakon terapije, ma koje vrste ona bila [5, 6].

Upravo zbog toga precizno i potpuno estetski opravdano pitanje promenjene percepcije facijalne privlačnosti nakon završene ortodontske terapije je merenje parametara anfasa na fotografijama pacijenta [7, 8].

Forma i sklad lica su direktno povezani sa idealnim proporcijama. Međutim, kada se govori o anfasu i proporcijama lica, danas je diskutabilno da li se približavanjem idealnim proporcijama sledstveno menja i percepcija lepote lica. Iako je u mnogim studijama dokazano da ove proporcije igraju ulogu u promeni atraktivnosti lica, rezultati se moraju gledati sa rezervom ukoliko poredimo isključivo rezultate ortodontske terapije [9, 10, 11].

Cilj rada bio je da se antropometrijskim merenjem parametara i proporcija na fotografijama anfasa dve grupe pacijenata ispita koliko je odstupanje vrednosti parametara od idealnih proporcija, posebno u donjoj trećini lica, kao i da se ispita razlika u antropometrijskim linearnim parametrima lica pre i nakon završene ortodontske terapije.

MATERIJAL I METOD

U ovom istraživanju korišćene su anfas fotografije 50 pacijenata sa malokluzijom II klase, podeljenih na dve grupe. Prva grupa od 25 pacijenata je lečena ekstrakcionom metodom (fiksni aparati sa intermaksilarnim gumicama II klase) – slika 1a, a druga grupa od 25 pacijenata neekstrakcionom (fiksni aparati sa fiksnim funkcionalnim Herbst aparatom) metodom – slika 1b.

Na fotografijama svakog pacijenta pre i posle terapije iscrtavane su tačke, a zatim su njihovim spajanjem dobijene linije na osnovu kojih su se određivali potrebni linearni parametri.

Tačke, odnosno facijalna obeležja, merena na fotografijama su:

Ch (cheilion) – tačka koja se nalazi na samom uglu usana;

Lc (lateral canthus) – tačka koja se nalazi na spoljašnjem, odnosno unutrašnjem uglu oka, koja se može označiti i pojedinačnim tačkama:

Ln (lateral nose) – tačka na spoljnoj ivici nosnog krilca u visini vrha nosa, sa leve i desne strane;

Lchk (lateral cheek) – najlateralnija tačka obraza;

Ts (temporal soft tissue) – najlateralnija tačka na čelu u nivou obrva;

Tr (trichion) – početak kosmatog dela glave;

N (nasion) – tačka na prelazu gornje u srednju trećinu lica;

Sn (subnasale) – tačka na granici donjeg dela nosa i gornje usne;

Sto (stomion) – tačka na granici gornje i donje usne;

Me (menton) – najniža tačka na bradi.

Na fotografijama anfasa su zatim mereni sledeći parametri:

I. Horizontalne trećine lica (Slika 2):
   1) prva trećina od tačke trichion do tačke nasion (gornja trećina)
   2) druga trećina od tačke nasion do tačke subnasale (srednja trećina)
   3) treća trećina od tačke subnasale do tačke menton (donja trećina)

II. Dužine – proporcije linearnih parametara (Slika 3):
   1) Me–Sto
   2) Sto–Lc
   3) Me–Ln
   4) Ln–Tr

III. Dimenzije lica (Slika 4):
   1) Trichion–Menton (dužina odnosno visina lica)
   2) Lchk–Lchk (širina lica)

Nakon ucrtavanja tačaka i spajanja tačaka potrebnih za dobijanje odgovarajućih dužina, mere se proporcije. Za svaki od ovih parametara postoje idealne proporcije, tako da stvarna dužina različitih parametara nema nikakvu ulogu i samim tim je mogućnost greške svedena na minimum.

Horizontalne trećine lica treba da budu jednake, čeoni, nosni i vilični sprat treba da budu iste dužine na svakoj fotografiji ponaosob; dakle, porede se dužine i njihova jednakost pojedinačno na svakoj fotografiji, tako da stvarna dužina ovih parametara nema značaj, već samo njihov odnos. (Slika 2)

Gornji deo viličnog sprata lica, rastojanje između tačaka subnasale i stomion treba da bude 1/3 ukupne dužine viličnog sprata lica, tj. rastojanja između tačaka subnasale–menton (Slika 5).

Odnos dužina rastojanja menton–stomion i stomion–lateral canthus treba da bude 1 : 1,618 (Slika 3).

Odnos dužina rastojanja menton–lateral nose i lateral nose–trichion treba da bude 1 : 1,618 (Slika 3).

Ako je širina lica odnosno rastojanje između dve tačke lateral cheek (lchk) 1, dužina lica, tj. rastojanje između tačaka trichion i menton treba da bude 1,618 (Slika 5).

REZULTATI

Rezultati ovog istraživanja su prikazani u tabelama 1 i 2.

U tabeli 1. prikazani su rezultati pre i posle terapije za obe grupe pacijenata, i statistički značajna razlika (Sig = < 0,01) dobijena je za parametre srednje i donje trećine lica (N–subN i subN–Me) kod obe grupe pacijenata. Odnos srednje i donje trećine lica je promenjen, i u prvoj grupi pacijenata posle terapije iznosio je 0,9, a u drugoj grupi pacijenata 0,92.

U tabeli br. 2 prikazani su parametri dužina. Za parametre Me–Ln i Tr–Me dobijena je statistički značajna razlika u vrednostima pre i posle terapije (Sig = < 0,01).Kada uporedimo odnos srednje i donje trećine lica, primećujemo da je pre terapije on iznosio 1,1 (dužina N–subN bila je 46,77 mm, a dužina subN–Me 52,20 mm), dok je posle terapije taj odnos iznosio 0,9 (dužina N–subN je 43,72 mm, a dužina subN–Me 48,44 mm). U prvoj grupi pacijenata odnos dužina Me–Ln i Ln–Tr je pre terapije iznosio 1 : 1,36 (dužina Me–Ln bila je 61,93 mm, a dužina Ln–Tr 84,38 mm), dok je posle terapije ovaj odnos iznosio 1 : 1,5 (dužina Me–Ln iznosila je 55,71 mm, a dužina Ln–Tr 83,76 mm).U drugoj grupi pacijenata odnos ova dva parametra je pre terapije iznosio 1,45 (dužina Me–Ln bila je 48,72 mm, a dužina Ln–Tr 70,72 mm), dok je nakon terapije ovaj odnos promenjen i iznosi 1,62 (dužina Me–Ln je nakon terapije iznosila 48,57 mm, a dužina Ln–Tr 78,77 mm).

DISKUSIJA

Analiza anfasa i mekotkivnog profila ispitanika i poređenje rezultata sa standardnim, prosečnim vrednostima, neophodna je u svim specijalnostima medicine i stomatologije koje mogu, u bilo kojoj meri, menjati karakteristike – obeležja lica [12, 13, 14]. Takve su npr. ortognatska i plastična hirurgija, ortodoncija, stomatološka protetika. Podrazumeva se da je važno, kada kliničar pokušava da poboljša izgled pacijenta, to da li će se njegova mentalna slika poboljšanja poklopiti sa slikom samog pacijenta, ali i njegove porodice i, naravno, širokog kruga poznanika i šire javnosti.

Razmišljanje o individualnoj proceni lepote kao merodavnoj pa do varijacija kolektivnih shvatanja koja se menjaju zajedno sa modnim trendovima mogu se pronaći u radovima mnogobrojnih autora [15]. Naravno, podrazumeva se da ortodonti i kolege hirurzi dele isto mišljenje o tome šta bi trebalo da budu objektivni klinički ciljevi koji se odnose na poboljšanje pacijentovog izgleda [16, 17].

U ovom istraživanju merili su se parametri anfasa koji nam govore kako o simetriji i prisustvu pravila podele lica na jednake delove, tako i o usklađenosti proporcija lica i, već pomenutih, idealnih proporcija. Kao što smo naglasili, lepota je u načelu nemerljiva stvar i nalazi se u oku posmatrača, tako da je kvantitativno i numeričko poređenje parametara dvaju različitih grupa pacijenata izostalo iz objektivnih razloga [18].

Gornja, srednja i donja trećina lica bi trebalo kod usklađenog, harmoničnog lica da budu usaglašene, u odnosu 1:1. Donja trećina lica se još može podeliti pomoću linije koja prolazi kroz tačku dodira gornje i donje usne – stomion, na gornji i donji deo, koji stoje u odnosu 2 : 1 [19].

U grupi pacijenata kod kojih su izvršene ekstrakcije statistički značajna razlika dobijena je za parametre srednje i donje trećine lica. Međutim, kao što je već pomenuto, sama dužina i promena vrednosti dužine parametara nam ne znači mnogo dok ne poredimo njihov međusobni odnos. Zanemarujući iznose u milimetrima, i posmatrajući odnos ovih parametara, dolazimo do zaključka da se donja trećina lica, ili donji sprat povećao u odnosu na srednji sprat nakon završene ortodontske terapije. Razlog i objašnjenje treba tražiti u promeni parametara vertikalnog rasta, i povećanja vertikalne dimenzije usled primene intermaksilarnih gumica II klase. Međutim, posmatrajući ove vrednosti i poredeći ih sa zlatnim proporcijama, odnosno idealnim, jednakim odnosom trećina lica, možemo konstatovati da su ovi parametri jednako bliski zlatnim proporcijama bili i pre i posle terapije, te da razlika ne može biti opisana kao približavanje idealnim vrednostima i promeni fa-

cijalne atraktivnosti koja se time objašnjava. Naravno, razlika je prisutna u samom kliničkom nalazu i u oku posmatrača. Do istih rezultata došli su i Shell i sar. [20], koji su upoređivali zlatne proporcije kod pacijenata sa II klasom malokluzija, koji su lečeni ili aktivatorom, tj. fiksnim funkcionalnim aparatom u periodu rasta, ili nakon završenog rasta lečeni fiksnim aparatom i ortognatskom hirurgijom. Upoređivana je i povezanost zlatnih proporcija sa percepcijom facijalne atraktivnosti, što je dodatno potvrdilo konstataciju da je diskutabilno to koliko se sama promena načinjena ortodontskom terapijom, iako menja izgled pacijenata, može poistovetiti sa približavanjem idealnim proporcijama lica [21].

U grupi pacijenata lečenih neekstrakcionom metodom, poredeći parametre horizontalne podele lica na jednake trećine, dobijena je statistička značajnost za parametar Tr–N. Kako se značajno promenio parametar gornje trećine lica, moramo utvrditi i tumačiti koji je novonastali odnos ovakve podele lica na trećine i da li on utiče na promenu percepcije pacijentovog lica u skladu sa mogućim približavanjem zlatnim proporcijama. I kod ove grupe pacijenata dobijen je sličan nalaz promene odnosa donje i srednje trećine lica u odnosu na gornju. Naime, prvobitni odnos od 1,1 je smanjen na 0,92, što se tumači, kao i kod ekstrakcione grupe pacijenata, blagom promenom vertikalne dimenzije donje trećine lica [22].

Odnos pojedinih dužina i njihovo približavanje idealnim proporcijama određen je numerički, 1 na prema 1,618. Odnos dužina Me–Ln i Ln–Tr je pre terapije iznosio 1 : 1,36, dok je posle terapije ovaj odnos iznosio 1 : 1,5. Kako je idealan racio 1 : 1,618, primećujemo da je nakon terapije međusobni odnos ovih parametara malo bliže idealnim vrednostima, usled promene parametra Me–Ln, koji označavaju dužinu donjeg dela lica i entiteta koji se menjaju tokom ortodontske terapije.

Ako posmatramo parametar Tr–Me, odnosno dužinu lica, iako je dobijena značajna promena, odnos dužine i širine lica, koji bi trebalo da odgovara zlatnom odnosu 1 : 1,618, neznatno je promenjen. Naime, odnos dužine (Tr–Me) i širine (lchk–lchk) lica je pre terapije iznosio 1,47, a nakon terapije 1,45. Kako su ovo vrednosti koje govore o širem aspektu izgleda anfasa i mogu se povezati sa oblikom lica, očekivano je da se percepcija izgleda u ovom segmentu neće puno promeniti nakon terapije.

