HISTOLOGICAL EVALUATION OF DIRECT PULP CAPPING WITH NOVEL NANOSTRUCTURAL MATERIALS BASED ON ACTIVE SILICATE CEMENTS AND BIODENTINE® ON PULP TISSUE

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The aim of this study was to examine the effect of Biodentine® and two new nanostructured materials based on active silicate cements on exposed tooth pulp of Vietnamese pigs.

The study comprised 40 teeth in two Vietnamese pigs (24 months old). After class V cavity preparation, the pulp on each tooth was exposed using a small round bur. The following materials were applied on pulp exposures: Biodentine® (10 teeth), ALBO MPCA-I (10 teeth), and ALBO MPCA-II (10 teeth). In the control group, exposed pulp was covered with ProRoot MTA® (10 teeth). After the observation period of 28 days, the animals were sacrificed and the teeth prepared for histological analysis. Light microscope was used for the analysis of dentin bridge formation, tissue reorganization and inflammation, and the presence of bacteria in the pulp.

In the group of Biodentine®, a complete dentin bridge was noted in 3 cases, while incomplete dentin bridge in the form of dental islets was detected in 4 cases. Nanostructured material ALBO-MPCA I provided complete dentin bridge formation in 5 teeth, in 3 teeth the formed dentin bridge was incomplete. ALBO MPCA-II showed complete closure of the pulp opening by dentin bridge in 4 samples, while in the same number of teeth it was incomplete. In the control group, 4 teeth showed a complete dentin bridge, whereas in 6 teeth it was incomplete.

Histological analysis indicated favourable therapeutic effects of Biodentine® and the two materials ALBO-MPCA I and ALBO-MPCA II after teeth pulp capping in Vietnamese pigs. Pulp reaction was similar to that caused by ProRoot MTA®.

Key words: biodentine, cement, pulp, tooth

INTRODUCTION

Direct pulp capping procedure is the therapeutic application of the drug on exposed tooth pulp in order to ensure the closure of the pulp chamber and allow healing processes. Numerous studies have presented calcium hydroxide as the gold standard in direct pulp capping from its introduction to dental practice by Hermannin in 1920. High pH of calcium hydroxide and its stimulative effect on odontoblasts allows tertiary dentin formation and pulp vitality preservation. However, the percentage of success of calcium hydroxide in the published articles varies from 31-100% (Schröder 1978, Waterhouse 1995, Waterhouse *et al.*, 2000a; Percinoto *et al.*, 2006) indicating the need to develop new materials. Inadequate bonding to the exposed pulp (Schröder, 1978; Heilig *et al.*, 1984; Waterhouse *et al.* 2000a; Tunç *et al.*, 2006) and internal resorption (Schröder, 1978; Waterhouse, 1995; Waterhouse *et al.*, 2000b) are the main disadvantages of calcium hydroxide.

In the recent years, much attention has been given to the mineral trioxide aggregate ProRoot MTA® (Dentsply Tulsa Dental, Johnson City, TN, USA) as a material for direct pulp capping due to its ability to assist complete dentin bridge formation, with no signs of pulp inflammation (Faraco & Holland 2004, Simon et al. 2008). It has also been confirmed that the thickness of the newly formed dentin bridge was greater after pulp capping with MTA than with calcium hydroxide (Aeinehchi et al., 2003; Camilleri and Pitt Ford, 2006; Nair et al., 2008). MTA consists of tricalcium silicate, di-calcium silicate, tricalcium aluminate, calcium sulfate dihydrate and bismuth oxide. Numerous studies have confirmed its biocompatibility, antimicrobial activity, good sealing and excellent physical and chemical properties (Parirokh and Torabineiad, 2010a: Torabineiad and Parirokh, 2010). Its great advantage is application in wet conditions. On the other hand, its disadvantages are long setting time (longer than 3 hours) (Parirokh and Torabinejad, 2010a), instability of the powder immediately after opening the package (must be used within 4 weeks after opening) even when kept in vacuum containers (British Cement Association BS EN 197-1: 2000.NF.3), as well as its high price on the market. Current research has been focused on developing new materials for direct pulp capping that will have the same good qualities of MTA, but also to overcome its shortcomings.

