UDC: 6 ; 616.314.18-085 ; 615.46:616.314-7 DOI: 10.2298/SGS1402065P

Direct Pulp Capping Using Biodentine

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SUMMARY

Introduction Direct pulp capping is therapeutic method of applying medication on exposed pulp in order to allow bridge formation and healing process. The aim of this study was to investigate the effect of Biodentine on exposed dental pulp of Vietnamese pigs.

Material and Methods The study was conducted on 20 teeth of Vietnamese pigs (Sus scrofa domesticus). On buccal surfaces of incisors, canines and first premolars, class V cavities were prepared and pulp was exposed. In the experimental group (six incisors, two canines and two premolars) the perforation was covered with Biodentine® (Septodont, Saint-Maur-des-Fosses, France). In the control group, the perforation was covered with MTA® (Dentsply Tulsa Dental, Johnson City, TN, USA). 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, an 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 both 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 Biodentine for direct pulp capping in teeth of Vietnamese pigs. Findings were similar with Biodentine and MTA.

Keywords: direct pulp capping; Biodentine; MTA; dentin bridge

INTRODUCTION

Direct pulp capping is therapeutic method of placing a medication on exposed pulp to induce formation of dentin bridge and healing. Preservation of pulp vitality is very important process, especially in young patients and in teeth with complicated 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 with 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 was shown to induce complete dentin bridge formation with no signs of pulp inflammation [9, 10]. MTA consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium sulfate dihydrate, and bismuth oxide. 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 was also confirmed that the thickness of dentinal bridge increased after pulp capping using MTA as compared to calcium hydroxide [13, 14, 15]. MTA shows excellent properties as material for direct pulp capping and successfully replaces calcium hydroxide because it does not cause local necrosis of the pulp, while chronic inflammation of the pulp rarely occurs [15]. MTA causes functional and cytological changes in pulp cells as well as their transformation to odontoblast like cells which produce reparative dentin on the surface of the pulp [16, 17]. However, MTA has inferior antibacterial effect than calcium hydroxide, it is non stable as powder immediately after package opening, other disadvantages include high price on the market and long setting time: setting time of 10 minutes is specified by the manufacturer, a study of Torabinejad et al. [18] found setting time between 2 and 4 hours.

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

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Biodentine® (Septodont, Saint-Maur-des-Fosses, France) is a new generation material based on calcium silicate synthesized by bioactive technology. It is indicated as filling in all cases of damaged dentin, in the coronal part of root dentin, but can be also used for direct pulp capping. It consists of powder (tricalcium silicate, calcium carbonate and zinc oxide) and liquid (water, calcium chloride, modified polycarboxylate) and has setting time of 12 minutes [19]. Biodentine stimulates release of TGF-β from pulpal cells, stimulating reparative dentin formation in a very short period of time. Using extracted human wisdom teeth, direct pulp capping using Biodentine showed good results in healing and pulp regeneration [20]. Experimental study of Tran et al. [21] in rats showed similar effect of Biodentine and MTA in direct pulp capping in terms of creating dentin bridge (its thickness, continuity, porosity), but significantly inferior performance was achieved with calcium hydroxide. Recent studies have also demonstrated favorable therapeutic results of Biodentine and MTA in direct pulp capping of primary teeth of pigs [22]. Despite the fact that this new cement has already been used in dentistry as restorative material, its effect on the pulp tissue of humans has not yet been demonstrated [22].

The aim of this study was to examine the effect of Biodentine in direct pulp capping of exposed pulp in Vietnamese pigs (*Sus scrofa domesticus*).

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 20 teeth of Vietnamese pigs (*Sus scrofa domesticus*), aged 24 months and weight about 25 kg. The study procedure complied with the protocol of the European Good Laboratory Practice (86/609/EEC), which 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 at 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 according to the manufacturer's instructions and applied on the perforation. Teeth in the right quadrants of upper and lower jaw (6 incisors, two canines and 2 premolars) received Biodentine. The same number of teeth in left quadrants of upper and lower jaw received MTA. All cavities were restored with glass ionomer cement (GC Fuji VIII, GC Corporation, Tokyo, Japan). Observational period was 28 days.