U grupi parametara dužina lica određenih idealnim proporcijama, kod pacijenata lečenih fiksnim funkcionalnim aparatom bez ekstrakcije, statistički značajna razlika dobijena je za parametar dužine Ln–Tr, koji se zajedno sa parametrom Me–Ln odnosi u vidu zlatnih proporcija. Odnos ova dva parametra je pre terapije iznosio 1,45, dok je nakon terapije ovaj odnos promenjen i iznosi 1,62, što praktično predstavlja dostizanje idealnog odnosa između ova dva parametra. Dakle, možemo reći da je u ovoj grupi pacijenata došlo do postizanja idealnih proporcija u odnosu dva parametra, od kojih je jedan direktno povezan sa rezultatom ortodontske terapije, odnosno novim položajem brade i tačke Menton (koja u analizi parametara anfasa predstavlja najnižu tačku na bradi). Ovi rezultati su u skladu sa istraživanjem Scolozzija i sar. [23] koji su dobili značajno približavanje idealnim proporcijama nakon završene terapije za isti parametar u donjoj trećini lica.

Baker i sar. [24] vršili su ispitivanja povezanosti idealnih facijalnih proporcija nakon ortodontsko-hirurške terapije sa percepcijom atraktivnosti, uzimajući pritom u obzir i udaljene entitete lica koji utiču na sveukupni utisak lepote lica. Koristeći anketu, dolaze do rezultata koji govore o signifikantnom napretku i poboljšanju facijalne atraktivnosti, ali bez stroge povezanosti sa približavanjem, odnosno udaljavanjem od vrednosti idealnih, zlatnih proporcija. Oni preporučuju upotrebu ove metode merenja kao dopunsku, pomoćnu uz ostale kefalometrijske i antropometrijske analize.

Očekivano, ortodontska terapija malokluzija II klase, bez obzira na metod kojim je završena, ograničeno utiče na promenu parametara anfasa, a samim tim na približavanje ovih vrednosti idealnim, zlatnim proporcijama. Međutim, značajnije promene se dešavaju na mekotkivnom profilu, tako da je merenje ovih parametara na profilnim fotografijama predstavljalo srž mnogobrojnih istraživanja vezanih za ovu tematiku, i kao takvo predstavlja neizostavnu dopunu kompletne analize mekotkivnih facijalnih parametara [25].

ZAKLJUČAK

Antropometrijskim merenjem linearnih parametara i proporcija na fotografijama anfasa može se zaključiti da pacijenti sa malokluzijama II klase, 1. odeljenja, odstupaju od idealnih vrednosti facijalnih proporcija, pogotovo u donjoj trećini lica. Nakon završene ortodontske terapije, vrednosti antropometrijskih parametara u donjoj trećini lica se približavaju idealnim vrednostima, u obe grupe pacijenata.

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Distribution of *Aggregatibacter actinomycetemcomitans* in deep caries lesions

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SUMMARY

Introduction Deep caries is a reversible process where caries lesion has affected bigger part of dentin and only thin layer of softened dentin that separates lesion from the pulp is remained. The objective of this study was to identify and determine serotypes of *Aggregatibacter actinomycetemcomitans* in teeth with deep caries lesions at the beginning of their treatment.

Material and methods Clinical research included 29 patients of both genders, aged 16 to 40 and 45 permanent teeth with diagnosed deep caries lesions based on medical history, clinical and radiographic examination. After cavity preparation and removal of softened dentin, microbiological swab was taken from the bottom of the cavity. Swabs were disposed in special sterile micro tubes and stored at the temperature of -80°C until serotyping was done (determination of serotypes of *A. actinomycetemcomitans* bacterium).

Results In one of the 3 samples two serotypes of *A. actinomycetemcomitans* (b and c) were identified which is relatively rare finding, while in the second and third sample serotypes (a) and serotype (b) was identified, respectively.

Conclusion In the three samples the 3 serotypes were found (a, b and c) and one of the samples was carrying even two different serotypes, which is a rare phenomenon. For more serious epidemiological study of *A. Actinomycetemcomitans* serotypes at the population level incomparably larger starting material is necessary, at least few hundred of samples.

Keywords: deep caries lesions; *Aggregatibacter actinomycetemcomitans*; serotypes; PCR

INTRODUCTION

Dental caries is a chronic complex bacterial infection that results in milligram loss of minerals from infected tooth. Some authors have defined caries as a disease of hard dental tissues (enamel, dentin and cementum) with characteristic processes of demineralization and remineralisation. Deep caries can be classified as clinically visible lesion in dentin characterized by close topographic relation to the pulp, followed by weakening of the sidewalls due to the progression of caries both in width and depth [1, 2].

One of the factors affecting the occurrence of caries is dental plaque that represents adhered deposits of bacteria and their products and it exists on every surface of teeth. Dental plaque contains pyogenic bacteria such as *Porphyromonas gingivalis, Prevotella intermedia* and *Aggregatibacter actynomycetemcomitans* [3]. According to the new classification based on phylogenetic similarity, *A. actynomycetemcomitans* was in 2006 grouped together with *Haemophilus aphrophilus* and *H. paraphrophilus* in the new Aggregatibacter genus. *A. actynomycetemcomitans* was the first time described in 1912 by Klinger as coccobacillary bacteria isolated together with *Actinomyces* from actinomycotic lesions of man and it was therefore originally grouped into the *Actinomyces* genus.

A. actynomycetemcomitans is a non-motile, slow-growing, capnophilic Gram-negative coccobacilus. It grows slowly at 37°C, both aerobically and anaerobically. Five stereotypic groups of *A. actinomycetemcomitans* were classified based on surface polysaccharide, where serotypes a, b and c are the most prevalent in the oral cavity. A particular clone of serotype b shows enhanced leukotoxic activity. It is predominantly associated with the cases of localized aggressive periodontitis, while the serotype c is usually found in healthy subjects [4–7].

Takahashi et al. demonstrated in an animal model that SPA serotype b has an accentuated ability to stimulate interleukin-1 release from macrophages. The latest data based on molecular genetic analysis indicated significant divergent evolutions of genomes of bacteria serotype a in relation to serotypes b/c, and differences in genomes implied accentuated phenotypic differences [8]. Pajukanta et al. showed that the response of *A. Actynomycetemcomitans* to antimicrobials can vary depending on the serotype [9].

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Table 1. PCR conditions
Tabela 1. Uslovi PCR-a

Initial denaturation Inicijalna denaturacija	35 cycles 35 ciklusa			Final elongation Finalna elongacija
3 min.	3 steps 3 koraka			7 min.
	Denaturation Denaturacija	Hybridization Hibridizacija	Elongation Elongacija	
	15 sec.	30 sec.	1 min.	

It is assumed that patients are usually infected with one serotype that is usually maintained over time, i.e. indicates stability [10]. However, more recent research based on molecular genetic testing and not on serological tests, indicated that serotype changes are possible over time. In the study of van der Reijden et al. performed on the population of Indonesian island Java, after 8 years (time interval of the study) there was a change noticed in the prevalence of serotypes at the population level [11]. Among the people who were tested at the beginning and at the end of the study, after 8 years 58% of them had the same bacterial serotypes, and 42% of them had other serotypes. They also reported rare cases of multiple serotype infections, around 10% while during the 8 years of research multiple infections increased from 12% to 17% [12].

Another characteristic of *A. actynomycetemcomitans* is that distribution of serotypes significantly fluctuates depending on geographic region of analysed population as well as periodontal status of teeth. Thus, for example, in the USA, in patients with localized juvenile periodontitis the serotype b is more common than serotypes a and c. The situation is similar in Finland population where serotype b dominates among the patients with periodontal disease, whereas serotype c is more commonly found among patients with no periodontal disease. In Japanese population, serotypes a, c and e were the most common [13].

The aim of this study was to determine and identify serotypes of *Aggregatibacter actynomycetemcomitans* in teeth with deep caries lesions at the beginning of deep caries treatment.

MATERIAL AND METHODS

The clinical study was conducted on 45 permanent teeth of patients, aged 16 to 40. Different morphology groups of permanent teeth with deep caries lesions were included in the study. Deep lesions considered dental caries followed by sensitivity to thermal stimuli, affecting more than ¾ of the tooth crowns with lots of softened dentin. After cavity preparations and removal of softened dentin, the swab was taken from the bottom of the cavity. Taken swabs were disposed in special sterile micro tubes and stored at the temperature of -80°C until serotyping was performed (determination of serotypes of *A. actinomycetemcomitans*).

The samples were tested at the Institute for Human Genetics, Faculty of Dentistry, University of Belgrade using the multiplex PCR method that enables simultaneous amplification of various gene sequences using multiple pairs of primers. Familiar sequences of primers were used for PCR reactions. Serotyping of *A. actynomycetemcomitans* was also based on the multiplex PCR reaction that included the use of five pairs of primers specific for a, b, c, d and e serotypes of this microorganism, as well as highly specific amplification conditions that are appropriate for all oligonucleotide primers.

The length of products of gene amplification for certain serotypes, with upper pairs of primers were as follows: serotype a: 428 bp, serotype b: 258 bp, serotype c: 559 bp, serotype d: 690 bp and serotype e: 211 bp. Reactions were conducted in the total volume of 25 microliters.

PCR conditions are given in the Table 1.

RESULTS

Oligonucleotide primers specific for the group of genes involved in the biosynthesis of bacterial serotype-specific polysaccharide antigens were designed to be able to identify five main serotypes of *A. actynomycetemcomitans* (a, b, c, d and e) by using the multiplex PCR. In laboratory conditions, multiplex PCR optimization has proven to be technically demanding and that is why it was possible to serotypically define only a small percentage of samples. A serotype was conditionally established after a number of repeated attempts in only 3 samples. The interesting fact is that two serotypes (b and c) were found in one of the 3 samples, which is relatively rare finding. Figure 1 shows that gel was given after one attempt of serotyping where only one out of 10 samples showed the corresponding strips (10b sample). In the samples # 18 and # 23 arrows represent strips that do not correspond to any known serotype and which could be the PCR artefacts.

One of repeated serotyping successfully identified serotypes in 2 more samples: 7c (serotype a) and 6 (serotype c). During repeated multiplex PCR reaction, a nonspecific strip that was present in the first experiment was now lost in the sample 23 (Figure 2).

DISCUSSION

A. actynomycetemcomitans, ie its serotype c, is a part of normal flora of the oral cavity in healthy patients. This bacterium can also be pathogen because it has significant virulence factors (one of them is adhesion) that enable colonisation of bacteria and intensify its destructive potential in oral diseases [14]. Serotyping of bacteria is a suitable typization method for epidemiological studies. Primarily it is easy to perform and more sensitive compared to other methods that require additional equipment and are more costly [15].

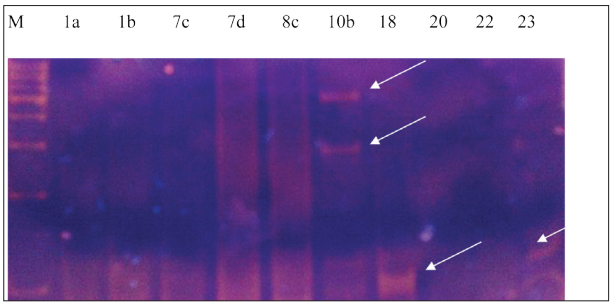

Figure 1. Multiplex PCR-based serotyping. In the sample 10b the arrows correspond to the familiar serotypes: b (298 bp) and c (559 bp). Strips in the samples no. 18 and no. 23 were nonspecific. M indicates molecular scale.