One of the new materials for direct pulp capping is Biodentine® (Septodont, Saint-Maur-des-Fosses, France). This new generation material consists of a powder (tricalcium silicate, calcium carbonate and zinc oxide) and liquid phase (water, calcium chloride, modified polycarboxylate) and it has a setting time of 12 min (Goldberg et al. 2009). Direct pulp capping in human teeth using Biodentine® showed positive results in pulp healing and regeneration and favourable effects compared to other current materials (Laurent et al., 2012). Another recent study showed that Biodentine® and MTA provided favourable therapeutic results after direct pulp capping of deciduous teeth in pigs (Shayegan et al., 2012).

The aim of this study was to evaluate the effect of Biodentine® and the two new nanostructured materials based on active silicate cements (ALBO-MPCA I

and ALBO-MPCA II) in exposed tooth pulp of Vietnamese pigs (Sus scrofa scrofa Vietnamese).

MATERIALS AN METHODS

Experimental animals

Experiments were conducted at the Faculty of Veterinary Medicine, University of Belgrade after obtaining the approval of the Ethic Committee of the School of Dental Medicine. Forty teeth and two Vietnamese pigs (*Sus scrofa domesticus*), aged 24 months, average weight of 25 kg were included in the experiment. The rules of the European Good Laboratory Practices (86/609/EEC) that include basic principles of asepsis and antisepsis, the realization of experiments for the minimum time needed without physical and mental suffering of animals (International Organization for Standardization, 1997) were followed.

Materials used

Biodentine® (Septodont, Saint-Maur-des-Fosses, France) and two newly developed nanostructured materials based on active silicate cements (ALBO-MPCA I and ALBO-MPCA II) were used. New materials were synthesized according to the original recipe of Prof. Jokanović at the Institute for Nuclear Research, Vinca, Serbia. ALBO-MPCA I contains a mixture of dicalcium and tricalcium silicate, calcite (gypsum) and bismuth oxide in ratio of 1:2:2, while ALBO-MPCA II contains barium sulfate instead of bismuth oxide in the same ratio. These materials consist of three particle sizes: crystals of 20 nm, particles of 300-500 nm and agglomerate particles of 3 μ m. Crystals – the particles of the smallest size allow shorter setting time of materials due to the larger surface of the total number of particles. Therefore, the setting time begins 3 minutes after mixing the powder with distilled water, and ends after 10 minutes allowing convenient clinical use. Particle size provides ideal hydration of cements, contributing to the hardness of materials. Agglomerates prevent tissue destruction due to their larger particle sizes which cannot pass through the pores of the cell membrane. ProRoot MTA® (Dentsply Tulsa Dental, Johnson City, TN, USA) was used in the control group since its effects on the pulp are already known (Shayegan et al., 2012).

Experimental procedure

Both animals were premedicated with atropine in a dose of 0.03-0.04 mg/kg, and after 15 minutes they were introduced into general anesthesia by xylazine 1.5-2 mg/kg i.m. and ketamine 20-25 mg/kg i.m. Teeth were isolated with a rubber dam and cleaned with 70% ethanol. On the vestibular surfaces of the incisors, canines and first premolars, class V cavity was prepared using a round carbide bur and continuous cooling with saline. The pulp chamber was exposed using a small round bur. Bleeding was controlled with a sterile cotton ball. Previously prepared material according to the manufacturer's instructions was applied on the perforation. Biodentine® was applied on the teeth in the upper and lower right quadrants (total of 6 incisors, 2 canines, 2 premolars) in the first Vietnamese pig (Table 1). ProRoot MTA® (control group) was applied on the same number of

teeth in the upper and lower left quadrants. Following the same principles and the same number of teeth, materials ALBO-MPCA I and ALBO-MPCA II, previously mixed with distilled water in the ratio 2:1 were applied on pulp exposures in the second Vietnamese pig. All cavities were restored with glass ionomer cement (GC Fuji VIII, GC Corporation, Tokyo, Japan). Observation period was 28 days.

Table 1. Materials applied on pulp perforations in experimental animals

	Vietnam	ese pig I	Vietnamese pig II				
	Right	Left	Right	Left			
Upper jaw	Biodentine®	ProRoot MTA®	ALBO-MPCA I	ALBO-MPCA II			
Lower jaw	Biodentine®	ProRoot MTA®	ALBO-MPCA I	ALBO-MPCA II			

After the completion of experimental procedures animals were given butorphanol at a dose of 0.1-0.2 mg/kg. Recovered animals were kept in individual cages in farm conditions. After 4 weeks they were sacrificed by introduction into general anesthesia and pentobarboton sodium i.v. at a dose of 100 mg/kg. After the animals were sacrificed, the jaws were dissected and the tissue samples were fixed for light microscopy analysis.

