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The effect of hydroxyapatite and growth factors on reparative dentine formation in the therapy of injured pulp

Efekat hidroksiapatita i faktora rasta na stvaranje reparativnog dentina u terapiji ledirane pulpe

Zorana Veličković*, Dušan Živković*, Marija Bubalo†‡, Milan Živković*, Aleksandar Mitić§, Milan Miladinović*, Miloš Duka†‡, Dragoslav Lazić*

University of Priština/Kosovska Mitrovica, Faculty of Medicine, *Clinic for Dental Medicine, Kosovska Mitrovica, Serbia; Military Medical Academy Belgrade, †Clinic for Dental Medicine, Belgrade, Serbia; University of Defence, ‡Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; University of Niš, Faculty of Medicine, §Clinic for Dental Medicine, Niš, Serbia

Abstract

Background/Aim. The studies of hydroxyapatite (HAp) and growth factors as the materials used for direct pulp capping have produced conflicting results for both the issue of the inflammatory response and the issue of calcified bridge formation. Hap/poly (lactide-co-glycolide) (HAp/PLGA) is a bioresorbable polymer with demonstrated good characteristics as the carrier for the bone morphogenetic protein necessary in bone tissue regeneration. The role of growth factors in dental tissue reparation (in both reactionary and reparative dentinogenesis) represents the new foundation and provides a different approach to dental pulp treatment. Growth factors – transforming growth factor beta-1 (TGF-β-1) – directly induce morphological and functional differentiation of neo-odontoblasts. The aim of this study was to investigate the effect of calcium HAp/PLGA and growth factors (TGF β-1) in the formation of a calcified tissue – dentine bridge – on the teeth of our experimental model. **Methods.** In this experimental study, rodent (rabbit) teeth were used as the animal model. After the trepanation of pulp space with sterile steel drills, the pulp was capped with calcium HAp/PLGA (experimental group I; n = 60); calcium HAp/PLGA combined with TGF β-1 growth factor (experimental group II; n = 60), and there was a control group of intact teeth (n = 20). The experiment was performed in general anesthesia. The animals were kept alive for 1, 3, and 6 months. The extracted teeth were adequately prepared for scanning electron microscopy. **Results.** Scanning electron microscopy demonstrated that the number of teeth with calcified tissue in the form of dental bridges in the HAp/PLGA+TGF β-1 group, 6 months after the treatment, was statistically significantly greater (66.67%) than after 3 months (26.67%), at the statistical significance level of $p < 0.05$. **Conclusion.** Direct pulp capping covers the artificially exposed dental pulp and makes possible the formation of a dentine bridge (a tubular structure composed of reparative dentine) in the period of 3 months.

Key words:
dental pulp; dental pulp capping; dentine; rabbits; minerals.

Apstrakt

Uvod/Cilj. Studije o hidroksiapatitu (HAp) i faktorima rasta kao materijalima za direktno prekrivanje pulpe daju potpuno oprečne rezultate i po pitanju zapaljenskog odgovora i po pitanju formiranja kalcifikovanog mosta. Kalcijum hidroksiapatit/poli (laktid-ko-glikolid) je bioresorbilni polimer koji je pokazao dobra svojstva kao nosač koštanog morfogenog proteina neophodnog za regeneraciju koštanog tkiva. Uloga faktora rasta u reparaciji zubnih tkiva (bilo da se radi o reaktivnoj ili reparativnoj dentinogenezi) daje nove osnove i drugačiji pristup tretmanu pulpe. Faktori rasta – transformišući faktor rasta beta-1 (TGF β-1) direktno indukuju morfološku i funkcionalnu diferencijaciju neoodontoblasta. Cilj ove eksperimentalne studije bio je da se ispita uticaj kalcijum HAp/poli (laktid-ko-glikolida) (HAp/PLGA) i faktora rasta TGF β-1 u stvaranju kalcifikovanog tkiva – dentinskog mosta na zubima eksperimentalnog modela. **Metode.** U eksperimentalnoj studiji kao animalni model korišćeni su zubi glodara (kunića). Nakon trepanacije pulpnog prostora sterilnim čeličnim svrdlom, pulpa je prekrivena kalcijum HAp/PLGA (I eksperimentalna grupa zuba, n = 60), kalcijum HAp/PLGA u kombinaciji sa faktorom rasta TGF β-1 (II eksperimentalna grupa, n = 60),