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. After 4 weeks animals were sacrificed after introducing general anesthesia and iv 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% of formalin, decalcified in 10% of 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. [24]. Formation of dentin bridge (A), morphological reorganization of pulp cells (B), inflammatory reaction of pulp (C) and presence of bacteria (D) were analyzed using the following scale:

A) Dentin bridge (thickness, localization, structure, continuity with surrounding dentin):

0 – no dentin bridge

1 – incomplete presence of dentin bridge in the area of exposed pulp

2 – presence of lateral dentin bridge

3 – presence of dentin bridge which completely encloses exposed pulp;

B) Morphological reorganization:

0 – normal pulp tissue

1 – disorganization of odontoblast like cells, odontoblast hyperactivity and proliferation of blood vessels beneath the exposed pulp

2 – complete disorganization of the pulp tissue

3 – pulp necrosis;

C) Inflammatory reaction of the pulp (chronic or acute, intensity and localization of inflammation) – it was monitored through the International Organization for Standardization and published criteria of Mjör [25] as:

0 – no inflammation or there are few inflammatory cells in the area of exposure

1 – mild inflammation and presence of inflammatory cells only at the site of exposure

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

Material Materijal	Dentin bridge Dentinski mostić				Tissue reorganisation Reorganizacija tkiva				Pulp inflammation Inflamacija pulpe				Presence of bacteria Pisustvo bakterija			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Biodentine	0	4	3	3	1	8	1	0	0	8	2	0	8	2	0	0
MTA	0	6	0	4	2	8	0	0	0	7	3	0	7	3	0	0

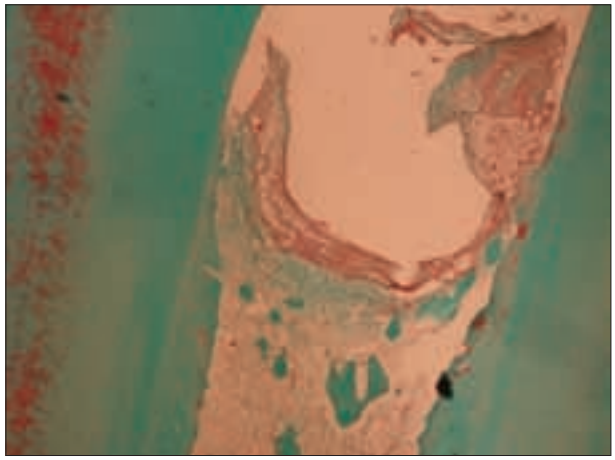

Figure 1. Dentin bridge in the area of perforation covered with Biodentine. Shown in the form of islands of mineralized tissue (green). Biodentine particles cover the entire surface of the defect (Goldner Trichrome, 40×).
Slika 1. Dentinski mostić u predelu perforacije pulpe prekrivene biodentinom. Uočava se dentinski mostić u obliku ostrvaca mineralizovanog tkiva (zeleno). Iznad dentinskog mostića vide se partikule biodentina koje prekrivaju celu površinu oštećenja (Goldner Trichrome, 40×).

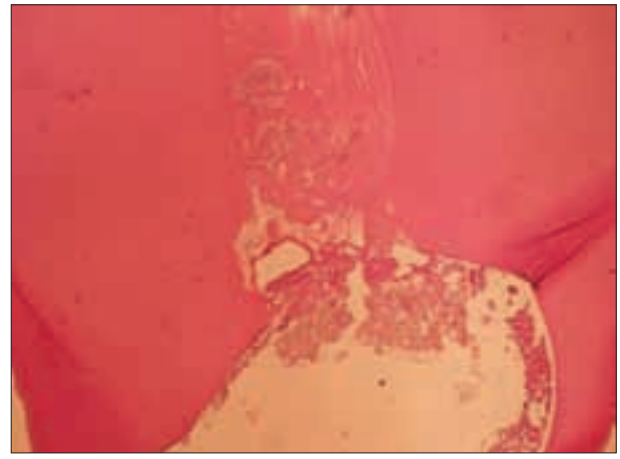

Figure 2. Complete closure of exposed pulp by newly formed dentin bridge after direct pulp capping using MTA. MTA particles incorporated in dentin bridge can be observed (HE, 40×).
Slika 2. Potpuno zatvaranje eksponirane pulpe novostvorenim dentinskim mostićem nakon prekrivanja sa MTA. Uočavaju se partikule MTA inkorporirane u dentinski mostić (HE, 40×).