Hystological procedure

The tissue for histological analysis was taken en block containing experimental teeth with surrounding bone tissue. Teeth were collected following ISO usage guidelines (Technical Report 7405) 28 days after pulp exposure and direct capping. The material for histological analysis was fixed in 10% buffered formalin, decalcified in 10% formic acid (pH=5) and embedded in paraffin. Serial sections 4 μ m thick were cut in mesio/distal direction, mounted on glass slides, stained with haematoxylin and eosin, Goldner trichrome, as well as with Gram for identification of bacteria microscopically (LMLeica). The quality of materials used in this study was analysed in the light of the biological response of pulp tissue.

Histological analysis was performed according to the following criteria:

1. Dentin bridge formation (thickness, localization, morphological aspects, continuity with surrounding dentin). The dentin bridge was identified as the formation of tertiary dentine by newly differentiated odontoblast like cells, at the site of pulp exposure (Murray et al., 2003). Dentine bridge is a specialized type of reparative dentine secretion because it has a tubular continuity with newly differentiated odontoblast – like cells. This is not the case with physiological secondary dentine or reactionary dentine, produced by original odontoblasts (Smith et al., 2002). Reactionary dentine, sometimes named irregular dentine, is identified in areas of increased tertiary dentine secretion with a tubular continuity with the physiological secondary dentine (Mjör, 1983). Differentiation between reactionary and reparative dentine was made during the examination of the sections.

Dentin bridge formation was scaled from:

- 0- no presence of dentin bridge formation
- 1- presence of incomplete dentin bridge in the region of pulp injury
- 2- presence of dentin bridge lying at the lateral surfaces of pulp
- 3- presence of complete dentin bridge formation with complete closure of the dental pulp chamber.
- 2. Morphological integrity of odontoblasts and odontoblast like cells, as well as the integrity of deeper pulp tissue, according to criteria established by Shayegan et al. (2009), Murray et al. (2003), Cox et al. (1996), Tziafas (1994) and Mjör et al. (1991).

Morphological and functional integrity of deeper pulp tissue was categorized from:

- 0- normal pulp tissue
- 1- disorganisation of odontoblast like cells, proliferation and hyperactivity
- 2- complete disorganisation of pulp tissue
- 3- pulp necrosis.
- 3. Inflammatory response of each pulp (chronic or acute, intensity and localization). Inflammatory reaction was categorized in increasing order of severity from none, slight, moderate and severe according to the Federation Dentaire Internationale and International Organization for Standardization Standards and published criteria (Mjör, 1983).

Inflammatory reaction was scored as:

- 0- none, characterized with either complete absence of inflammatory cells or presence of very few cells
- 1- mild inflammation, inflammatory cells were observed just beneath the capping material
- 2- moderate inflammation, more than one third of the pulp tissue was infiltrated with inflammatory cells
- 3- severe inflammation, dental pulp was diffusely infiltrated with mononuclear cells, with complete destruction of normal histological organization of pulp tissue.
 - 4. Presence of bacterial cells

Bacterial cells were scaled from:

- 0- no presence of bacteria either in pulp tissue, or dentinal tubules
- 1- presence of bacteria in dentinal tubules, but not in dental pulp
- 2- presence of bacteria along lateral dentin surfaces
- 3- presence of bacteria in dental pulp and along lateral dentin surfaces.

RESULTS

The results are presented in Table 2 and Figures 1-6.

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Table 2. Histological analysis of dental pulp tissue after the application of tested materials.

Material	Dentine bridge			Tissue reorganization			Pulp inflammation			Bacterial presence						
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Biodentine	0	4	3	3	0	8	2	0	0	8	2	0	8	2	0	0
ALBO-MPCA I	1	3	1	5	1	8	1	0	1	8	1	0	5	5	0	0
ALBO-MPCA II	1	4	1	4	0	9	1	0	1	7	2	0	6	4	0	0
MTA	0	6	0	4	2	8	0	0	0	7	3	0	7	3	0	0

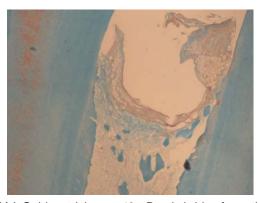


Figure 1. ALBO-MPCA I. Goldner trichrome 40x. Dentin bridge formation in the area of pulp injury. Dentin bridge appears as an island of mineralized tissue (green). Particles of capping material covering the area of the defect