Correspondence to: Milan Miladinović, University of Priština/Kosovska Mitrovica, Faculty of Medicine, Clinic of Dental Medicine, Anri Dinana bb., 38 220 Kosovska Mitrovica, Serbia. E-mail: milanbetter@gmail.com

kontrolna grupa zuba (intaktni zubi, n = 20). Eksperiment je obavljen u opštoj anesteziji. Životinje su održavane u životu 1, 3 i 6 meseci. Ekstrahovani zubi su pripremljeni za posmatranje skenirajućom elektronskom mikroskopijom. **Rezultati.** Skenirajućom elektronskom mikroskopijom dokazano je da je broj zuba sa kalcifikovanim tkivom u vidu dentinskog mosta bio veći 6 meseci nakon tretmana u odnosu na 3 meseca nakon tretmana. **Zaključak.** Direktnim prekrivanjem pulpe dolazi do zatvaranja artificijelno otvorene pulpe i stvaranja dentinskog mosta reparatornog dentina tubularne strukture u toku perioda posmatranja od 3 meseca.

Ključne reči:
zub, pulpa; zub, pulpa, prekrivanje; dentin; zečevi; minerali.

Introduction

Direct pulp capping, as one of the essential endodontic modalities, is often used as a therapeutic procedure for dental pulp vitality preservation. It is usually defined as a treatment on exposed pulp tissue, where the pulp wound is covered (capped) with materials that stimulate reparative dentine formation [1]. Since the capping material comes into direct contact with the pulp tissue, it plays a key role in this treatment [2, 3].

In the selection of materials for vital pulp therapy, the following material properties should be sought: antibacterial action, ability to induce mineralization, adequate sealing of the pulp space in order to prevent the entry of bacteria from the mouth cavity [4]. Some studies have investigated the use of biomaterials such as hydroxyapatite (HAp) in dental pulp treatments within the technique of direct pulp capping or amputation of the crown portion of the dental pulp [5, 6]. The results suggested that their use in the observation period of 3 months speeds up the process of healing, i.e. the formation of dentine bridge and continued dental root growth. HAp is one of the most frequently used calcium phosphate bioceramics with osteoconductive properties. Since its structure is similar to bone minerals, it is capable of forming direct bonds with the bone tissue. HAp has got several clear advantages: it is well-accepted and incorporable into the host bone, but it also provides a solid base for new bone growth [7]. Its biocompatibility is excellent, and its surface layer has a key role in the formation, growth, and maintenance of the tissue/biomaterial bond [8]. Moreover, it does not contain any proteins and consequentially does not induce any allergic reactions or immune system responses [9, 10]. On the other hand, HAp has very poor mechanical properties [11].

Synthetic HAp belongs to the group of non-resorbable ceramic biomaterials. It could perhaps successfully replace bone tissue, facilitate new bone formation and exert an osteoconductive effect [12–15].

Poly (lactide-co-glycolide) (PLGA) is a copolymer of lactide and glycolide registered by the Food and Drug Administration as a material which can be used in medicine and pharmacy, and it belongs to the class of biodegradable and biocompatible polymers [14, 16–19].

Pulp capping with HAp-based materials requires the use of a mechanically more resilient material over the medicament, and only then a better quality of the dentine-HAp interface becomes prominent [19]. Novel insights in the role of growth factors in dental tissue reparation, in both reactive and reparative dentinogenesis, could represent the basis of different pulp treatment [20].

Growth factors are biological mediators that regulate key processes in tissue reparation, including cell proliferation, differentiation, extracellular matrix synthesis, and angiogenesis [21].

There have been attempts to use HAp and growth factors for the same purposes, although with a low success rate. A number of these investigations are still ongoing and attract much attention [22, 23].

Transforming growth factor beta-1 (TGF β-1) is a member of the superfamily of homologous disulfide-bound hemodynamic proteins, regulating proliferation and differentiation of normal and transforming cells. Human TGF β-1 is a 25.0 kDa protein that contains 2 identical polypeptide chains of 112 amino acids interconnected by one disulfide bond [24].

Growth factors are present in the dentine matrix, and they can play an important role in mediating pulp responses to an injury or restorative procedure. Since they may be released during the tooth decay process, this could represent the basis of a novel biological approach to dental tissue reparation [25–28].

The aim of this study was to investigate the dentinogenetic effectiveness of bioactive materials calcium HAp/PLGA and growth factor TGF β-1 in reparative dentine formation in the cases of injured pulp during the standard procedure of dental pulp capping.