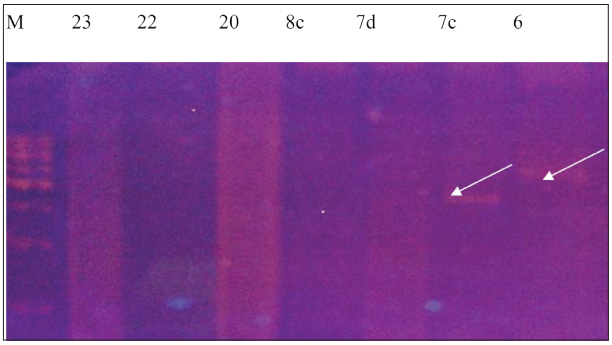

Figure 2. Repeated series of serotyping. In the samples 7c and 6, the expected length of strips was found, 428 bp for the serotype a and 559 bp for the serotype c. M indicates molecular scale.
Slika 2. U uzorcima 7c i 6 uočavaju se trake očekivanih dužina 428 bp za a, i 559 bp za c serotip. M označava molekulsku lestvicu.

Dental caries is multibacterial disease but serotyping is the only method to determine certain serotypes of bacteria that play role in aetiology. Number of studies has shown that certain bacteria detected in dental plaque are closely associated with the occurrence of caries while large caries lesions often communicate with subgingival biofilm bacteria. *Streptococcus mutans* and *Aggregatibacter actynomycetemcomitans* are oral pathogenic bacteria associated with caries and periodontal disease. Psoter et al. (2011) aimed to determine colonization of these two microorganisms in dental plaque of adolescents from rural areas of Haiti by using two different methods of polymerase chain reactions (PCR): Standard PCR and Quantitative real-time PCR (qPCR). This study included 152 plaque samples from 104 patients aged 12 to 19 years. Total genomic DNA of these bacteria was isolated from the samples while dental caries or periodontal changes were found in all subjects during clinical examination. The results showed moderate to high prevalence of *S. mutans* and *A. actinomicetemcomitans* in all samples [16-20].

A large number of teeth affected with deep caries in both, upper and lower jaw (regardless the morphological group of teeth) could primarily be explained with socioeconomic and health conditions, not only in war but also in the post-war period in our region. Certainly the most important factor is the level of health care that does not meet basic health needs of the population. Also, difficult social situation and struggle for pure existence often put dental health as second priority. More frequent occurrence of deep caries on the upper and lower molars could be explained by the fact that occlusal surfaces of these teeth is susceptible to caries, due to their morphology and the existence of fissures and cusps. It is also well-known fact that nutrition, use of fluorides and oral hygiene have dominant influence on the occurrence of caries.

During clinical examination in this study, poor oral hygiene was noticed among the respondents. Sofrata et al. (2008) tested the antibacterial effect of Miswak sticks for oral hygiene against bacteria involved in the occurrence of periodontal disease and caries including *A. actynomycetemcomitans.* The Miswak sticks were standardized by size and weight (0.07 and 0.14 g) and they were tested against *S. mutans, Lactobacillus acidophilus, A. actynomycetemcomitans*, *Porphiromonas gingivalis* and *Haemophilus influenzae*. The inhibitory effect of the pieces of Miswak sticks embedded in the agar plate was most pronounced on *P. gingivalis, A. actynomycetemcomitans, H. influenzae* and less on *S. mutans* and *L. acidophilus* [21].

The literature indicates that it is impossible to determine the serotype in 3 to 8% of samples of *A. Actynomycetemcomitans* [22]. Unfortunately, this percentage was significantly higher in our study. We tried to overcome technical problems in many ways by changing the number of experimental parameters but we got unsatisfactory results. Multiplex PCR was performed with different amounts of starting material and different duration of particular steps of reaction. Also, the number of cycles was modified (25, 30, 35) as well as $MgCl_2$ concentrations and hybridization temperature. However, even after all the effort and repeated attempts the success of serotyping was moderate. We were able to determine *A. Actynomycetemcomitans* serotypes in only 21% of the samples.

Numerous studies have come to conclusion that *A. actynomycetemcomitans* is most frequently associated with periodontal diseases [23]. The study of Cortelli et al. (2005) showed the presence of *A. actynomycetemcomitans* in 41.6% of the subjects with chronic periodontal disease and 72% of subjects with acute periodontal disease [24]. Tinoco et al. also found *A. actynomycetemcomitans* in 80% of young patients with periodontal disease and suggested that the presence of this bacterium in the oral cavity may serve as an indicator of risk for future tests of acute periodontal disease [25].

Based on research of deep caries lesions Simon-Soro and Mira found diverse ecosystem made of a large number of bacteria affecting the spreading of caries lesions. The results showed that *S. mutans* was present in a small percentage and that other bacteria including *A. actynomycetemcomitans* affect spreading of caries lesions [26].

CONCLUSION

Given the small initial number of teeth, relatively small percentage of samples positive for *A. actynomycetemcomitans* and finally, poor achievement of the multiplex reaction of serotyping, it is impossible to talk about the prevalence of certain serotypes in our population. In the 3 samples three serotypes of *A. actinomycetemcomitans* (a, b and c) were identified and two different serotypes were

identified in one of the samples that is a rare phenomenon. For more serious epidemiological study of serotypes of *A. Actinomycetemcomitans* at the population level and their relation to the formation of dental caries an incomparably larger starting material is necessary, at least a few hundred of samples.

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Zastupljenost bakterije *Aggregatibacter actinomycetemcomitans* u dubokim karijesnim lezijama

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KRATAK SADRŽAJ

Uvod Duboki karijes je reverzibilni proces kod kojeg je karijesna lezija zahvatila veći deo dentina i samo tanak sloj razmekšalog dentina razdvaja leziju od pulpe.
Cilj ovog rada je bio da se na početku terapije utvrde i odrede serotipovi bakterije *Aggregatibacter actynomycetemcomitans* kod zuba sa dubokim karijesnim lezijama.
Materijal i metod rada Kliničko ispitivanje je obuhvatalo 29 pacijenata, oba pola, uzrasta od 16 do 40 godina i 45 stalnih zuba kod kojih je na osnovu anamneze, kliničkog i radiografskog pregleda dijagnostikovan duboki karijes. Posle preparacije kaviteta i uklanjanja razmekšalog dentina, sa dna kaviteta je uziman bris, odlagan u posebne sterilne mikrotubice i čuvan na temperaturi od -80°C do postupka serotipizacije (utvrđivanja serotipova bakterije *Aggregatibacter actynomycetemcomitans*) primenom metode multipleks PCR.
Rezultati Serotipizacija je registrovana u samo tri uzorka. U jednom od tri uzorka identifikovana su dva serotipa *A. actynomycetemcomitans* – b i c, što je relativno redak nalaz, dok su u drugom i trećem uzorku identifikovani serotipovi a, odnosno serotip b.
Zaključak U tri uzorka nađena su tri serotipa – a, b i c, a jedan od uzoraka je nosio čak dva različita serotipa, što je redak fenomen. Za ozbiljniju epidemiološku studiju serotipova *A. Actynomycetemcomitans* na nivou populacije neophodan je neuporedivo veći uzorak i to reda veličine nekoliko stotina.
Ključne reči: duboka karijesna lezija; *Aggregatibacter actinomycetemcomitans*; serotipovi PCR

UVOD

Zubni karijes je hronična kompleksna bakterijska infekcija koja dovodi do miligramskih gubitaka minerala iz zuba koji je zahvaćen infekcijom. Pojedini autori su definisali karijes kao oboljenje tvrdih zubnih tkiva (gleđi, dentina i cementa) koje karakterišu naizmenični procesi demineralizacije i remineralizacije. Duboki karijes predstavlja klinički vidljivu leziju u dentinu i karakterišu je vrlo blizak topografski odnos lezije sa pulpom i oslabljeni bočni zidovi kaviteta usled napredovanja karijesa u širinu i dubinu [1, 2].

Jedan od faktora koji utiče na nastanak karijesa je dentalni plak, koji predstavlja adherirani depozit bakterija i njihovih produkata, koji se formira na svim površinama zuba. U zubnom plaku je moguće detektovati brojne bakterije, ali i prisustvo piogenih bakterija kao što su *Porphyromonas gingivalis*, *Prevotella intermedia* i *Aggregatibacter actynomycetemcomitans* [3].

Aggregatibacter actynomycetemcomitans je bakterija koja je po novoj klasifikaciji svrstana sa bakterijama *Haemophilus aphrophilus* i *H. paraphrophilus* u novi *Aggregatibacter* rod. Klinger je 1912. godine ovu bakteriju prvi put opisao kao kokobacil koji je bio izolovan zajedno s bakterijama roda *Actinomyces* iz aktinomikotičkih lezija čoveka. To je nepokretni, spororastući, kapnofilni gram-negativni kokobacil koji raste sporo na temperaturi od 37°C, u aerobnoj ili anaerobnoj sredini. Klasifikovano je pet sero skupina *A. actinomycetemcomitans* na osnovu površinskih polisaharida, od čega su serotipovi a, b i c najčešće prisutni u usnoj šupljini. Specifični klon b serotipa pokazuje povećanu leukotoksičnu aktivnost i predominantno se povezuje sa slučajevima lokaliziranog agresivnog parodontitisa, dok se serotip c najčešće nalazi kod zdravih osoba [4–7].

Takahashi i saradnici su pokazali na animalnom modelu da SPA serotipa b imaju izraženiju sposobnost da stimulišu oslobađanje interleukina-1 iz makrofaga od SPA sojeva a i c. Najnoviji podaci zasnovani na molekularno-genetičkim analizama ukazuju na značajnu divergentnu evoluciju genoma bakterija serotipa a u odnosu na serotipove b/c, a razlike u genomima podrazumjevaju i izražene fenotipske razlike [8].

Pajukanta i saradnici su pokazali da je odgovor *A. actynomycetemcomitans* na antimikrobna sredstva podložan varijacijama u zavisnosti od serotipa [9].

Smatra se takođe da su pacijenti najčešće inficirani jednim serotipom koji se po pravilu održava tokom vremena, jer je vrlo stabilan [10]. Međutim, novija istraživanja, zasnovana na molekularno genetičkim testovima, ukazuju na to da su moguće serotipske promene tokom vremena. U studiji Van der Reijdena i saradnika [11] na stanovništvu indonežanskog ostrva Java, nakon osam godina (vremenski interval studije) došlo je do promene prevalence serotipova na populacionom nivou. Od osoba koje su ispitane na početku i na kraju studije (nakon osam godina) 58% je zadržalo iste bakterijske serotipove, a kod 42% su se pojavili drugi. Zanimljiva je i činjenica da su uočeni retki slučajevi infekcija multiplim serotipovima (oko 10%), dok je u pomenutoj studiji holandskih naučnika tokom osam godina istraživanja zapažen porast multiplih infekcija, sa 12 na 17% [12].

Još jedna karakteristika *A. actynomycetemcomitans* je da serotipovi pokazuju prilične varijacije u distribuciji u zavisnosti od geografskog regiona analiziranih populacija, odnosno od parodontalnog stanja ispitivanih grupa. Tako npr. u SAD kod pacijenata sa lokalizovanim juvenilnim periodontitisom češći je serotip b od serotipova a i c. Slično je i u Finskoj populaciji, gde serotip b dominira među pacijentima sa parodontopatijama,

dok se serotip c često sreće kod osoba koje su parodontalno zdrave. U japanskoj populaciji kod obolelih su najučestaliji serotipovi a, c i e [13].

Cilj ovog rada je bio da se na početku terapije utvrde i odrede serotipovi bakterije *Aggregatibacter actynomycetemcomitans* kod zuba sa dubokim karijesnim lezijama.

MATERIJAL I METOD RADA

Kliničko istraživanje je sprovedeno na 45 stalnih zuba pacijenata dobi od 16 do 40 godina. U istraživanje su bili uključeni stalni zubi različitih morfoloških grupa sa dubokim karijesnim lezijama. Duboka lezija je podrazumevala karijes zuba kod pacijenata koji je praćen osetljivošću na termičke nadražaje i koji je zahvatio više od ¾ krunice zuba sa dosta razmekšalog dentina. Posle preparacije kaviteta i uklanjanja razmekšalog dentina, sa dna kaviteta je uzet bris koji je odlagan u posebne sterilne mikrotubice i čuvan na temperaturi od -80°C do serotipizacije (utvrđivanja serotipova bakterije *Aggregatibacter actynomycetemcomitans*).