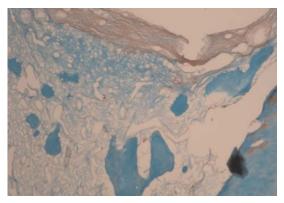


Figure 2. ALBO-MPCA I. Goldner trichrome 100x. Dentin bridge formation in the area of pulp injury. Dentin bridge appears as an island of mineralized tissue (green). Particles of capping material covering the area of the defect



Figure 3. ALBO-MPCA I. Goldner trichrome 100x. Dentin bridge appears as partly mineralized island (red) surrounded by normal pulp tissue with increased number of newly formed blood vessels

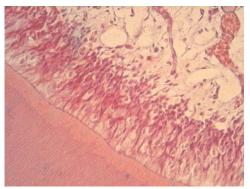


Figure 4. Biodentine®. HE. 400x. Odontoblast like cells and well organized subjacent pulp tissue

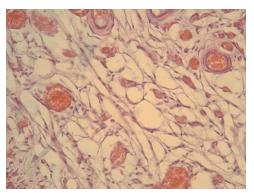


Figure 5. ALBO-MPCA I. HE. 400x. Signs of neoaniogenesis and stasis in dental pulp. This finding is probably related to chemical properties of the capping material and mechanical trauma during cavity preparation, rather than the presence of bacterial infection



Figure 6. Control. HE.40x. Complete closure of pulp chamber by newly formed mineralized dentin bridge. Particles of capping material are incorporated in dentin bridge formation

Histological analysis of pulp tissue in the experimental groups

The results of histological analysis showed that dentin bridge was created in almost all samples in all three experimental groups (not observed in one sample only in the group ALBO-MPCA I and one sample in the group ALBO-MPCA II). Newly formed dentin had characteristics of reparative dentin with none or a small number of irregular dentinal tubules that were in continuity with the surrounding dentin.

Dentin bridge that completely enclosed pulp tissue in the region of pulp perforation was detected in 3 cases with Biodentine®, 5 cases with ALBO-MPCA I and 4 cases with MPCA ALBO-II. Odontoblast-like cells were observed below complete dentin bridge in continuity with tubular dentine bridge secretion. Original odontoblasts were positioned peripherally to the site of exposure. They were identified as primary pallisade columnar cells with an eosinophilic cytoplasm and a nucleus located in the basal position, adjacent to predentin. Incomplete dentin bridge in the form of dentin islets was observed in 4 teeth in the group of Biodentine®, 3 teeth in the group ALBO-MPCA I and 4 teeth in the group ALBO-MPCA II (Figures 1 and 2). Continuous reparative dentin that extends along the lateral dentin walls was recorded in 3 cases in the Biodentine® group and one case in each of the two groups of new nanostructured materials.

The results of microscopic analysis revealed that subjacent pulp tissue underwent a reorganization process. Reorganizing tissue comprised of a mixed range of cells of different morphologies, including fibroblasts and inflammatory cells. Reorganization of subjacent area correlated with the number of odontoblast like cells and it was more prominent in ALBO-MPCA I group (Figure 3). Although the role of the subjacent area cells remains unclear, it could be presumed that these cells have a supporting role to odontoblast-like cells.

Intact pulp tissue was found only in one case in the group ALBO-MPCA I. Disorganization of pulp tissue in terms of appearance of odontoblast-like cells and their hyperactivity (Figure 4), was observed in most of the samples (8 teeth in the

groups Biodentine® and ALBO-MPCA I and 9 in the group ALBO-MPCA II). Signs of neoangiogenesis with proliferation of existing and development of new blood vessels were recorded in most of cases indicating a healing process and complete revascularization (Figure 5). Complete disorganization of pulp tissue was registered in one sample only in each of the groups of new nanostructured materials and 2 samples in the group of Biodentine®. Necrosis was not detected in any case.

Histologic analysis of pulp tissue 4 weeks after direct pulp capping showed in most cases mild or moderate chronic inflammation. Complete absence of inflammation was observed in one sample in the group ALBO-MPCA I and ALBO-MPCA II. Mild inflammation was present in 8 samples per group of Biodentine® and ALBO-MPCA I and 7 in the group ALBO-MPCA II. Moderate inflammation with cellular infiltration of coronal and the part of radicular pulp was present in 2 teeth from the groups of Biodentine® and ALBO-MPCA II and one tooth from the group ALBO-MPCA I. Marked inflammation with numerous inflammatory cells and the appearance of the abscess was not detected in any case.