2 – moderate inflammation with infiltration cells in the coronal and radicular pulp

3 – severe inflammation with cell infiltration which covers the entire pulp with complete disorganization of normal pulp tissue;

D) The presence of bacteria:

0 – absence of bacteria in the pulp and dentinal tubules

1 – bacteria present in dentinal tubules, but not in the pulp

2 – bacteria present along the side walls of pulp chamber

3 – bacteria present in the whole pulp, along the side walls of the pulp chamber and dentinal tubules.

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 irregular dentinal tubules that were in continuity with surrounding dentin. Complete dentin bridge which closed pulp perforation was noted in 3 cases with Biodentine (Figure 1) and 4 cases with MTA (Figure 2). Odontoblast like cells associated with newly formed dentin were found below complete dentin bridge. The original odontoblasts were positioned peripherally. They are identified through their regular palisade arrangement, eosinophilic cytoplasm and basal nucleus alignment.

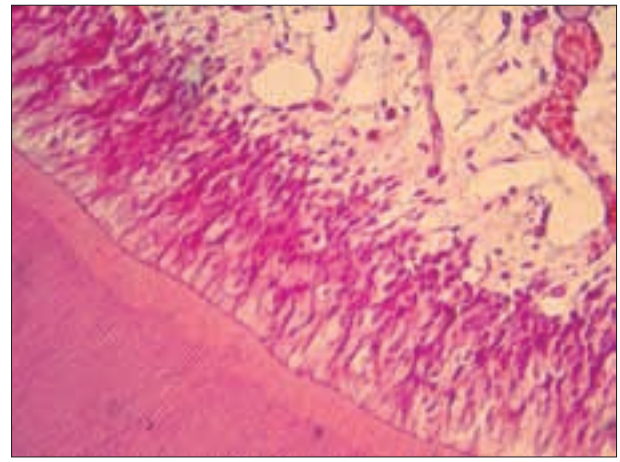

Figure 3. Reorganization of the pulp tissue after covering with Biodentine. Odontoblast like cells that produce dentin bridge are present (HE, 40×).
Slika 3. Reorganizacija pulpnog tkiva nakon prekrivanja biodentinom. Uočavaju se ćelije slične odontoblastima koje stvaraju dentinski mostić (HE, 40×).

Incomplete dentin bridge in the form of dental islets was observed in 4 teeth in the group of Biodentine and 6 teeth in the group with MTA. Continuous reparative dentin that extends along the lateral walls of dentin was recorded in 3 cases in the group of Biodentine while this form of dentin was not registered in the samples with MTA.

Fully preserved pulp tissue was observed in only one case in the group of Biodentine and two cases with MTA. Pulp disorganization characterized by the appearance of odontoblast like cells and their hyperactivity was observed in most samples (8 teeth in the experimental and control