Uzorci su ispitani na Institutu za humanu genetiku Stomatološkog fakulteta Univerziteta u Beogradu, primenom metode multipleks PCR, koja omogućava simultanu amplifikaciju različitih genskih sekvenci uz korišćenje više parova prajmera. Za izvođenje PCR reakcije korišćene su poznate sekvence prajmera. Serotipizacija *Aggregatibacter actynomycetemcomitans* je takođe bila zasnovana na multipleks PCR reakciji koja je podrazumevala korišćenje pet parova prajmera specifičnih za serotipove a, b, c, d i e ovog mikroorganizma, kao i usko specifične uslove amplifikacije koji odgovaraju svim oligonukleotidnim prajmerima.

Produkti amplifikacije gena za pojedine serotipove, sa gornjim parovima prajmera, bili su sledećih dužina: serotip a – 428 bp, serotip b – 258 bp, serotip c – 559 bp, serotip d – 690 bp i serotip e – 211 bp.

Reakcije su rađene u ukupnoj zapremini od 25 mikrolitara, a uslovi PCR-a dati su u tabeli 1.

REZULTATI

Oligonukleotidni prajmeri specifični za grupu gena uključenih u biosintezu bakterijskih serotip-specifičnih polisaharidnih antigena dizajnirani su tako da mogu da identifikuju pet glavnih serotipova bakterije *A. actynomycetemcomitansa* (a, b, c, d i e) i to koristeći multipleks PCR. U laboratorijskim uslovima optimizacija multipleks PCR se pokazala tehnički zahtevnom te je mali procenat uzoraka mogao da bude serotipski definisan. U svega tri uzorka, i to iz više ponovljenih pokušaja, uslovno je ustanovljen serotip. Zanimljivo je to što su u jednom od ta tri uzorka uočena čak dva serotipa (b i c), što je relativno redak nalaz. Na slici 1 dat je gel nakon jednog od pokušaja serotipizacije, gde je od 10 uzoraka samo jedan pokazao odgovarajuće trake (uzorak 10b). U uzorcima broj 18 i 23 strelicama su označene trake koje ne odgovaraju poznatim serotipovima, a koje bi mogle biti artefakti PCR-a.

Jedan od ponovljenih pokušaja serotipizacije uspeo je da identifikuje serotipove u još dva uzorka: 7c i 6. U uzorku 7c serotip je a, a u uzorku 6 serotip je c. Tokom ponovljene reakcije multipleks PCR-a izgubila se nespecifična traka u uzorku 23 koja je bila prisutna u prvom eksperimentu (Slika 2).

DISKUSIJA

Bakterija *A. actynomycetemcomitans*, odnosno njen serotip c, predstavlja deo normalne flore usne šupljine kod zdravih pacijenata. Ova bakterija može biti i patogena jer poseduje značajan faktor virulencije – adheziju, koja omogućava kolonizaciju bakterija, čime se pojačava njen destruktivni potencijal u oralnim oboljenjima [14].

Serotipizacija bakterija je adekvatna tipizaciona metoda za epidemiološka ispitivanja jer ima mnoge prednosti u odnosu na druge metode ispitivanja bakterija. Prvenstveno je jednostavnija za izvođenje i osetljivija u odnosu na druge metode koje zahtevaju dodatnu opremu i finansijski su zahtevnije [15].

Mnoge bakterije učestvuju u nastanku karijesa pa je i serotipizacija kao postupak određivanja pojedinih bakterija vrlo značajna u razjašnjavanju etiologije. Veliki broj istraživanja je pokazao da su pojedine bakterije koje su detektovane u dentalnom plaku usko povezane sa nastankom karijesa, a velike karijesne lezije često komuniciraju sa bakterijama subgingivalnog biofilma. *Streptococcus mutans* i *Aggregatibacter actynomycetemcomitans* su oralne patogene bakterije koje se dovode u vezu i sa karijesom i sa parodontopatijom. Upravo zbog toga su Psoter i njegovi saradnici (2011) imali za cilj da utvrde kolonizaciju ova dva mikroorganizama u dentalnom plaku adolescenata iz ruralne oblasti Haitija pomoću dve različite metode lančane reakcije polimeraze (PCR), tj. standardne PCR i kvantitativne real-time PCR (kPCR). Ova studija je obuhvatala 152 uzorka plaka od 104 pacijenta starosne dobi od 12 do 19 godina. Ukupna genomska DNK ovih bakterija izolovana je iz uzoraka, a kod svih ispitanika je pronađen karijes ili parodontološka promena tokom kliničkog pregleda. Rezultati su pokazali umerenu do visoke prevalence *S. mutans* i *A. actinomicetemcomitans* u uzorcima [16–20].

Veliki broj zuba zahvaćenih dubokim karijesom i u gornjoj i u donjoj vilici (bez obzira na morfološku grupu zuba) mogao bi se objasniti pre svega socijalnoekonomskim i zdravstvenim uslovima ne samo u ratu nego i u posleratnom periodu na ovim prostorima. Ipak, najvažniji faktor bi mogao biti stepen zdravstvene zaštite koji ne zadovoljava osnovne zdravstvene potrebe stanovništva. Takođe, teška socijalna situacija u borbi za čistu egzistenciju često stavlja zdravlje zuba u drugi plan. Češća pojava dubokog karijesa na molarima gornje i donje vilice mogla bi se objasniti činjenicom da je okluzalna površina ovih zuba prijemčiva za karijes, zbog njihove morfologije odnosno postojanja fisura i kvržica. Takođe, poznato je da dominantan uticaj na pojavu karijesa imaju ishrana, primena fluorida i oralna higijena.

U ovoj studiji, tokom kliničkog pregleda, takođe je uočena lošija oralna higijena kod svih ispitanika. Sofrata i saradnici (2008) ispitivali su antibakterijski efekat Misvak štapića za održavanje oralne higijene na bakterije koje učestvuju u nastanku parodontopatije i karijesa, među kojima i na *Aggregatibacter actynomycetemcomitans*. Dejstvo Misvak štapića je nakon njihove standardizacije po veličini i težini (0,07 i 0,14 g) proveravano na *Streptococcus mutans*, *Lactobacillus acidophilus*, *Aggregatibacter actynomycetemcomitans* (ranije *Actinobacillus actinomicetemcomitans*), *Porphiromonas gingivalis*, i na *Haemophilus influenzae*. Komadi Misvak štapića su ugrađeni u agar podloge te je uočen njihov najveći inhibitorni efekat kod *P. gingivalis*, *A. actynomycetemcomitans*, *H. influenzae*, a znatno manji efekat na *S. mutans* i *L. acidophilus* [21].

U literaturi se navodi podatak da je za 3 do 8% uzoraka *A. actynomycetemcomitans* nemoguće odrediti serotip [22]. Nažalost, u našoj studiji taj procenat je neuporedivo veći. Pokušaj da se na razne načine prevaziđu tehnički problemi, menjanjem eksperimentalnih parametara, nije dalo rezultate. Multipleks PCR je rađen sa različitom količinom polaznog materijala i u različitom trajanju pojedinih koraka reakcije. Takođe, menjan je i broj ciklusa (25, 30, 35), a modifikovane su i koncentracije $MgCl_2$, kao i temperature hibridizacije. Međutim, i posle svih napora i ponovljenih pokušaja, uspeh u serotipizaciji je bio veoma skroman. Kod samo 21% uzoraka određen je serotip bakterija *A. actynomycetemcomitans*. Mnogobrojnim studijama se došlo do zaključka da je *A. actynomycetemcomitans* ipak najčešće povezan sa parodontološkim oboljenjima [23].

Cortelli i saradnici su u svom istraživanju (2005) utvrdili zastupljenost *A. actynomycetemcomitans* kod 41,6% ispitanika sa hroničnom parodontopatijom i kod 72% pacijenata sa akutnom parodontopatijom [24].

Takođe, Tinoco i saradnici su pronašli bakteriju *A. actynomycetemcomitans* kod 80% uzoraka mladih ispitanika sa parodontopatijom i predložili da zastupljenost ove bakterije u usnoj šupljini može poslužiti kao indikator rizika za buduća ispitivanja akutne parodontopatije [25].

Simon-Soro i Mira su na osnovu istraživanja dubokih karijesnih lezija otkrili raznovrstan ekosistem, sačinjen od velikog broja bakterija, koji utiče na širenje karijesne lezije. Rezultati su ukazali na to da je *S. mutans* bio zastupljen u manjem procentu, a da veliki broj bakterija, a samim tim i *A. actynomycetemcomitans* ima uticaj na širenje karijesne lezije [26].

ZAKLJUČAK

S obzirom na mali uzorak zuba i relativno mali procenat pozitivnih uzoraka *A. actynomycetemcomitans*, odnosno slabiji uspeh multipleks reakcije serotipizacije, teško je govoriti o prevalenciji pojedinih serotipova u našoj populaciji. U tri uzorka nađena su tri serotipa (a, b i c), a jedan od uzoraka je nosio čak dva različita serotipa, što je redak fenomen. Za ozbiljniju epidemiološku studiju serotipova *A. actynomycetemcomitans* na nivou populacije i njihovu vezu za nastankom karijesa neophodan je neuporedivo veći uzorak, i to reda veličine nekoliko stotina.

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Direct pulp capping with novel nanostructural materials based on calcium silicate systems and hydroxyapatite

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SUMMARY

Introduction Direct pulp capping is an important therapeutic method that has goal to provide formation of dentin bridge and healing process of the pulp. The aim of this study was to investigate the effects of new nanostructural materials based on calcium silicate systems and hydroxyapatite on exposed dental pulp in Vietnamese pigs.

Material and Methods The study was conducted on 30 teeth of two Vietnamese pigs (*Sus scrofa verus*). On buccal surfaces of incisors, canines and first premolars, class V cavities were prepared with a small round bur and pulp horn was exposed. In the first experimental group (10 teeth) the perforation was covered with new nanostructural material based on calcium silicate systems (CS). In the second experimental group, the perforation was covered with compound of calcium silicate systems and hydroxyapatite (HA-CS) (10 teeth). In the control group, exposed pulp was covered with Pro Root MTA® (Dentsply Tulsa Dental, Johnson City, TN, USA) (10 teeth). All cavities were restored with glass ionomer cement (GC Fuji VIII, GC Corporation, Tokyo, Japan). Observation period was 28 days. After sacrificing the animals, histological preparations were done to analyze the presence of dentin bridge, inflammatory reaction of the pulp, pulp tissue reorganization and the presence of bacteria.

Results Dentin bridge was observed in all teeth (experimental and control groups). Inflammation of the pulp was mild to moderate in all groups. Neoangiogenesis and many odontoblast like cells responsible for dentin bridge formation were detected. Necrosis was not observed in any case, neither the presence of Gram-positive bacteria in the pulp.

Conclusion Histological analysis indicated favorable therapeutic effects of new nanostructural materials based on calcium silicate systems and hydroxyapatite for direct pulp capping in teeth of Vietnamese pigs.

Keywords: direct pulp capping; calcium silicate; hydroxyapatite; MTA; dentin bridge

INTRODUCTION

Direct pulp capping and preservation of pulp vitality are very important therapeutic mathods, especially in young patients and teeth with complex multi canal systems [1, 2]. Numerous studies have confirmed calcium hydroxide as gold standard for direct pulp capping since its introduction in dental practice in 1920 [2]. High pH provides stimulating effect on odontoblasts that initiate production of tertiary dentin and pulp vitality preservation. However, the success rate of calcium hydroxide as direct pulp capping medicament in published papers varies from 31% to 100% [3–6]. Due to inadequate bond of calcium hydroxide to exposed pulp that degrades over time, porosity of new dentin bridge and appearance of internal resorption there is need to find more efficient material [3, 7, 8].