Methods

The experimental study took place at the Institute of Biomedical Research, Faculty of Medicine in Niš, and at the Faculty of Medicine in Priština, temporarily seated in Kosovska Mitrovica, with the approval of the Ethics Committee of the Faculty of Medicine in Niš (number: 05-603/1 of 2011).

The experiment included five chinchilla rabbits that were 6 months old and of mean weight of 3–4 kg. The animals were anesthetized by intramuscular Zoletil 100 administration (Virbac S.A. 1ère avenue 2065 M - L.I.D. 06516 Carros, France) at a dose of 10 mg/kg of body weight and ketamine hydrochloride (1–4.5 mg/kg body weight). After the induction of anesthesia and placement of cofferdam rubber insulation, the teeth were cleaned using 70% ethanol. Small cavities were created on the occlusal surfaces of the teeth with small round drills. The cavities were washed with saline for the removal of debris, created during cavity

preparation. After the trepanation of pulp space, the lesions were covered with biomaterial and growth factor, and cavities were definitively closed with glass-ionomer cement and amalgam. For the purpose of this study, we used calcium HAp/PLGA and autogenic TGF β-1.

The teeth were divided into three groups: experimental group I (n = 60), composed of the left lower jaw teeth, where calcium HAp/PLGA biomaterial was applied; experimental group II (n = 60), composed of the left upper jaw teeth, where calcium HAp/PLGA was applied in combination with TGF β-1; calcium HAp/PLGA biomaterial served as a carrier, 80:20 (0.5 g), manufactured by the ITN SANU, Belgrade; intact right upper jaw teeth and right lower jaw teeth served as our control group (n = 20).

After this phase of the study, our animals were kept alive for 1, 3, and 6 months. After these periods of time, they were sacrificed with a lethal dose of ketamine hydrochloride. Jawbones were disarticulated and each tooth was individually extracted. Material preparation involved tooth storage in sterile saline at 40°C without any fixation agents.

Occlusal surfaces of dental crowns, 2–3 mm thick, were cut in a circular manner using the finest diamond fissure bur. Dental roots were cut longitudinally using separating discs, producing longitudinal separation into the oral and vestibular surfaces. In order to eliminate superficial debris produced by cutting, the samples were washed in distilled water and dried with compressed air. Occlusal surfaces were separated first using the separation pliers, and then the roots were longitudinally separated along the already prepared grooves. Each half of the sample was placed onto an appropriate mount; the samples thus fixed were gold vapour treated in a vacuum evaporator and viewed under scanning electron microscopy JEOL-JCM-5300.

The entry and tabular data representation were done using the MS Office Excel 2007 software package, and calculations were performed using the SPSS, 15.0 version. The results of the statistical analysis were presented in tables.

The differences in the observed parameters, both between the groups and within them, in different intervals of time, were established using the Mantel-Haenszel χ^2-test or Fisher's test of the exact probability of the null hypothesis (when some of the expected frequencies were less than 5).

Results

In total, 140 teeth of 5 sacrificed experimental animals (rabbits) were used in the study – 20 teeth (4 teeth × 5) in the control group, 60 teeth (12 teeth × 5) in the experimental group I, and 60 teeth (12 teeth × 5) in the experimental group II.

As the measures of effect of the studied material types and treatment modalities, we tried to observe the formation of new hard dental tissue – dentine bridges (reparative dentine) in the studied specimens. This parameter was monitored using scanning electron microscopy 1, 3, and 6 months after the treatment.

The presence of dentine bridges in experimental groups with direct pulp capping and the control group (using scanning electron microscopy) is presented in Table 1.

Comparing the number of teeth among the studied groups, it was established that the number of teeth with formed dentine bridges in the HAp/PLGA+TGF β-1 group, 6 months after the treatment, was statistically significantly greater (66.67%) than after 3 months (26.67%), at the statistical significance level of $p < 0.05$.

Table 1

The presence of dentine bridges in the experimental groups with direct pulp capping and in the control group

Group	n	Period (months)		
		1	3	6
Control	20	0 (0.00%)	0 (0.00%)	0 (0.00%)
TGF β-1 + HAp/PLGA	60	9 (26.67%)	17 (33.33%)*	29 (66.67%)†‡
HAp/PLGA	60	5 (13.33%)	9 (26.67%)	15 (33.33%)

n – number of teeth; TGF β-1 – transforming growth factor beta-1; HAp/PLGA – hydroxyapatite/poly (lactide-co-glycolide).
***$p < 0.01$ vs. control group; †$p < 0.05$ vs. the same group one month after the treatment; ‡$p < 0.001$ vs. control group.**