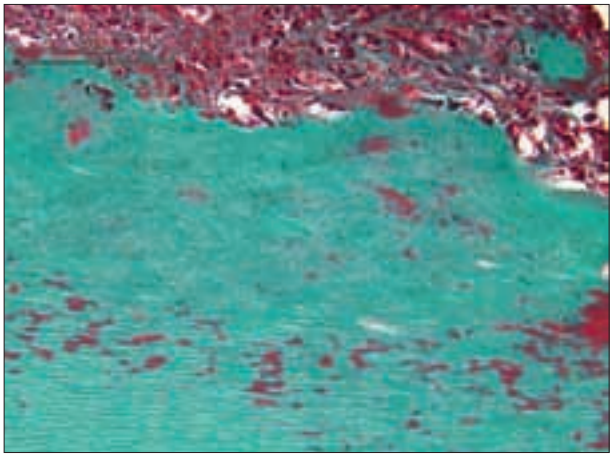

Figure 4. Reparative dentin in the group treated with MTA. Dentin bridge is separated from surrounding dentin with clearly visible demarcation line. Pulp tissue beneath the dentin bridge has normal structure (Goldner Trichrome, 100×).
Slika 4. Reparativni dentin u grupi uzoraka tretiranih sa MTA. Dentinski mostić je odvojen od okolnog dentina jasno vidljivom demarkacionom linijom. Pulpno tkivo ispod dentinskog mostića je normalne strukture (Goldner Trichrome, 100×).

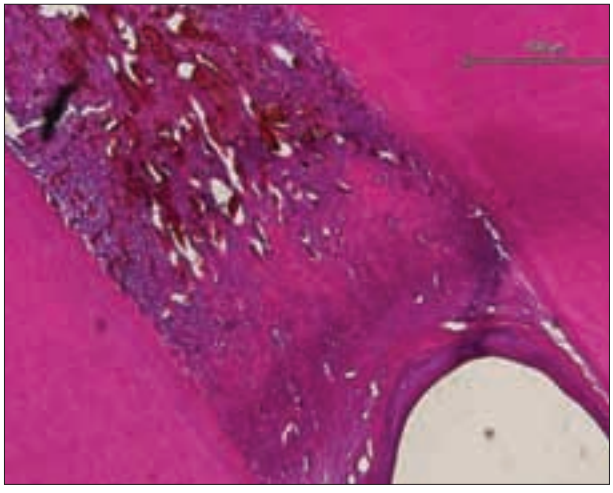

Figure 5. In majority of samples in both groups inflammatory cell s were either absent or present in small numbers (HE, 40×).
Slika 5. U većini uzoraka obe grupe nisu uočene ćelije zapaljenjskog infiltrata ili je njihov broj bio veoma mali (HE, 40×).

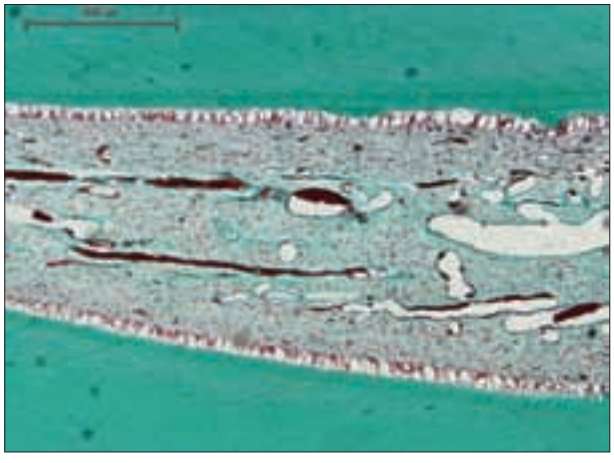

Figure 6. Dilated blood vessels with signs of venous stasis after pulp capping with MTA. Inflammatory cells are present (HE, 100×).
Slika 6. Dilatirani krvni sudovi sa znacima venske staze posle prekrivanja pulpe sa MTA. Ćelije zapaljenja ne postoje (HE, 100×).

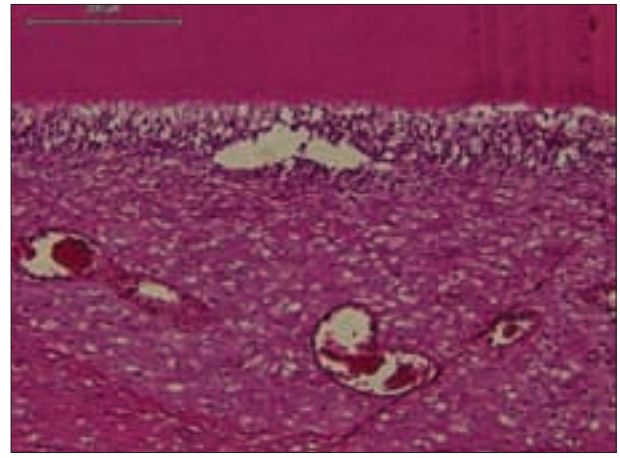

Figure 7. Venous stasis in the pulp after covering with Biodentine and appearance of dilated blood vessels. No bacterial cells in the pulp (HE, 40×).
Slika 7. Venska staza u pulpi zuba posle prekrivanja biodentinom i dilatirani krvni sudovi, ali bez bakterijskih ćelija u pulpi (HE, 40×).