Gram staining showed the absence of gram-positive bacteria in the pulp of all samples. The minimum number of bacteria in dentinal tubules was registered in 2 teeth in the group of Biodentine®, 5 teeth in the group ALBO-MPCA I and 4 teeth in the group ALBO-MPCA II. It is difficult to explain whether these bacteria invaded dentin during the capping procedure, or they came into dentinal tubules through the cavity margins later on, during the healing process, due to microleakage of the restorative material.

Histological analysis of pulp tissue in the control group

Histological analysis of pulp tissue 4 weeks after direct pulp capping with ProRoot MTA® showed that the healing process proceeded without complications. Complete dentin bridge was formed in 4 teeth (Figure 6). The presence of incomplete dentin bridge in the form of dentin islands that only partially closed the pulp space was revealed in 6 samples. Some particles of used material and components of connective tissue were observed in the dentin bridges (Figure 6). Intact pulp tissue was detected in two teeth. Reorganization of pulp tissue was observed in 8 teeth where the presence of venous stasis, hemorrhage and inflammation was found in the central part of the pulp.

Mononuclear cells were observed in the pulp tissue of all analyzed samples suggesting the presence of chronic inflammation scored from mild (7 teeth) to moderate (3 teeth), according to previously established criteria. Unexpectedly, inflammatory cells were more numerous in the central, than in peripheral parts of the pulp and were not related to the presence of bacterial cells. Bacteria were identified in dentinal tubules of 3 samples, while no bacteria were found in the dental pulp. In 7 teeth bacteria were detected only in the cavity.

DISCUSSION

The current experimental study was performed on permanent teeth of Vietnamese pigs which are by their morphology very similar to human teeth.

Earlier studies with new materials were conducted on dog teeth (Tabarsi *et al.*, 2010; Tziafas, 2002), deciduous and permanent teeth of pigs (Shayegan *et al.*, 2012; Nakamura *et al.*, 2002) and monkeys (Danilovic, 2008). A significant advantage in working with experimental animals is that a large number of teeth can be included and the effect of different materials can be observed in the same time period.

The results of this study showed similar findings on teeth in three experimental and one control group. The process of reparative dentinogenesis, and complete or partial closing of perforations with dentin bridges, was considered as a good therapeutic result. Dentin bridge was observed in all teeth were direct pulp capping was performed using Biodentine®. Similar results were obtained in the study of Laurent *et al.* (2012). Favorable therapeutic effects of Biodentine® were explained by a significant increase of TGF-B1 release from human pulp cells that stimulated odontoblasts and increased their secretory activity and reparative dentinogenesis. In the control group where ProRoot MTA® was applied, dentin bridge was also observed in all cases. This finding is in agreement with experimental studies in pigs (Shayegan *et al.* 2009).

Just below the newly formed dentin bridge, odontoblasts with minor or major structural changes that ranged from mild to complete disorganization were observed in most of the teeth from all three experimental groups. These are most probably not true odontoblasts, but odontoblast-like cells (although for their definitive identification additional immunohistochemical analysis is required). Similarly to true odontoblasts, they have an elongated shape, palisade orientation and a nucleus in the basal position (Murray et al., 2003). They have the ability to secrete an extracellular matrix that after mineralization forms reparative dentin in the form of complete or incomplete dentin bridge or islets that tend to establish contact with the side walls of dentin and close and preserve exposed dental pulp. Tissue reorganization in the form of hyperactivity of odontoblast like cells and altered cell morphology in relation to odontoblasts was observed below the perforation in the most of the samples from the MTA group in the study performed in dogs (Tziafas et al., 2002). There was a correlation between the number of odontoblast like cells, bridge thickness and the preservation of deeper areas of the pulp. The greater number of these cells increases the thickness of the dentin bridge, while radicular pulp retains its physiological morphology (Orhan et al., 2012).

Acute inflammation and pulp necrosis were not found in any sample. This can be explained by good cavity sealing using glass ionomer cements in aseptic working conditions. In an experimental study in dogs after direct pulp capping with MTA, necrosis was present in 22.7% of samples (Tabarsi *et al.*, 2010). Different findings can be explained by the fact that in this study MTA was applied after pulpotomy whereas in the current study MTA was applied directly on the perforation.