Comparing the number of teeth among the studied groups in the same time intervals, it was found that 3 months after the treatment, the number of teeth with dentine bridges in the TGF β-1 + HAp/PLGA group was statistically significantly greater than in the control group ($p < 0.01$). Six months after the treatment, the number of teeth with dentine bridges in the TGF β-1 + HAp/PLGA group (66.67%) was statistically significantly greater than among controls at an even higher level of statistical significance ($p < 0.001$). In the same period, comparing the TGF β-1 + HAp/PLGA and HAp/PLGA groups 6 months after the treatment, it was established that the number of teeth with dentine bridges in the TGF β-1 + HAp/PLGA group was twice the number observed in the HAp/PLGA group (66.67% vs. 33.00%), but a statistically significant difference was not established due to small sample size.

Results of the SEM analysis

After an observation period of one month, the results of SEM analysis with direct pulp capping after the application of HAp/PLGA showed the formation of fibrodentine of atubular structure (Figure 1), and after TGF β-1 and HAp/PLGA application, the results showed a regular structure of reparative dentine from the cavum towards the periphery (Figure 2).

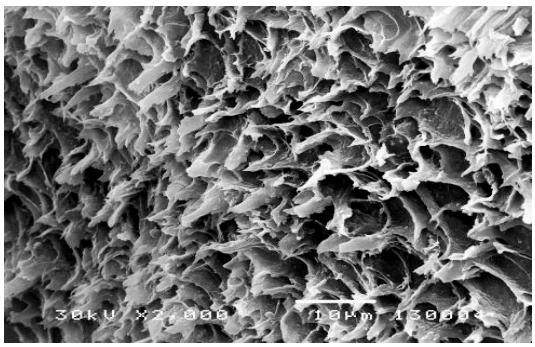


Fig. 1 – Calcium hydroxyapatite/poly (lactide-co-glycolide): atubular irregular dentine (fibrodentine) – scanning electron microscopy finding (magnification is labeled inside the figure).

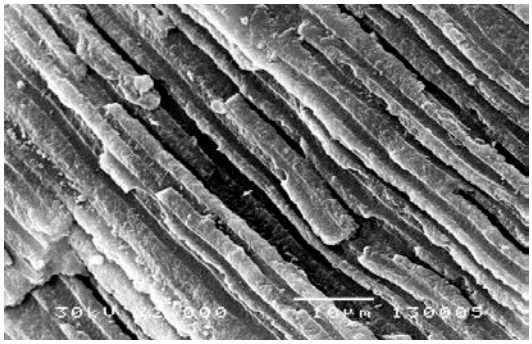


Fig. 2 – Transforming growth factor beta-1 and calcium hydroxyapatite/poly (lactide-co-glycolide): regular structure of reparative dentine at the periphery – scanning electron microscopy finding (magnification is labeled inside the figure).

After an observation period of three months, the results of SEM analysis with direct pulp capping after the application of HAp/PLGA revealed the formation of fibrodentine of atubular structure (Figure 3), and after TGF β-1 and HAp/PLGA application, the results showed the presence of newly formed dentine with numerous channels and dentine bridge formation – tubular dentine (Figure 4).

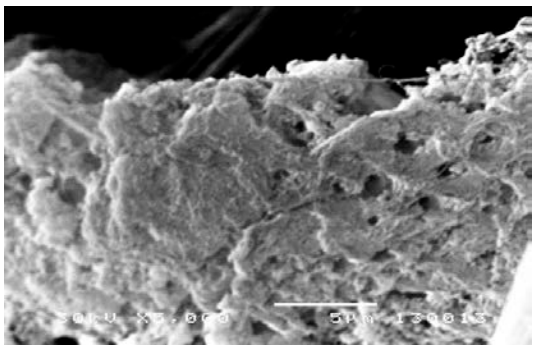


Fig. 3 – Calcium hydroxyapatite/poly (lactide-co-glycolide): a detail of the dentine bridge (fibrodentine with atubular structure finding) – scanning electron microscopy finding (magnification is labeled inside the figure).