group), where in the central part of the pulp the presence of venous stasis, hemorrhage and inflammation was observed (Figure 3).

In most of these cases neoangiogenesis with proliferation of existing and creation of new blood vessels was observed indicating healing process and complete revascularization. Complete disorganization of the pulp tissue was registered in the samples of the experimental group, while it was not seen in samples of the control group (Figure 4). Necrosis was not observed in any case.

Histological analysis after 4 weeks revealed that the following experimental pulp capping material 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 experimental group (Figure 6) and 7 samples in the control group with MTA (Figure 7). Moderate inflammation where cellular proliferation was present both in coronal and radicular pulp was observed in 2 teeth of the Biodentine group and 3 teeth in the MTA group.

Gram staining showed the absence of gram -positive bacteria in the pulp of all samples (Figure 7). Small number of bacteria in dentinal tubules was observed in 2 teeth with Biodentine and 3 teeth with MTA.

DISCUSSION

Previous studies with new materials were conducted on teeth of dogs [17, 26], deciduous and permanent teeth of pigs [24, 27] as well as monkeys [28]. An important advantage in working with animals is that experiment can be carried out on large number of teeth in the same time period and the effect of various materials can be checked at the same time.

Results of the current study showed similar effects of Biodentine and MTA. The process of reparative dentinogenesis and complete or partial closure of perforations with dentin bridge was considered as good therapeutic result. Dentin bridge was observed in all teeth where

perforations were capped using Biodentine. Similar results were confirmed by the study of Laurent et al. [20], where a favorable therapeutic effect of Biodentine was explained by significant release of TGF-β1 in pulp cells that stimulated odontoblasts to increase their activity and activate reparative dentinogenesis. Results of the current study where the pulp was capped by MTA also showed presence of dentin bridge in all samples which is consistent with similar experimental study in pigs done by Shayegana et al. [24].

In most teeth of both groups, odontoblasts were observed just below the 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 [29]. 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 of the samples in the MTA group reorganization of the tissue below the perforation was observed (hyperactivity of odontoblast like cells and altered cell morphology as 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 [30].

In the group with MTA necrosis was not observed in any sample. In the experimental study of Tabarsi et al. [26] in 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 group of Biodentine in most teeth mild inflammation was observed, suggesting biocompatibility of the material [31]. Acute inflammation and necrosis of the pulp was not observed in any of samples. This can be explained by good marginal seal achieved with glass ionomer cements and aseptic conditions as well as good immune status of experimental animals. The results of the current 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 [24, 26]. Therapeutic effects were similar in the experimental and control groups, indicating that Biodentine has favorable effects on reparative activities of the pulp primarily due to their physical and chemical properties.

After application of both materials 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 [29].

CONCLUSION

Reparation of pulp exposure was successful in the experimental and control group. 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 Biodentine which was similar to MTA after direct pulp capping of pulp in Vietnamese pigs.

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Received: 15/03/2014 • Accepted: 20/05/2014