In the past twenty years, great attention was given to mineral trioxide aggregate ProRoot MTA® (Dentsply Tulsa Dental, Johnson City, TN, USA) for direct pulp capping and it has been shown to induce complete dentin bridge formation with no signs of pulp inflammation [9, 10]. Numerous studies have confirmed its biocompatibility, antimicrobial effect, good sealing ability and good physical and chemical properties [11, 12]. It is hydrophilic and therefore can be used in wet operating field and the presence of blood. It has also been confirmed that the thickness of dentinal bridge increased after pulp capping using MTA compared to calcium hydroxide [13, 14, 15]. MTA shows excellent properties as material for direct pulp capping and successfully replaces calcium hydroxide. It does not cause local necrosis of the pulp, while chronic inflammation of the pulp occurs [15]. MTA causes functional and cytological changes in pulp cells as well as their transformation to odontoblast like cells that produce reparative dentin on the surface of the pulp [16, 17]. However, MTA is non-stable as powder immediately after package opening, and other disadvantages include high price on the market and long setting time. The manufacturer specifies setting time of 10 minutes, but study of Torabinejad et al. [18] founds setting time between 2 and 4 hours. Con-

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temporary research aimed to find material for direct pulp capping that would have all good properties of MTA, but also overcome its limitations.

Hydroxyapatite is one of the most commonly used calcium phosphate materials in medicine and dentistry. Biocompatibility of hydroxyapatite is closely related to its chemical composition, similar to dental and bony tissues. However, inferior mechanical properties limit the use of hydroxyapatite as an endodontic material. Recent studies have focused on new and modified formulations of calcium-phosphate-based biomaterials with improved mechanical and favorable biological properties [19–23].

Recently, two non-commercial new materials based on calcium silicates and hydroxyapatite have been synthesized using original recipe of V. Jokanović. One material is a calcium silicate system that consists of tricalcium and dicalcium silicates (CS) and the other one is a mixture of this calcium silicate system and hydroxyapatite in 1:2 ratio (HA-CS). Barium sulphate is added for radiopacity. These materials are synthesized using two combined techniques – a hydrothermal sol-gel method and self-propagating combustion waves. This way two nanostructural materials containing agglomerates, several micrometers in size, formed by smaller particles, 117-447 nm in size, that contain even smaller building blocks, 20 nm-sized crystallites were obtained [24]. Particle size affects cement hydration and consequently setting time and final quality of the cement. Nanoparticles improve particle activity and shorten setting time to 10 minutes (CS) and 15 minutes (HA-CS) [24].

The aim of this study was to examine the effect of new nanostructural materials based on active calcium silicate systems and hydroxyapatite (CS and HA-CS) on direct pulp capping of exposed pulp in Vietnamese pigs.

MATERIAL AND METHODS

The research was conducted at the Faculty of Veterinary Medicine, University of Belgrade, with the approval of the Ethics Committee of the School of Dental Medicine, University of Belgrade. The experiment included 30 teeth of two Vietnamese pigs (Sus scrofa verus), aged 24 months and weight about 25 kg. The study procedure complied with the protocol of the European Good Laboratory Practice (86/609/EEC) that involved the implementation of main principles of asepsis and antisepsis, conducting the experiment in the minimum required time without physical and mental suffering of animals (International Organization for Standardization, 1997). ProRoot MTA® (Dentsply Tulsa Dental, Johnson City, TN, USA) was used as control material.

Experimental procedure

All animals were premedicated with atropine in the dose of 0.03-0.04 mg/kg, and after 15 minutes they were introduced to general anesthesia using xylazine in the dose 1.5-2 mg/kg and ketamine 20-25 mg/kg intramuscularly. After rubber dam placement, the teeth were cleaned with 70% ethanol.

On the buccal surfaces of incisors, canines and first premolars class V cavities were prepared using round carbide burs and continuous cooling with saline. Using a small round bur pulp chamber was exposed and bleeding controlled with sterile cotton pellet. Material was prepared and applied on the perforation. Teeth in the right quadrants of upper jaws in both experimental animals (6 incisors, two canines and 2 premolars) received CS. The same number of teeth in left quadrants of upper jaws in both animals received HA-CS. MTA was applied in the right quadrants of lower jaws in both Vietnamese pigs on 6 incisors, two canines and two premolars. All cavities were restored with glass ionomer cement (GC Fuji VIII, GC Corporation, Tokyo, Japan). At the end of the procedure, the animals were given an analgesic dose of butorphanol in the dose of 0.1-0.2 mg/kg. After recovery the animals were kept in individual cages in breeding system. Observational period was 28 days.

After 4 weeks animals were sacrificed after introducing general anesthesia and i.v. administration of sodium phenobarbital in the dose of 100 mg/kg. The jaws were cut into block sections and tissue was fixed and prepared for microscopic analysis.

Histological procedure

Tissue for histological analysis was taken from every block of the experimental sample including the tooth and surrounding bone. Samples were collected respecting the ISO criteria (Technical Report 7405) 28 days after the exposure of the pulp and direct capping. The material for histological analysis was fixed in 10% formalin, decalcified in 10% formic acid (pH=5) and molded in paraffin. Serial sections were made in mesio-distal direction in the thickness of 4 μm. The samples were stained with hematoxylin and eosin, by method of Goldner and Gram (for the microscopic identification of bacteria) and analyzed using light microscope.

Histological criteria for the evaluation of pulp reaction were used by the methodology of Shayegan et al. [25]. Formation of dentin bridge (A) (thickness, localization, structure, continuity with surrounding dentin), morphological reorganization of pulp cells (B), inflammatory reaction of pulp (C) (chronic or acute, intensity and localization of inflammation) and presence of bacteria (D) were analyzed. These parameters were monitored according to the International Organization for Standardization (International Organization for Standardization) and published criteria by Mjör in 1983 [26].

RESULTS

Histological analysis showed that dentin bridge was formed in all samples of the experimental and control groups (Table 1). Newly formed dentin had characteristics of reparative dentin with or without small number of ir-

Table 1. Histological analysis of dental pulp after application of the test materials
Tabela 1. Rezultati histološke analize stanja zubne pulpe nakon primene testiranih materijala

Material Materijal	A. Dentin bridge Dentinski mostić				B.Tissue reorganization Reorganizacija tkiva				C. Pulp inflammation Inflamacija pulpe				D. Bacterial presence Prisustvo bakterija			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
CS	0	5	1	4	1	9	0	0	0	8	2	0	6	4	0	0
HA-CS	0	3	1	6	1	9	0	0	0	7	1	0	5	5	0	0
MTA	0	6	0	4	2	8	0	0	0	7	3	0	7	3	0	0

CS – calcium silicate
HA-CS – hydroxyapatite and calcium silicate
MTA – mineral trioxide aggregate

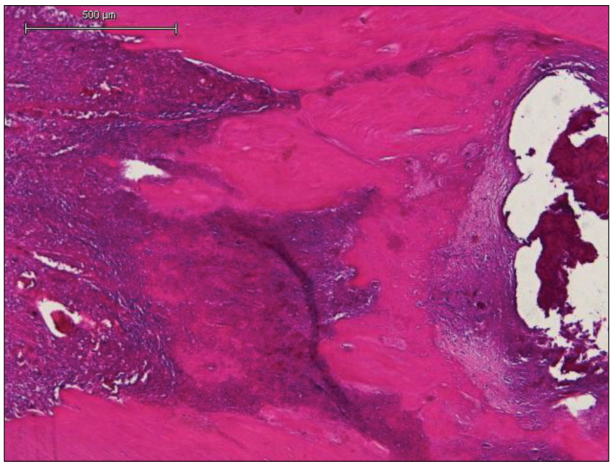

Figure 1. Complete dentin bridge after direct pulp capping with HA-CS. Newly formed dentin completely closes preparation region of the pulp chamber and it is very similar to original dentin. Particles of HA-CS in the area of perforation are visible (HE, 40×).
Slika 1. Kompletan dentinski most nakon direktnog prekrivanja pulpe materijalom HA-CS. Novostvoreni dentin potpuno zatvara predeo perforacije komore pulpe i nalik je pravom dentinu. Na slici se mogu uočiti ostaci materijala HA-CS u predelu perforacije cavuma dentis (HE, 40×).

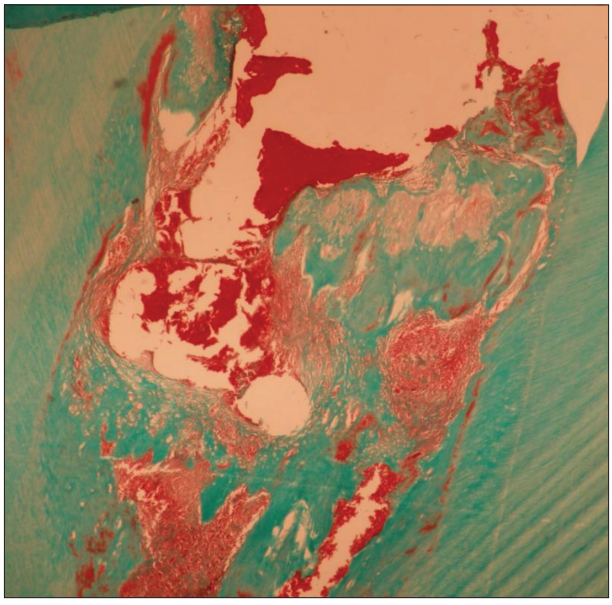

Figure 2. Complete dentin bridge after direct pulp capping with CS. Newly formed dentin completely closes the preparation region of the pulp chamber. Particles of HA-CS are visible in the area of perforation (Goldner trichrome, 40×)
Slika 2. Kompletan dentinski most nakon direktnog prekrivanja pulpe materijalom CS. Novostvoreni dentin potpuno zatvara predeo perforacije komore pulpe. Na slici se mogu uočiti ostaci materijala HA-CS u predelu perforacije cavuma dentis (Goldner trihrom, 40×)

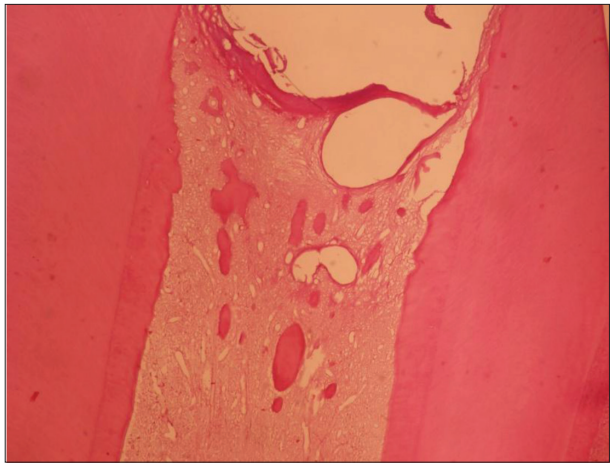

Figure 3. Dentin islets after direct pulp capping with CS. Newly formed dentin is in the form of dentin islands and tends to close perforation of cavum dentis. Completely preserved pulp tissue without inflammatory reaction can be seen (HE, 40×).
Slika 3. Dentinska ostrvca nakon direktnog prekrivanja pulpe materijalom CS. Novostvoreni dentin u vidu dentinskih ostrvaca teži da zatvori perforaciju cavuma dentis. Vidi se potpuno očuvano pulpno tkivo bez zapaljenske reakcije (HE, 40×).

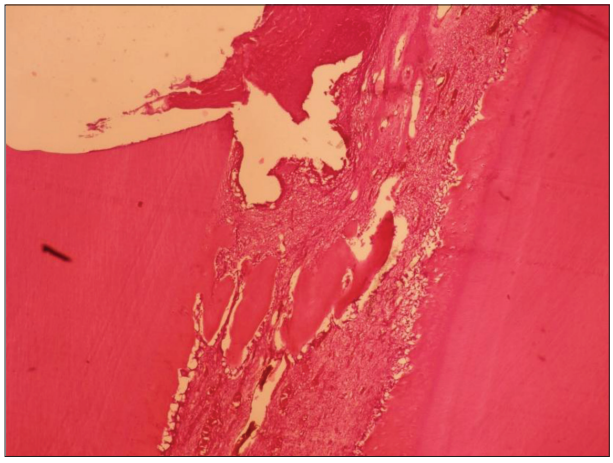

Figure 4. Dentin islets after direct pulp capping with HA-CS. Three dentin islets that almost completely closed cavum dentis and signs of neoangiogenesis in the form of newly created blood vessels can be seen (HE, 40×).
Slika 4. Dentinska ostrvca nakon direktnog prekrivanja pulpe materijalom HA-CS. Na slici se mogu uočiti tri dentinska ostrvca koja skoro u potpunosti zatvaraju perforaciju cavuma dentis i znaci neoangiogeneze u vidu novostvorenih krvnih sudova (HE, 40×).