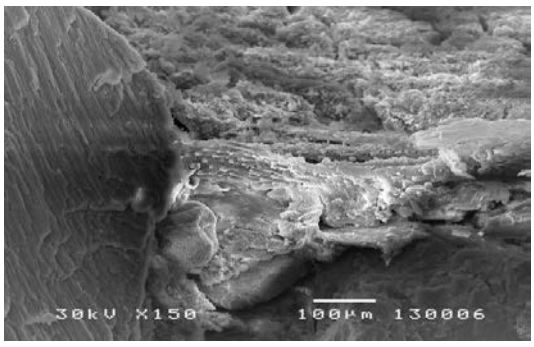
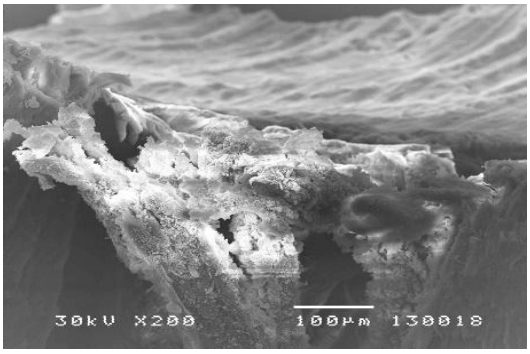
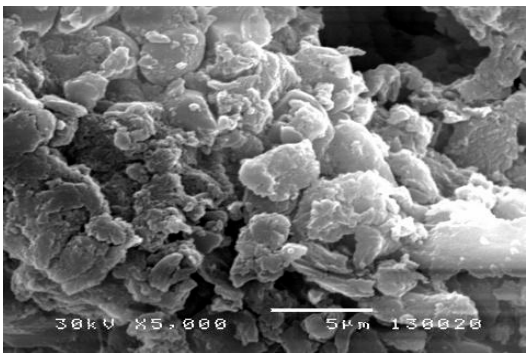


Fig. 4 – Transforming growth factor beta-1 and calcium hydroxyapatite/poly (lactide-co-glycolide): dentine bridge (tubular dentine) – scanning electron microscopy finding (magnification is labeled inside the figure).

After an observation period of six months, the results of SEM analysis with direct pulp capping after the application of HAp/PLGA showed the presence of dentine bridges, amorphous fibrodentine, dentine bridges with large hydroxyapatite crystals, fibrodentine rich in bioactive proteins (Figure 5), and after TGF β-1 and HAp/PLGA application, the results indicated the formation of dentine bridges with tubular structure, reparative dentine, calcified dentine, calcified Tomes fibers and calcified pulp within dental roots (Figure 6). Figure 7 shows regular dentine in an intact tooth.



a)



b)

Fig. 5 – Calcium hydroxyapatite/poly (lactide-co-glycolide): a) dentine bridge (amorphous dentine); b) dentine bridge structure (amorphous fibrodentine) – scanning electron microscopy finding (magnifications are labeled inside the figures).

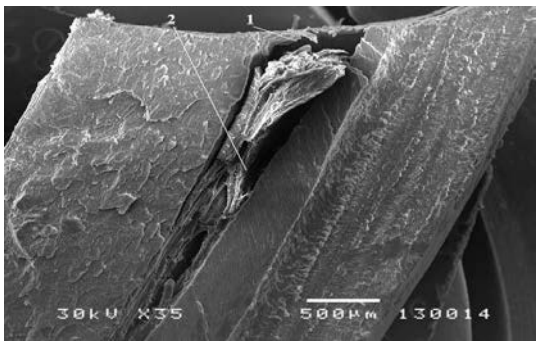


Fig. 6 – Transforming growth factor beta-1 and calcium hydroxyapatite/poly (lactide-co-glycolide): 1) dentine bridge with tubular structure; 2) portion of the calcified pulp within dental root canal – scanning electron microscopy finding (magnification is labeled inside the figure).

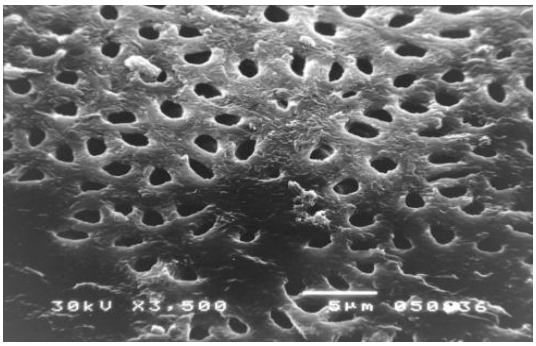


Fig. 7 – Regular dentine (an intact tooth) – scanning electron microscopy finding (magnification is labeled inside the figure).

Discussion

Direct pulp capping is a therapeutic procedure of tooth vitality preservation, whereby adequate materials are applied onto the exposed pulp tissue in order to stimulate reparative dentine formation [1]. Since the material used for direct pulp capping comes into direct contact with the pulp tissue, it, therefore, plays a key role in this kind of treatment [24].