Direktno prekrivanje pulpe biodentinom

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KRATAK SADRŽAJ

Uvod Direktno prekrivanje pulpe je terapijski postupak primene leka na eksponiranu pulpu zuba radi zatvaranja pulpne komore i omogućavanja procesa zarastanja. Cilj ovog rada je bio da se ispita efekat biodentina na eksponiranu pulpu zuba vijetnamske svinje.
Materijal i metode rada Istraživanje je izvršeno na 20 zuba vijetnamske svinje (*Sus scrofa domesticus*). Na vestibularnim površinama sekutića, očnjaka i prvih premolara urađene su preparacije kaviteta V klase, pri čemu je napravljena namerna eksploracija komora pulpe. U eksperimentalnoj grupi (šest sekutića, dva očnjaka i dva premolara) perforacija je prekrivana preparatom *Biodentine*® (*Septodont*, Sent Mor de Fos, Francuska). U kontrolnoj grupi perforacija je prekrivana sa *MTA*® (*Dentsply Tulsa Dental*, Džonson Siti, Tenesi, SAD). Svi kaviteti su restaurirani glasjonomer-cementom (*GC Fuji VIII, GC Corporation*, Tokio, Japan). Opservacioni period trajao 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 eksperimentalne i kontrolne grupe zabeleženo je stvaranje dentinskog mostića. Zapaljenje pulpe je bilo blago do umereno i u eksperimentalnoj i u kontrolnoj grupi uzoraka. 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 biodentina u direktnom prekrivanju pulpe zuba vijetnamskih svinja. Reakcija pulpe bila je slična onima koje je izazvao MTA.
Ključne reči: direktno prekrivanje pulpe; biodentin; MTA; dentinski mostić

UVOD

Direktno prekrivanje pulpe je terapijski postupak primene leka na eksponiranu pulpu zuba radi zatvaranja pulpne komore i omogućavanja procesa zarastanja. Očuvanje vitaliteta pulpe je izuzetno značajan postupak, posebno kod karijesnih zuba mladih osoba i zuba s komplikovanim višekanalnim sistemima [1, 2]. Brojna istraživanja su potvrdila da je kalcijum-hidroksid tzv. 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]. MTA se sastoji od: trikalcijum-silikata, dikalcijum-silikata, trikalcijum-aluminata, kalcijum-sulfata dihidrata i bizmut-oksida. 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 vlažnom operativnom polju i prisustvu 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]. Međutim, MTA ima manji antibakterijski efekat od kalcijum-hidroksida, a nedostaci su mu nestabilnost praha neposredno nakon otvaranja, visoka cena na tržištu i dugo vreme vezivanja: proizvođač navodi vreme vezivanja od 10 minuta, a studija Torabinedžada (*Torabinejad*) i saradnika [18] je utvrdila da je vreme vezivanja između dva i četiri sata.

Cilj aktuelnih istraživanja je pronalaženje materijala za direktno prekrivanje pulpe zuba koji bi zadržao sve dobre osobine MTA, ali i prevazišao njegove mane.

Biodentine® (*Septodont*, Sent Mor de Fos, Francuska) je materijal novije generacije na bazi kalcijum-silikata koji je sintetisan bioaktivnom tehnologijom. Indikovan je kao ispun u svim slučajevima oštećenog dentina, kako u kruničnom, tako i u korenskom delu zuba, ali se može koristiti i za direktno prekrivanje pulpe. Sastoji se od praha (trikalcijum-silikat, kalcijum-karbonat i cink-oksid) i tečnosti (voda, kalcijum-hlorid, modifikovani polikarboksilat) i ima vreme vezivanja od 12 minuta [19]. Biodentin stimuliše oslobađanje TGF-β iz pulpnih ćelija, što dovodi do stvaranja reparativnog dentina u vrlo kratkom vremenskom roku. Na modelu humanih ekstrahovanih trećih molara direktno prekrivanje pulpe biodentinom pokazalo je dobre rezultate u zarastanju i regeneraciji pulpe [20]. Eksperimentalna studija Trana (*Tran*) i saradnika [21] na pacovima pokazala je sličan efekat MTA i biodentina na regeneraciju pulpe nakon direktnog prekrivanja pulpe u pogledu stvaranja dentinskog mostića (njegove debljine, kontinuiranosti, poroznosti), ali i znatno slabije rezultate dejstva kalcijum-hidroksida. Novija istraživanja su takođe pokazala povoljne terapijske rezultate biodentina i

MTA kod direktnog prekrivanja pulpe mlečnih zuba svinja [22]. Uprkos činjenici da se ovaj novi cement već uveliko koristi u stomatologiji kao restaurativni materijal, njegov efekat na pulpno tkivo ljudi još nije dokazan [22].