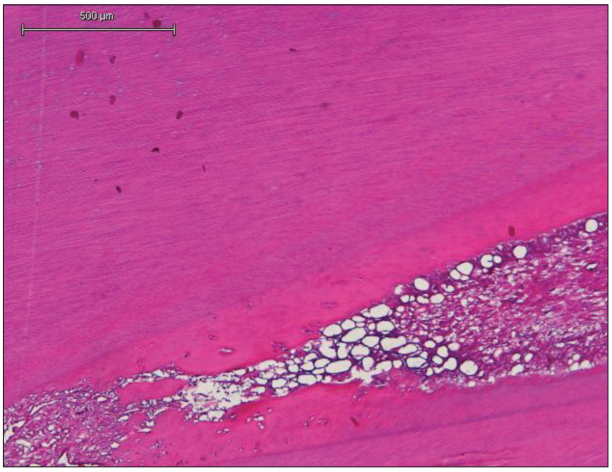

Figure 5. Lateral dentin bridge after direct pulp capping with HA-CS. Newly laterally deposited dentin that narrows pulpal space and mild inflammatory reaction with the presence of inflammatory cells can be found in the area of the perforation only (HE, 40×).
Slika 5. Lateralni dentinski most nakon direktnog prekrivanja pulpe materijalom HA-CS. Na slici se može videti novostvoreni dentin lateralno deponovan koji sužava pulpni prostor. Blaga inflamatorna reakcija sa prisutnim ćelijama zapaljenja samo u predelu perforacije (HE, 40×).

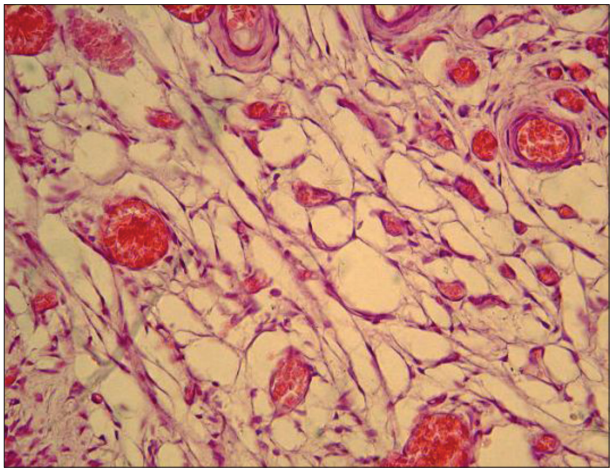

Figure 6. Signs of neoangiogenesis and stasis in dental pulp. This finding was probably related to the chemical properties of the capping material and mechanical trauma during cavity preparation, rather than the presence of bacterial infection (HE, 400×).
Slika 6. Znaci neoangiogeneze i staze u pulpnom tkivu. Ovakav nalaz je verovatno posledica hemijskog sastava materijala za direktno prekrivanje i mehaničke traume tokom preparacije, pre nego posledica prisustva bakterija (HE, 400×).

regular dentinal tubules that were in continuity with surrounding dentin. Complete dentin bridge that closed pulp perforation was noted in 6 cases with HA-CS (Figure 1). Complete dentin bridge was noted in 4 cases with CS and 4 teeth with control material (MTA) (Figure 2). Odontoblasts like cells associated with newly formed dentin were found below complete dentin bridge. The original odontoblasts were positioned peripherally. They were identified through their regular palisade arrangement, eosinophilic cytoplasm and basal nucleus alignment. Incomplete dentin bridge in the form of dentin islets was observed in 5 teeth in the group of CS (Figure 3) and 3 teeth in the group with HA-CS (Figure 4). In the control group dentin islets were noted in 6 teeth. Continuous reparative dentin that extends along the lateral walls of dentin was recorded in 1 case in the group of CS and 1 case in the group of HA-CS (Figure 5), while this form of dentin was not registered in the samples with control material (MTA).

Fully preserved pulp tissue was observed in only one case in each experimental group and two cases with MTA (Figure 3). Pulp disorganization characterized by the appearance of odontoblast like cells and their hyperactivity was observed in most samples (9 teeth in each experimental group and 8 teeth in the control group), where in the central part of the pulp the presence of venous stasis, hemorrhage and inflammation was observed (Figure 5). In most of these cases neoangiogenesis with proliferation of existing and creation of new blood vessels was observed indicating healing process and complete revascularization (Figure 6). Complete disorganization of the pulp tissue was not registered in the samples of either experimental or control groups. Necrosis was not observed in any case.

Histological analysis after 4 weeks revealed that the experimental pulp capping materials in most cases caused mild to moderate chronic inflammation of pulp tissue (Figure 5). Severe inflammation or abscesses were not observed in any sample. Mild inflammation was present in 8 samples in the group with CS and 7 samples in the group with HA-CS and MTA (Figure 5). Moderate inflammation with cellular proliferation present both in coronal and radicular pulp was observed in 2 teeth of the CS group and 1 tooth in the HA-CS group. Similar number of inflammatory cells was registered in 3 samples after direct pulp capping with the control material (MTA).

Gram staining did not detect any gram-positive bacteria in the pulp of all samples. Small number of bacteria in dentinal tubules was observed in 4 teeth with CS, 3 teeth with HA-CS, while bacteria in dentin tubules were registered in 3 samples with MTA.

DISCUSSION

The current experimental study was performed on permanent teeth of Vietnamese pigs that are by their morphology very similar to human teeth. Previous studies with new materials were conducted on teeth in dogs [17, 27], deciduous and permanent teeth of pigs [25, 28] as well as monkeys [29]. An important advantage in working with animals is that experiment can be carried out on large number of teeth for the same time period and the effect of various materials can be checked at the same time.

Results of the current study showed similar findings in the control and experimental groups. The process of reparative dentinogenesis and complete or partial closure of perforations with dentin bridge was considered good therapeutic result. Dentin bridge was observed in all teeth where perforations were capped using new synthesized nanostructural materials based on calcium silicate systems and hydroxyapatite. Similar results were confirmed by the study of Laurent et al. [30], where favorable therapeutic effect of calcium silicate systems was explained

by significant release of TGF-β1 from the pulp cells that stimulated odontoblasts to increase their activity and activate reparative dentinogenesis.

Results of the current study where the pulp was capped with MTA also showed the presence of dentin bridge in all samples, which is consistent with similar experimental study in pigs done by Shayegana et al. [25].

In most teeth of both groups, odontoblasts were observed just below dentin bridge with major or minor structural changes that ranged from mild to complete disorganization. It is likely that these cells are not true odontoblasts, but odontoblast like cells (although for definitive identification additional immunohistochemical analysis is required). These cells, similarly to true odontoblasts have elongated shape, palisade orientation and basal nucleus alignment [31]. They have ability to produce extracellular matrix that after mineralization becomes complete or incomplete dentin bridge or islands that tend to establish contact with side dentin walls to close and preserve exposed pulp.

In most samples in the MTA group reorganization of the tissue below the perforation was observed (hyperactivity of odontoblast like cells and altered cell morphology compared to odontoblasts). This is also confirmed by the study of Tziafas et al. [17] performed on dogs. Correlation between the number of odontoblast like cells, the thickness of the bridge and preservation of deeper parts of the pulp was found. With increased number of these cells, the thickness of dentin bridge is increasing and radicular part of the pulp remains vital [32]. In the experimental groups and in the group with MTA necrosis was not observed in any sample. In the experimental study of Tabarsi et al. [27] on dogs after direct capping with MTA necrosis was present in 22.7% of samples. Different findings of these two studies can be explained by the fact that in the study of Tabarsi et al. MTA was placed after pulpotomy was performed whereas in the current study only small exposed surface of the pulp was covered with MTA.

In the most teeth of both experimental groups mild inflammation was observed, suggesting biocompatibility of the materials [33]. Acute inflammation and necrosis of the pulp was not observed in any sample. Good marginal seal achieved with glass ionomer cements and aseptic conditions as well as good immune status of experimental animals can explain this.

The results of our study demonstrated the presence of inflammatory cells in the coronal and radicular part of the pulp. In the control group where pulp was capped with MTA only few samples showed the presence of lymphocytes, plasma cells and macrophages, which is consistent with the findings of other authors [25, 27].

Therapeutic effects were similar in the experimental and control groups, indicating that new nanostructured materials based on calcium silicate cements and hydroxyapatite have favorable effects on reparative activities of the pulp primarily due to their physical and chemical properties.

After application of new materials (CS and HA-CS) and MTA neoangiogenesis was observed, indicating regenerative processes in the pulp and successful tissue remodeling. Similar results obtained by both tested materials can be explained by similar chemical composition (dicalcium and tricalcium silicate make the most of the material). On the other hand Murray et al. suggested that for dentinogenesis the most important is preservation of pulp and odontoblasts, absence of infection and necrosis but not the type of material [31].

CONCLUSION

Reparation of pulp exposure was successful in the experimental and control groups. In most teeth reparative dentinogenesis resulted in dentin bridge formation and preservation of functional and morphological integrity of the pulp. Histological analysis indicated favorable therapeutic effects of new nanostructured materials based on active calcium silicate systems and hydroxyapatite that were similar to MTA after direct pulp capping of pulp in Vietnamese pigs.

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Direktno prekrivanje pulpe novim nanostrukturnim materijalima na bazi kalcijum-silikatnih sistema i hidroksiapatita

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KRATAK SADRŽAJ

Uvod Direktno prekrivanje pulpe je važan terapijski postupak koji ima zadatak da obezbedi zatvaranje pulpne komore i omogući proces zarastanja. Cilj ovog rada je bio da se ispita efekat novih nanostrukturnih materijala na bazi kalcijum-silikatnih sistema i hidroksiapatita na eksponiranu pulpu zuba vijetnamskih svinja.

Materijal i metode rada Istraživanje je sprovedeno na 30 zuba kod dve vijetnamske svinje (Sus scrofa verus). Na vestibularnim površinama sekutića, očnjaka i prvih premolara urađene su preparacije kaviteta V klase, pri čemu je malim okruglim svrdlom napravljena namerna eksploracija komore pulpe. U prvoj eksperimentalnoj grupi (10 zuba) perforacija je prekrivana novim nanostrukturnim materijalom na bazi kalcijum-silikatnih sistema (CS). U drugoj eksperimentalnoj grupi (10 zuba) perforacija je prekrivana smešom kalcijum-silikatnog sistema i hidroksiapatita (HA-CS). U kontrolnoj grupi (10 zuba) perforacija je prekrivana sa MTA® (Dentsply Tulsa Dental, Džonson Siti, Tenesi, SAD). Svi kaviteti su restaurirani glas-jonomer cementom (GC Fuji VIII, GC Corporation, Tokio, Japan). Opservacioni period iznosio je 28 dana. Nakon žrtvovanja životinja napravljeni su histološki preparati na kojima su analizirani postojanje dentinskog mostića, inflamatorna reakcija pulpe, reorganizacija pulpnog tkiva i prisustvo bakterija.

Rezultati Na svim zubima eksperimentalnih grupa i kontrolne grupe zabeleženo je stvaranje dentinskog mostića. Zapaljenje pulpe je bilo blago do umereno i u eksperimentalnim i u kontrolnoj grupi. Uočeni su znaci neoangiogeneze i mnoštvo ćelija sličnih odontoblastima koje su odgovorne za stvaranje dentinskog mostića. Nekroza nije zabeležena ni u jednom slučaju, kao ni prisustvo gram-pozitivnih bakterija u pulpi.

Zaključak Histološka analiza je ukazala na povoljne terapijske efekte novih nanostrukturnih materijala na bazi kalcijum-silikatnih sistema i hidroksiapatita u direktnom prekrivanju pulpe zuba vijetnamskih svinja.