Our study dealt with the advantages of applying hydroxyapatite calcium HAp/PLGA alone or combined with growth factors. A high biological potential of the pulp, including optimal conditions for the tissue function, with adequate vascularization and absence of inflammation, was the primary criterion in the interpretation of obtained results. The tested materials were applied in accordance with the manufacturers' manuals. HAp/PLGA powder was mixed with saline in order to obtain the consistency easy to manipulate [29].

In the *in vitro* and *in vivo* studies, the biocompatibility of HAp/PLGA, composite beta carotene/PLGA, and its effect on dental pulp cells have been demonstrated. Histological analysis has demonstrated the presence of cellular infiltration and dentine bridge formation after 60 days. In all studied groups, fibroblast development and growth and survival of macrophages were identified [30, 31].

In recent years, growth factors and their role in the initiation of reparatory processes in pulp injury have attracted much attention, which was partially the subject of our study as well. These bioactive molecules promote proliferation and differentiation of cells, matrix synthesis, and angiogenesis. Growth factors are necessary for tissue regeneration, and they have an important role in inducing angiogenesis, i.e. oxygenation and supply of nutrients essential for biological functioning.

Numerous authors have identified various difficulties in clinical manipulation, application, and retention of the materials at the site of application [30]. As the potentially suitable growth factor carriers, calcium-phosphate-based materials have been suggested, like those in our study, the porous structure of which enables gradual release and diffusion of growth factors. In our study, HAp was shown to be a good growth factor carrier.

Since synthetic biomaterials have been shown to be successful in the restoration of bone tissue, with their well-known biocompatibility and bioconductivity, the intention of this study was to investigate the use of HAp as a synthetic biomaterial and growth factors in direct pulp capping.

The results obtained by Tziafas et al. [32] have shown that the use of some of the growth factors, especially TGF β, stimulates odontoblast differentiation and leads to the release of endogenous growth factors contained in the organic dentine matrix, which further stimulates dentinogenesis [33, 34].

The results of the study by Popović Bajić et al. [35] have shown the highest regularity in the organization of deeper pulp layers in the zones of the thickest dentine bridges. These results have also shown the farthest deviation from the statement concerning platelet-rich fibrin in direct pulp capping in animals compared to other materials. In their study, they proved the formation of calcified tissue in the pulp in all the samples, which partly agreed with our research. However, it is still unclear which growth factor concentration in platelet-rich fibrin is optimal for the processes of reparation and regeneration.

In all their cases, Hebling et al. [36] have demonstrated dentine bridge formation with direct pulp capping using autogenous growth factors combined with a HAp-based material. In two observation periods, increased dentine bridge thickness was noticed, although without statistically significant differences, compared to the groups where HAp alone was applied. In this study, similar results were obtained: in a shorter observation period, dentine bridge formation occurred more rapidly and more regularly in the samples in which HAp combined with growth factors was applied, although without any statistically significant differences. At the end of a 12-month observation period, the results were the same for both HAp-treated samples and those treated with HAp combined with thrombocyte-rich plasma, suggesting that growth factors produced more rapid healing, i.e. dentine bridge formation, which agreed with our results.

Numerous studies have stressed the importance of adequate cavity sealing after the therapy and prevention of superimposed bacterial contamination, which was provided

for this study by the application of glass-ionomer cement and amalgam for cavity sealing [37].

Formation, quality, and thickness of the calcified bridge, presence of inflammatory cells, and pulp tissue preservation are all important indicators of tissue response in the therapy with direct pulp capping [38]. According to Accorinte et al. [39], these parameters have been considered relevant and used for histological assessment of treatment success with the nanomaterials tested in this study. The formation of the dentine bridge at the interface of pulp and material used for direct capping is an issue for further discussion since a dentine bridge is not necessarily the sign of dental pulp preservation (health). The presence of dentine bridges may be interpreted as a sign of healing or as a reaction (response) to irritation. In this study, it was interpreted as a sign of biocompatibility and bioactivity of the material, which agrees with other authors' opinions [26, 28]. Dentine matrix is not just a scaffold serving to mobilize and support the development of mineralized tissue; it is also the pool of growth factors excreted by odontoblasts and pulp fibroblasts [40]. These growth factors hypothetically produce signals for proliferation, differentiation, and recruitment of pulp cells at the sites of injured pulp tissue and initiate tissue regeneration [41, 42].