Cilj rada je bio da se proveri efekat biodentina na eksponiranu pulpu zuba vijetnamskih svinja (*Sus scrofa domesticus*).

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 20 zuba vijetnamske svinje (*Sus scrofa domesticus*) starosti od 24 meseca i telesne mase od 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 životinje je izvršena premedikacija atropinom u dozi 0,03–0,04 mg/kg i.m., a nakon 15 minuta životinja je uvedena 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 prema uputstvu proizvođača. Na zubima desnog kvadranta gornje i donje vilice (ukupno šest sekutića, dva očnjaka i dva premolara) primenjen je biodentin.

Na istom broju zuba u levom kvadrantu gornje i donje vilice postavljen je kontrolni materijal MTA. Svi kaviteti su restaurirani glasjonomer-cementom (*GC Fuji VIII, GC Corporation*, Tokio, Japan). Opservacioni period trajao je 28 dana.

Posle prestanka dejstva anestezije životinji je dat analgetik butorfanol u dozi 0,1–0,2 mg/kg, a nakon oporavka svinja je čuvana i gajena u individualnom kavezu u farmskim uslovima. Posle četiri nedelje životinja je žrtvovana 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 [24]. Analizirani su dentinski mostić (A), morfološka reorganizacija ćelija pulpe (B), inflamatorna reakcija pulpe (C) i prisustvo bakterija (D) prema sledećoj skali:

A) Dentinski mostić (debljina, lokalizacija, struktura, kontinuitet s okolnim dentinom):

0 – nema dentinskog mostića

1 – nepotpun dentinski mostić u predelu eksponirane pulpe

2 – lateralni dentinski mostić

3 – potpuni dentinski mostić koji u celosti zatvara eksponiranu pulpu;

B) Morfološka reorganizacija:

0 – normalno pulpno tkivo

1 – dezorganizacija ćelija sličnih odontoblastima, hiperaktivnost odontoblasta i proliferacija krvnih sudova ispod eksponirane pulpe

2 – potpuna dezorganizacija pulpnog tkiva

3 – nekroza pulpe;

C) Inflamatorna reakcija pulpe (hronična ili akutna, intenzitet i lokalizacija zapaljenja) – praćena je zahvaljujući Međunarodnoj organizaciji za standardizaciju (*International Organization for Standardization*) i objavljenim kriterijumima Mjora (*Mjör*) [25] iz 1983. godine:

0 – nema zapaljenja ili postoji nekoliko ćelija zapaljenja u predelu eksponirane pulpe

1 – blago zapaljenje i postojanje ćelija zapaljenja samo na mestu eksponirane pulpe

2 – umereno zapaljenje gde ćelijska infiltracija doseže koronarnu pulpu i deo radiksne pulpe

3 – izrazito zapaljenje, gde ćelijska infiltracija zahvata celu pulpu s potpunom dezorganizacijom normalnog pulpnog tkiva;

D) Prisustvo bakterija:

0 – nema bakterija u pulpi i dentinskim kanalićima

1 – bakterije u dentinskim kanalićima, ali ih nema u pulpi

2 – bakterije duž bočnih zidova pulpne komore

3 – bakterije u celoj pulpi, duž bočnih zidova pulpne komore i u dentinskim kanalićima.

REZULTATI

Rezultati histoloških analiza su pokazali da je dentinski mostić stvoren kod svih uzoraka i u eksperimentalnoj i u kontrolnoj grupi (Tabela 1). Novostvoreni dentin imao je odlike reparativnog dentina bez nepravilno postavljenih dentinskih kanalića ili s malim brojem ovakvih kanalića, koji su bili u kontinuitetu s okolnim dentinom. Kompletan dentinski mostić koji je potpuno zatvarao pulpni prostor u predelu perforacije zabeležen je u tri uzorka na kojima je primenjen biodentin (Slika 1) i u četiri kod kojih je primenjen MTA (Slika 2). Ispod potpunog dentinskog mostića uočene 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 četiri zuba u grupi u kojoj je primenjen biodentin i kod šest zuba u grupi gde je primenjen MTA. Kontinuiran reparativni dentin koji se prostire duž lateralnih zidova dentina uočen je u tri uzorka u grupi zuba kod kojih je primenjen biodentin, dok takav vid dentina nije zabeležen u uzorcima kontrolne grupe (MTA).