Ključne reči: direktno prekrivanje pulpe; kalcijum-silikati; hidroksiapatit; MTA; dentinski mostić

UVOD

Direktno prekrivanje pulpe i očuvanje vitaliteta pulpe je izuzetno značajan terapijski postupak, posebno kod karijesnih zuba mladih osoba i zuba s komplikovanim višekanalnim sistemima [1, 2]. Brojna istraživanja su potvrdila da kalcijum-hidroksid predstavlja zlatni standard u direktnom prekrivanju pulpe još od momenta njegovog uvođenja u stomatološku praksu 1920. godine [2]. Za to su odgovorni visoka pH vrednost ovog preparata i stimulativni efekat na odontoblaste, koji dovodi do stvaranja tercijarnog dentina i očuvanja vitaliteta pulpe. Ipak, procenat uspešnosti primene kalcijum-hidroksida u publikovanim radovima je neujednačen – od 31% do 100% [3–6]. Zbog neodgovarajuće veze kalcijum-hidroksida sa eksponiranom pulpom, degradacije tokom vremena, poroznosti novostvorenog dentinskog mostića i pojave internih resorpcija, postoji potreba za pronalaženjem efikasnijih materijala [3, 7, 8].

Poslednjih dvadesetak godina velika pažnja se poklanja mineral-trioksid agregatu ProRoot MTA® (Dentsply Tulsa Dental, Džonson Siti, Tenesi, SAD), čija je jedna od indikacija i direktno prekrivanje pulpe, jer dovodi do stvaranja kompletnog dentinskog mostića i bez znakova inflamacije pulpe [9, 10]. Mnoge studije su potvrdile njegovu biokompatibilnost, antimikrobni efekat, dobro zaptivanje i dobre fizičke i hemijske osobine [11, 12]. Izuzetno je hidrofilan i zbog toga se može koristiti i u vlažnim uslovima i uz prisustvo krvi. Potvrđeno je takođe da je debljina novostvorenog dentinskog mostića veća posle prekrivanja pulpe sa MTA nego pri korišćenju kalcijum-hidroksida [13, 14, 15]. MTA pokazuje odlične osobine kao materijal za direktno prekrivanje zubne pulpe i uspešno zamenjuje kalcijum-hidroksid, jer ne prouzrokuje lokalnu nekrozu pulpe, dok se hronično zapaljenje zubne pulpe ređe pojavljuje [15]. MTA izaziva funkcionalne i citološke promene u ćelijama pulpe, kao i njihovu transformaciju u ćelije slične odontoblastima, koje izgrađuju fibrodentin, odnosno reparativni dentin na površini eksponirane pulpe [16, 17]. Nedostaci su mu nestabilnost praha neposredno nakon otvaranja, visoka cena na tržištu i dugo vreme vezivanja. Iako proizvođač navodi vreme vezivanja od 10 minuta, studija Torabinedžada (Torabinejad) i saradnika [18] utvrdila je da je vreme vezivanja između dva i četiri sata. Aktuelna istraživanja su usmerena ka pronalaženju materijala za direktno prekrivanje pulpe zuba koji bi zadržao sve dobre osobine MTA, ali i prevazišao njegove mane.

Hidroksiapatit je materijal na bazi kalcijum-fosfata koji se veoma često koristi u medicini i stomatologiji. Biokompatibilnost hidroksiapatita je usko povezana sa njegovim hemijskim sastavom koji je veoma sličan zubnim i koštanim tkivima. Ipak, loše mehaničke osobine hidroksiapatita onemogućavaju njegovu primenu u endodonciji. Skorašnje studije su se bavile ispitivanjima novih formulacija kalcijum-fosfata, koje su ukazale na povoljne mehaničke i biološke osobine ovih materijala [19, 20, 21, 22, 23].

Poslednjih godina na Institutu za nuklearna istraživanja u Vinči su sintetisana dva nova eksperimentalna materijala na bazi kalcijum-silikata po originalnoj recepturi V. Jokanovića i saradnika. Prvi kalcijum-silikatni sistem predstavljao je kombi-

naciju trikalcijum i dikalcijum silikata (CS), a drugi smešu CS i hidroksiapatita u odnosu 1:2 (HA-CS). Oba materijala su u svom sastavu imala barijum-sulfat kao rendgen-kontrastno sredstvo.

Radi se o materijalima koji su sintetisani kombinacijom dve tehnike: hidrotermalnom sol–gel metodom i metodom samosagorevajućih talasa. Na taj način dobijeni su materijali koji u svom sastavu imaju tri veličine čestica: aglomerati (veličine nekoliko mikrometara), partikule (veličine 117–447 nanometara) i kristaliti (veličine svega 20 nanometara) [24]. Čestice nanometarskih dimenzija omogućile su veću aktivnost čestica, a samim tim i bolju hidrataciju cementa i kraće vreme vezivanja (CS – 10 min., HA-CS – 15 min.) [24].

Cilj ovog rada je bio da se proveri efekat novih materijala na bazi aktivnih kalcijum-silikatnih sistema i hidroksiapatita (CS i HA-CS) nakon prekrivanja eksponirane pulpe zuba vijetnamskih svinja.

MATERIJAL I METODE RADA

Eksperimentalno istraživanje obavljeno je na Fakultetu veterinarske medicine Univerziteta u Beogradu uz saglasnost Etičkog komiteta Stomatološkog fakulteta Univerziteta u Beogradu. U eksperiment je bilo uključeno 30 zuba kod dve vijetnamske svinje (*Sus scrofa verus*) starosti 24 meseca i telesne mase 25 kg. Tokom rada poštovan je protokol Evropske dobre laboratorijske prakse (86/609/EEC), koji podrazumeva primenu glavnih principa asepse i antisepse – realizaciju eksperimenta u minimalnom potrebnom vremenu bez fizičkog i duševnog bola životinja (Međunarodna organizacija za standardizaciju, 1997). Kao kontrolni materijal korišćen je ProRoot MTA® (Dentsply Tulsa Dental, Džonson Siti, Tenesi, SAD), čiji su efekti na pulpu već poznati.

Eksperimentalni postupak

Kod životinja je izvršena premedikacija atropinom u dozi 0,03–0,04 mg/kg i.m., a nakon 15 minuta životinje su uvedene u opštu anesteziju davanjem ksilazina u dozi 1,5–2 mg/kg i.m. i ketamina u dozi 20–25 mg/kg i.m. Nakon anesteziranja i postavljanja koferdam-gume radi izolacije, zubi su očišćeni sedamdesetoprocentnim etanolom.

Na vestibularnim površinama sekutića, očnjaka i prvih premolara okruglim karbidnim borerom urađena je preparacija kaviteta V klase uz stalno hlađenje fiziološkim rastvorom. Potom je malim okruglim borerom eksponirana komora pulpe, a krvarenje je kontrolisano sterilnim kuglicama vate. Zatim je na perforaciju aplikovan prethodno pripremljen materijal. Na zubima desnog kvadranta gornje vilice kod obe vijetnamske svinje (ukupno šest sekutića, dva očnjaka i dva premolara) postavljen je CS. Na istom broju zuba u levom kvadrantu gornje vilice obe vijetnamske svinje postavljen je HA-CS. MTA je kao kontrolni materijal postavljen na istom broju zuba u donjoj vilici sa desne strane kod obe vijetnamske svinje (šest sekutića, dve očnjaka i dva premolara). Svi kaviteti su restaurirani glasjonomer-cementom (GC Fuji VIII, GC Corporation, Tokio, Japan). Opservacioni period je iznosio 28 dana. Posle prestanka dejstva anestezije životinjama je dat analgetik butorfanol u dozi 0,1–0,2 mg/kg, a nakon oporavka životinje su čuvane i gajene u individualnim kavezima u farmskim uslovima.

Posle četiri nedelje životinje su žrtvovane uvođenjem u opštu anesteziju i davanjem pentobarboton-natrijuma i.v. u dozi od 100 mg/kg. Vilice su isečene na blok-sekcije i tkivo je fiksirano i pripremano za mikroskopsku analizu.

Histološki postupak

Tkivo za histološku analizu uzeto je u bloku i svaki uzorak je sadržao eksperimentalni zub i okolnu kost. Uzorci su prikupljeni poštujući ISO kriterijume (Technical Report 7405) 28 dana nakon eksponiranja pulpe i direktnog prekrivanja. Materijal za histološku analizu je fiksiran u desetoprocentnom formalinu, dekalcifikovan u desetoprocentnoj mravljoj kiselini (pH = 5) i kalupljen u parafinu. Na staklenim pločicama su napravljene serijske sekcije u meziodistalnom smeru debljine 4 μm. Preparati su bojeni hematoksilin-eozinom metodom po Goldneru (Goldner) i metodom po Gramu (Gram) zbog mikroskopske identifikacije bakterija. Materijal je analiziran pod svetlosnim mikroskopom.

Histološki kriterijumi za procenu reakcije pulpe su korišćeni u skladu s metodologijom Šajegana (Shayegan) i saradnika [25]. Analizirani su dentinski mostić (A) (debljina, lokalizacija, struktura, kontinuitet s okolnim dentinom), morfološka reorganizacija ćelija pulpe (B) (hronična ili akutna, intenzitet i lokalizacija zapaljenja) inflamatorna reakcija pulpe (C) i prisustvo bakterija (D). Ovi parametri praćeni su zahvaljujući Međunarodnoj organizaciji za standardizaciju (International Organization for Standardization) i objavljenim kriterijumima Mjora (Mjör) [26] iz 1983. godine.

REZULTATI

Rezultati histoloških analiza su pokazali da je dentinski mostić stvoren kod svih uzoraka i u eksperimentalnim i u kontrolnoj grupi (Tabela 1). Novostvoreni dentin imao je odlike reparativnog dentina sa pravilno postavljenim dentinskim kanalićima ili s malim brojem nepravilnih dentinskih kanalića, koji su bili u kontinuitetu s okolnim dentinom. Kompletan dentinski mostić, koji je potpuno zatvarao pulpni prostor u predelu perforacije, zabeležen je u šest uzorka na kojima je primenjen HA-CS (Slika 1). U četiri uzorka nakon primene CS i istom broju uzoraka kontrolne grupe uočeno je takođe prisustvo kompletnog dentinskog mostića (Slika 2). Ispod novostvorenog dentina zabeležene su ćelije slične odontoblastima, koje su u vezi s novonastalim tubularnim dentinom. Originalni odontoblasti su bili pozicionirani periferno. Oni su prepoznati zahvaljujući njihovom regularnom palisadnom rasporedu, eozinofilnoj citoplazmi i bazalno postavljenim jedrom. Nepotpun dentinski mostić u vidu dentinskih ostrvaca uočen je kod pet zuba u grupi u kojoj je primenjen CS (Slika 3) i kod tri zuba u grupi gde je primenjen HA-CS (Slika 4). U kontrolnoj grupi dentinska ostrvca su bila zabeležena u šest uzoraka. Kontinuiran reparativni dentin koji se prostire duž lateralnih zidova dentina uočen je u po jednom uzorku eksperimentalnih grupa (Slika 5), a takav vid dentina nije registrovan u uzorcima kontrolne grupe (MTA).

Potpuno očuvano pulpno tkivo zabeleženo je samo u po jednom uzorku eksperimentalnih grupa i u dva uzorka kontrolne grupe (Slika 3). Dezorganizacija pulpnog tkiva u vidu pojave ćelija sličnih odontoblastima i njihove hiperaktivnosti uočena je kod najvećeg broja uzoraka (po devet zuba u eksperimentalnim i osam u kontrolnoj grupi), kod kojih su u centralnom delu pulpe uočeni venska staza, krvarenje i zapaljenje (Slika 5). U većini ovih uzoraka uočeni su znaci neoangiogeneze s proliferacijom postojećih i stvaranjem novih krvnih sudova, što je ukazivalo na proces zarastanja i potpunu revaskularizaciju (Slika 6). Potpuna dezorganizacija pulpnog tkiva i nekroza nisu zabeležene ni u jednom uzorku kako eksperimentalnih grupa tako i kontrolne grupe.