In all the experimental samples of our study, 1, 3, and 6 months after the treatment, a thin layer of calcified tissue – dentine bridge – was observed. These follow-up intervals matched those in the studies in which hard tissue formation was observed as early as 2 weeks after the treatment [28, 33].

Finally, it should be mentioned that these findings are the result of a healthy dental pulp response, without any inflammation, and that the performance of these materials in the presence of inflamed pulp will be assessed in further studies. Some authors have reported the absence of dentine bridges, while others report well-calcified bridges after the period of three months [43, 44].

In the study by Nowicka et al. [45], it has been histologically confirmed that Biodentin® (a calcium-silicate based material), applied as direct pulp capping of intact teeth planned for extraction, leads to the formation of calcified tissue and dentine bridges in 50% of samples after the period of 6 weeks.

Conclusion

Based on all of the above, a conclusion may be drawn that calcium HAp/PLGA combined with TGF β-1 yields better results after both shorter observation period (3 months) and longer period of time (6 months) compared to HAp/PLGA alone, which has been demonstrated as a good growth factor carrier.

It is reasonable to consider with all the necessary precautions the clinical use of growth factors, especially TGF β, which has been reported to be able to induce differentiation of the second generation of odontoblast cells.

TGF β directly induces morphological and functional differentiation of neo-odontoblasts. However, the clinical use of TGF β may lead to the "doubling" of its unchanged positive action. The clinical use of TGF β also involves paying special attention to the means of molecule transport, response dosing, and control of the degree of reparation processes, molecule half-life, and possible immune reactions.

R E F E R E N C E S

1. *Zhang S, Yang X, Fan M.* BioAggregate and iRoot BP Plus optimize the proliferation and mineralization ability of human dental pulp cells. Int Endod J 2013; 46(10): 923–9.
2. *Živojinović V.* Biological aspects of use of calcium hydroxyapatite and mineral trioxide aggregate in the treatment of diseased pulp. [dissertation]. Belgrade: University of Belgrade, Faculty of Dentistry; 2008. (Serbian)
3. *Teodorović N.* Primena keramičkih biomaterijala u lečenju kanala korena zuba. [dissertation]. Belgrade: University of Belgrade, Faculty of Dentistry; 1998. (Serbian)
4. *Witherspoon DE, Small JC, Harris GZ.* Mineral trioxide aggregate pulpotomies: a case series outcomes assessment. J Am Dent Assoc 2006; 137(5): 610–8.
5. *Petrović M.* Biological activity of composite cellular carriers based on highly porous hydroxyapatites and their impact in bone tissue engineering. [dissertation]. Belgrade: University of Belgrade, Faculty of Dentistry; 2012. (Serbian)
6. *Petrović V.* Modalities of use of hydroxyapatite in apexogenesis. [dissertation]. Belgrade: University of Belgrade, Faculty of Dentistry; 2008. (Serbian)
7. *Suwanprateeb J, Thammarakcharoen F, Wasoontararat K, Chokevivat W, Phanphiriya P.* Preparation and characterization of nanosized silver phosphate loaded hydroxyapatite by single step co-conversion process. Mat Sci Eng 2012; 32(1): 2122–8.
8. *Liu WN, Chang J, Zhu YQ, Zhang M.* Effect of tricalcium aluminate on the properties of tricalcium silicate-tricalcium aluminate mixtures: setting time, mechanical strength and biocompatibility. Int Endod J 2011; 44(1): 41–50.
9. *Song Y, Lin K, He S, Wang C, Zhang S, Li D*, et al. Nano-biphasic calcium phosphate/polyvinyl alcohol composites with enhanced bioactivity for bone repair via low-temperature three-dimensional printing and loading with platelet-rich fibrin. Int J Nanomedicine 2018; 13: 505–23.
10. *Khalil WA, Eid NF.* Biocompatibility of BioAggregate and mineral trioxide aggregate on the liver and kidney. Int Endod J 2013; 46(8): 730–7.
11. *Kong D, Chen Z.* Evaluation of the interaction between hydroxyapatite and bisphosphonate by nonlinear capillary electrochromatography. J Sep Sci 2017; 40(9): 2030–6.
12. *Gangolli RA, Devlin SM, Gerstenhaber JA, Lelkes PI, Yang M.* A Bilayered Poly (Lactic-Co-Glycolic Acid) Scaffold Provides Differential Cues for the Differentiation of Dental Pulp Stem Cells. Tissue Eng Part A 2019; 25(3-4): 224–33.
13. Popović Bajić M, Danilović V, Prokić BB, Prokić B, Jokanović V, Živković S. Biodentin used for direct pulp capping. Stom Glas S 2014; 61(2): 67–74.
14. *Katge FA, Patil DP.* Comparative Analysis of 2 Calcium Silicate-based Cements (Biodentine and Mineral Trioxide Aggregate) as Direct Pulp-capping Agent in Young Permanent Molars: A Split Mouth Study. J Endod 2017; 43(4): 507–13.
15. *Fuzinatto RN, Farina AP, Souza MA, Miyagaki DC, Randi Ferraz CC, Cecchin D.* Effects of an endodontic auxiliary chemical substance on the bond strength of two methacrylate-based en-