Potpuno očuvano pulpno tkivo zabeleženo je samo u jednom uzorku u grupi u kojoj je primenjen biodentin i u dva uzorka na kojima je primenjen MTA. Dezorganizacija pulpnog tkiva u vidu pojave ćelija sličnih odontoblastima i njihove hiperaktivnosti uočena je kod najvećeg broja uzoraka (po osam zuba i u eksperimentalnoj i u kontrolnoj grupi), kod kojih su u centralnom delu pulpe uočeni venska staza, krvarenje i zapaljenje (Slika 3).

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. Potpuna dezorganizacija pulpnog tkiva uočena je u jednom uzorku eksperimentalne grupe, dok se to nije moglo videti ni u jednom uzorku kontrolne grupe (Slika 4). Nekroza nije zabeležena ni u jednom slučaju.

Rezultati histoloških analiza nakon četiri nedelje pokazali su da je posle prekrivanja pulpe eksperimentalnim materijalom 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 eksperimentalne grupe (Slika 6) i u sedam uzoraka grupe sa MTA (Slika 7). Umereno zapaljenje kod koje ćelijska infiltracija zahvata koronarnu i deo radiksne pulpe uočeno je kod dva zuba iz grupe u kojoj je primenjen biodentin i kod tri zuba iz grupe gde je prekrivanje vršeno sa MTA.

Bojenjem preparata po Gramu uočen je potpuni izostanak Gram-pozitivnih bakterija u pulpi svih uzoraka (Slika 7). Najmanji broj bakterija u dentinskim kanalićima zabeležen je kod dva zuba eksperimentalne grupe, dok su u kontrolnoj grupi bakterije uočene u tri uzorka.

DISKUSIJA

Dosadašnje studije sa novim materijalima vršene su na zubima pasa [17, 26], mlečnim i stalnim zubima svinja [24, 27], odnosno na zubima majmuna [28]. 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 i potpuno ili delimično zatvaranje perforacione rane dentinskim mostićem u ovom istraživanju smatrao se dobrim terapijskim rezultatom.

Kod svih zuba kod kojih je pulpa prekrivena biodentinom uočen je dentinski mostić. Slične rezultate potvrdila je i studija Lorena (*Laurent*) i saradnika [20], gde autori povoljan terapijski efekat biodentina 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 [24].

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 [29]. 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 [30].

U grupi zuba na kojima je primenjen MTA nekroza nije uočena ni u jednom uzorku. U eksperimentalnoj studiji na psima Tabarsija (*Tabarsi*) i saradnika [26], 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 nije vršeno direktno prekrivanje male površine eksponirane pulpe kao u našoj studiji.

Primenom biodentina kod najvećeg broja zuba utvrđeno je blago zapaljenje, što govori u prilog biokompatibilnosti materijala [31]. 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 [24, 26]. Budući da je terapijski efekat bio vrlo sličan u eksperimentalnoj i u kontrolnoj grupi, to ukazuje na činjenicu da i biodentin ima povoljno dejstvo na reparatorne aktivnosti pulpe zuba vijetnamskih svinja zahvaljujući, pre svega, svojim fizičkim i hemijskim osobinama.

Nakon primene biodentina 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 [29] 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 zuba eksperimentalnih životinja u eksperimentalnoj i u kontrolnoj grupi bila je vrlo efikasna. Kod većine zuba proces reparativne dentinogeneze je ispraćen stvaranjem dentinskog mostića i očuvanjem funkcionalnog i morfološkog integriteta pulpe. Histološka analiza je ukazala na povoljne terapijske efekte biodentina u direktnom prekrivanju pulpe zuba vijetnamskih svinja. Reakcija pulpe bila je slična onima koje je izazvao MTA.