Rezultati histoloških analiza nakon četiri nedelje pokazali su da je posle prekrivanja pulpe eksperimentalnim materijalima u najvećem broju uzoraka zabeleženo blago ili umereno hronično zapaljenje (Slika 5). Izrazita upala sa mnoštvom ćelija zapaljenja i pojava apscesa nisu zabeleženi ni u jednom uzorku. Blago zapaljenje je ustanovljeno u osam uzoraka nakon prekrivanja zubne pulpe materijalom CS i u po sedam uzoraka nakon primene HA-CS i MTA (Slika 5). Umereno zapaljenje kod kojeg ćelijska infiltracija zahvata koronarnu i deo radiksne pulpe uočeno je kod dva zuba iz grupe u kojoj je primenjen CS i kod jednog zuba nakon prekrivanja sa HA-CS. Sličan broj ćelija zapaljenja je registrovan kod tri uzorka nakon direktnog prekrivanja pulpe kontrolnim materijalom (MTA).

Bojenjem preparata po Gramu uočen je potpuni izostanak gram-pozitivnih bakterija u pulpi svih uzoraka. Mali broj bakterija u dentinskim kanalićima zabeležen je kod četiri zuba nakon primene CS, pet zuba nakon primene HA-CS, dok su u kontrolnoj grupi bakterije u dentinskim kanalićima uočene u tri uzorka.

DISKUSIJA

Ovo eksperimentalno istraživanje je realizovano na stalnim zubima vijetnamskih svinja koji su po svojoj morfologiji veoma slični humanim zubima. Dosadašnje studije sa novim materijalima vršene su na zubima pasa [17, 27], mlečnim i stalnim zubima svinja [25, 28], odnosno na zubima majmuna [29]. Značajna prednost u radu s eksperimentalnim životinjama je u tome što se eksperiment može realizovati na velikom broju zuba i u istom vremenskom intervalu može proveravati efekat različitih materijala.

Rezultati ove studije su pokazali slične rezultate na zubima u kontrolnoj i eksperimentalnoj grupi. Proces reparativne dentinogeneze, tj. potpuno ili delimično zatvaranje perforacione rane dentinskim mostićem, u ovom istraživanju smatrao se dobrim terapijskim rezultatom. Kod svih zuba kod kojih je pulpa prekrivena novosintetisanim nanostrukturnim materijalima na bazi kalcijum-silikatnih sistema i hidroksiapatita uočen je dentinski mostić. Slične rezultate potvrdila je i studija Lorena (Laurent) i saradnika [30], gde autori povoljan terapijski efekat kalcijum-silikatnih sistema objašnjavaju značajnim povećavanjem oslobađanja TGF-β1 iz ćelija pulpe, koji deluje stimulativno na odontoblaste i pojačava njihovu sekretornu aktivnost, odnosno reparativnu dentinogenezu.

U kontrolnoj grupi, u kojoj je direktno na pulpu aplikovan MTA, takođe je uočen dentinski mostić u svim uzorcima, što je u saglasnosti sa sličnom eksperimentalnom studijom na svinjama Šajegana i saradnika [25].

U najvećem broju zuba obe grupe na mestu eksponirane pulpe, ispod novostvorenog dentinskog mostića, uočeni su odontoblasti s manjim ili većim strukturnim promenama, koje su varirale od vrlo blagih do potpune dezorganizacije. Verovatno je da to zapravo i nisu odontoblasti, već ćelije slične odontoblastima (iako su za njihovu konačnu identifikaciju potrebne dodatne imunohistohemijske analize). One, kao i pravi odontoblasti, imaju izdužen oblik, palisadnu orijentaciju i bazalno postavljena jedra [31]. Imaju sposobnost stvaranja i lučenja vanćelijskog matriksa, čijom mineralizacijom nastaje reparativni dentin u vidu potpunog ili nepotpunog dentinskog mostića, odnosno ostrvaca koja teže da uspostave kontakt sa bočnim zidovima dentina i tako zatvore i sačuvaju eksponiranu pulpu.

U najvećem broju uzoraka iz grupe MTA primećena je reorganizacija tkiva ispod perforacije u vidu hiperaktivnosti ćelija sličnih odontoblastima i izmenjene morfologije ćelija u odnosu na odontoblaste. Ovo potvrđuju i rezultati studije na psima Cjafasa (Tziafas) i saradnika [17]. Uočena je i povezanost između broja ćelija sličnih odontoblastima, debljine mostića i očuvanosti dubljeg dela tkiva pulpe. S povećanjem broja ovih ćelija raste debljina dentinskog mostića, pri čemu pulpa u radiksnom delu zadržava svoj fiziološki izgled [32]. U grupi zuba na kojima su primenjeni CS i HA-CS kao i kontrolni materijal MTA nekroza nije uočena ni u jednom uzorku. U eksperimentalnoj studiji na psima Tabarsija (Tabarsi) i saradnika [27], posle direktnog prekrivanja pulpe, nekroza je uočena u 22,7% uzoraka. Drugačiji nalazi se mogu objasniti činjenicom da je u njihovoj studiji urađen postupak pulpotomije i postavljen MTA, a ne direktno prekrivanje manje površine eksponirane pulpe kao u našoj studiji.

Primenom eksperimentalnih materijala kod najvećeg broja zuba utvrđeno je blago zapaljenje, što govori u prilog biokompatibilnosti materijala [33]. Akutno zapaljenje i nekroza pulpe takođe nisu zapaženi ni u jednom ispitivanom uzorku. To se može objasniti dobrim rubnim zatvaranjem kaviteta glasjonomer-cementom i aseptičnim uslovima rada, ali i dobrim imunološkim stanjem eksperimentalnih životinja.

Rezultati naše eksperimentalne studije su otkrili ćelije zapaljenja i u koronarnom i u radiksnom delu pulpe. Kod uzoraka kontrolne grupe u kojoj je primenjen MTA u samo nekoliko uzoraka uočeni su limfociti, plazmociti i makrofagi, što je u saglasnosti s nalazima drugih autora [25, 27].

Budući da je terapijski efekat bio vrlo sličan u eksperimentalnim i u kontrolnoj grupi, to ukazuje na činjenicu da i novi nanostrukturni materijali na bazi kalcijum-silikatnih sistema i hidroksiapatita imaju povoljno dejstvo na reparatorne aktivnosti pulpe zuba vijetnamskih svinja zahvaljujući, pre svega, svojim fizičkim i hemijskim osobinama.

Nakon primene novih materijala (CS i HA-CS) i MTA došlo je i do neoangiogeneze u pulpi, što ukazuje na regenerativne procese u pulpi i uspešnu remodelaciju tkiva. Slični rezultati dobijeni primenom ovih materijala mogu se objasniti sličnim hemijskim sastavom, jer oba materijala u najvećem procentu sadrže dikalcijum i trikalcijum-silikat. Naravno, o ovome ima i drugačijih mišljenja. Tako Mari (Murray) i saradnici [31] smatraju da su za započinjanje procesa dentinogeneze najvažniji očuvanost pulpe i odontoblasta, te nepostojanje infekcije i nekroze, a ne vrsta materijala.

ZAKLJUČAK

Reparacija veštački izazvanih oštećenja pulpe zuba eksperimentalnih životinja u eksperimentalnoj i u kontrolnoj grupi bila je vrlo efikasna. Kod većine zuba proces reparativne dentinogeneze je praćen stvaranjem dentinskog mostića i očuvanjem funkcionalnog i morfološkog integriteta pulpe. Histološka analiza je ukazala na povoljne terapijske efekte novih nanostrukturnih materijala na bazi aktivnih kalcijum-silikatnih sistema i hidroksiapatita u direktnom prekrivanju pulpe zuba vijetnamskih svinja. Reakcija pulpe bila je slična onima koje je izazvao MTA.

Da li ste pažljivo čitali radove?

1. Direktno prekrivanje pulpe zuba vijetnamskih svinja je proveravano:
   a) materijalima na bazi kalcijum-hidroksida
   b) materijalima na bazi kalcijum-silikata
   c) materijalima na bazi kalcijum-aluminata

2. Na zarastanje koštanih defekata kod eksperimentalnih životinja proveravan je:
   a) materijal za opturaciju
   b) materijal za irigaciju
   c) materijal za restauraciju

3. Serotipizacija je proveravana kod bakterija:
   a) *Lactobacillusa*
   b) *Enterococcus faecalisa*
   c) *Agregatibacter actinomycetem comitons*

4. Primena parametra anfasa nakon ortodontske terapije je korišćena kod:
   a) milokluzija I klase
   b) milokluzija II klase
   c) milokluzija II klase II odeljenje

5. Direktno prekrivanje nanomaterijalima je proveravano na zubima:
   a) pacova
   b) kunića
   c) svinja

6. Za proveru bioloških efekata materijala za opturaciju najčešće se koriste:
   a) materijali na bazi smola
   b) materijali na bazi cink-oksida
   c) materijali na bazi glas-jonomer cementa

7. Analizom rezultata na osnovu fotografija pre i posle terapije uočena je promena atropometrijskih parametara:
   a) srednje i gornje trećine lica
   b) gornje i donje trećine lica
   c) srednje i donje trećine lica

8. Direktno prekrivanje pulpe zuba je realizovano na:
   a) 30 zuba
   b) 40 zuba
   c) 50 zuba

9. Histološki odgovor koštanog tkiva na implantaciju materijala proveravan je na uzorku od:
   a) 30 pacova
   b) 20 pacova
   c) 16 pacova

10. Primena parametara anfasa nakon ortodontske terapije milokluzija II klase je uključila fotografije anfasa:
    a) 50 pacijenata
    b) 40 pacijenata
    c) 30 pacijenata

11. Kontrolnu grupu kod direktnog prekrivanja pulpe zuba vijetnamskih svinja činio je materijal:
    a) kalcijum-hidroksid
    b) kalcijum-aluminat
    c) MTA

12. Kliničko ispitivanje i zastupljenost bakterija AA je obuhvatilo:
    a) 29 pacijenata
    b) 39 pacijenata
    c) 49 pacijenata

13. Kod ortodontske terapije pacijenata korišćena je fotografija anfasa nakon ekstrakcije zuba:
    a) fiksnim HERBST aparatom
    b) fiksnim aparatom sa intermaksilnim gumicama
    c) mobilnim ortodontskim aparatom

14. Nekroza zubne pulpe nakon direktnog prekrivanja pulpe zuba vijetnamskih svinja je uočena u:
    a) dva slučaja
    b) u tri slučaja
    c) ni u jednom slučaju

15. Brisevi za postupak serotipizacije su uzimani
    a) pre terapije dubokog karijesa
    b) posle preparacije kaviteta
    c) posle završene terapije dubokog karijesa

16. Toksičnost materjala na bazi cink-oksida se najčešće pripisuje:
    a) eugenolu
    b) prahu cink-oksida
    c) prahu magnezijum-oksida

17. Uzeti brisevi za analizu serotipizacije su čuvani na temperaturi:
    a) -60°C
    b) -80°C
    c) -100°C

18. Za analizu na fotografijama su iscrtavane:
    a) mekotkivne tačke
    b) tačke na kostima
    c) tačke van fotografija

19. U kost donje vilice je implantiran:
    a) endometazon
    b) AH plus
    c) apeksit

20. Serotipovi kod bakterije AA su registrovani u:
    a) tri uzorka
    b) pet uzoraka
    c) osam uzoraka

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http://www.nlm.nih.gov/databases/databases_medline.html

OPERATIVE DENTISTRY
http://www.jopdent.org/journal/journal.php

JOURNAL OF CONTEMPORARY DENTAL PRACTICE
http://thejcdp.com/

JOURNAL OF THE AMERICAN DENTAL ASSOCIATION (JADA)
http://jada.ada.org/

BRITISH DENTAL JOURNAL
http://www.nature.com/bdj/index.html

JOURNAL OF DENTAL RESEARCH
http://www.iadr.com/

CANADIAN MEDICAL ASSOCIATION JOURNAL (CMAJ)
http://www.cmaj.ca/

JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION (JAMA)
http://jama.ama-assn.org/

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http://www.cda-adc.ca/jcda/

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