The results of the current experimental study showed the presence of inflammatory cells in the coronal and radicular pulp areas for all used materials. In the control group, where the pulp was covered with MTA, only a few samples revealed the presence of lymphocytes, plasma cells and macrophages, which is

in agreement with findings of other authors (Shahravan *et al.* 2011, Tabarsi *et al.* 2010). After the application of Biodentine® in most of the cases a mild inflammation of pulp tissue was observed suggesting the biocompatibility of this material (Costa *et al.*, 2003).

After the application of new nanostructured materials ALBO-MPCA I and ALBO-MPCA II, neoangiogenesis in the pulp suggested regenerative processes and successful tissue remodeling. Similar results obtained using Biodentine® and ProRoot MTA® can be explained by similar chemical compositions (dicalcium and tricalcium silicate in high percentages). ALBO-MPCA I and ALBO-MPCA II have similar compositions, but with different size of particles, which significantly improve the performance of these materials in terms of shorter setting time and workability in wet conditions. These properties affect the quality of bonding of these materials to exposed pulp and their favorable effects on odontoblast activation and dentin bridge formation.

There are also other opinions. Therefore, Murray *et al.* (2003) considered the beginning of dentinogenesis to be of primary importance for pulp and odontoblasts preservation, as well as the absence of infection and necrosis but not the type of material used. Having in mind that therapeutic effect was very similar in all four groups, it can be concluded that new nanostructured materials based on active silicate cements have favorable effects on reparative processes in the dental pulp of Vietnamese pigs primarily to their physical and chemical properties.

CONCLUSION

Reparation of artificially created dental pulp exposures in experimental animals in all three experimental groups was accompanied by the formation of dentin bridges and partial or complete closure of the pulp chamber and preservation of functional and morphological integrity of the pulp. That can be considered as reasonable therapeutic result. Histological analysis indicated favorable therapeutic effects of Biodentine® and the two materials ALBO-MPCA I and ALBO-MPCA II on the pulp of Vietnamese pigs after direct capping. Reaction of the pulp was similar to that caused by MTA.

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HISTOLOŠKA PROCENA STANJA ZUBNE PULPE NAKON DIREKTNOG PREKRIVANJA NOVIM NANOSTRUKTURNIM MATERIJALIMA NA BAZI AKTIVNIH SILIKATNIH CEMENATA I BIODENTINA

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SADRŽAJ

Cilj ovog rada je bio da se ispita efekat Biodentina i dva nova nanostrukturna materijala na bazi aktivnih cilikatnih cemenata na eksponiranu pulpu zuba vijentamskih svinja.

Istraživanje je obuhvatilo 40 zuba i dve vijetnamske svinje starosti 24 meseca. Na svima zubima je urađena preparacije kaviteta V klase i malim okruglim svrdlom eksponirana komora pulpe. Na perforaciju pulpe aplikovani su materijali: Biodentin (10 zuba), ALBO-MPCA I (10 zuba), ALBO-MPCA II (10 zuba). U kontrolnoj grupi pulpa je prekrivena MTA-om (10 zuba). Nakon observacionog perioda od 28 dana, životinje su žrtvovane i pripremljene za histološku analizu. Svetlosnom mikroskopijom analizirano je formiranje dentinskog mostića, postojanje reorganizacije tkiva, prisustvo inflamacije i bakterija u pulpi.

Kod Biodentina kompletan dentinski mostić je zabeležen u 3 slučaja, a inkompletan dentinski mostić u vidu dentinskih ostrvaca u 4 slučaja. Nanostrukturni materijal ALBO-MPCA I je doveo do stvaranja kompletnog dentinskog mostića kod 5 zuba, a kod 3 zuba dentinski mostić je bio inkompletan. ALBO-MPCA II je pokazao kompletno zatvaranje komore pulpe dentinskim mostićem u 4 uzorka,

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dok je kod 4 uzorka on bio inkompletan. Kontrolni materijal MTA je kod 4 zuba imao stvoren kompletan dentinski mostić, a u kod 6 zuba je on bio inkompletan. Histološka analiza je ukazala na povoljne terapijske efekte i Biodentina i dva novosintetisana materijala ALBO-MPCA I i ALBO-MPCA II u direktnom prekrivanju pulpe zuba vijentamskih svinja. Reakcija pulpe bila je slična onima koje je izazvao MTA.