dodontic sealers to dentin. Microsc Res Tech 2017; 80(6): 627–33.
16. *Lickorish D, Ramshaw JA, Werkmeister JA, Glattauer V, Howlett CR*. Collagen-hydroxyapatite composite prepared by biomimetic process. J Biomed Mater Res 2004; 68(1): 19–27.
17. *Unda FJ, Martín A, Hernandez C, Pérez-Nanclares G, Hilario E, Aréchaga J*. FGFs-1 and -2, and TGF beta 1 as inductive signals modulating in vitro odontoblast differentiation. Adv Dent Res 2001; 15: 34–7.
18. *Leong DJ, Setzer FC, Trope M, Karabucak B*. Biocompatibility of two experimental scaffolds for regenerative endodontics. Restor Dent Endod 2016; 41(2): 98–105.
19. *Gala-Garcia A, Texiera KI, Wykrota FH, Sinisterra RD, Cortes ME*. Bioceramic- Poly (glycolic)-poly (lactic acid) composite induces mineralized barrier after direct capping of rat tooth pulp tissue. Brazil Oral Res 2010; 24(1): 8–14.
20. *Li F, Liu X, Zhao S, Wu H, Xu HH*. Porous chitosan bilayer membrane containing TGF-β1 loaded microspheres for pulp capping and reparative dentin formation in a dog model. Dent Mater 2014; 30(2): 172–81.
21. *Oh SP, Seki T, Goss KA, Imamura T, Yi Y, Donahoe PK* et al. Activin receptor-like kinase 1 modulates transforming growth factor-beta 1 signaling in the regulation of angiogenesis. Proc Natl Acad Sci U S A 2000; 97(6): 2626–31.
22. *Popović- Bajić M.* Impact of amelogenin, growth factors and new nanostructural materials based on calcium silicate cements on pulp regeneration. [dissertation]. Belgrade: University of Belgrade, Faculty of Dentistry; 2016. (Serbian)
23. *Besinis A, van Noort R, Martin N*. Remineralization potential of fully demineralized dentin infiltrated with silica and hydroxyapatite nanoparticles. Dent Mater 2014; 30(3): 249–62.
24. *Luiz de Oliveira da Rosa W, Machado da Silva T, Fernando Demarco F, Piva E, Fernandes da Silva A*. Could the application of bioactive molecules improve vital pulp therapy success? A systematic review. J Biomed Mater Res A 2017; 105(3): 941–56.
25. *Tziafas D, Smith AJ, Lesot H*. Designing new treatment strategies in vital pulp therapy. J Dent 2000; 28(2): 77–92.
26. *Asgary S, Shahabi S, Jafarzadeh T, Amini S, Kheirieh S*. The properties of a new endodontic material. J Endod 2008; 34(8): 990–3.
27. *Melin M, Joffre-Romeas A, Farges JC, Couble ML, Magloire H, Bleicher F*. Effects of TGF-beta 1 on dental pulp cells in cultured human tooth slices. J Dent Res 2000; 79(9): 1689–96.
28. *Zarrabi MH, Javidi M, Jafarian AH, Joushan B*. Histologic assessment of human pulp response to capping with mineral trioxide aggregate and a novel endodontic cement. J Endod 2010; 36(11): 1778–81.
29. *Moon HJ, Kim KN, Kim KM, Choi SH, Kim CK, Kim KD*, et al. Bone formation in calvarial defects of Sprague-Dawley rats bytransplantation of calcium phosphate glass. J Biomed Mater Res A 2005; 74(3): 497–502.
30. *Pissiotis E, Spangberg LS*. Biological evaluation of collagen gels containing calcium hydroxide and hydroxyapatite. J Endod 1990; 16(10): 468–73.
31. *van Noort R, Brown D, Clarke R, Combe EC, Curtis R, Lloyd CH*, et al. Dental materials: 1992 literature review. J Dent 1994; 22(1): 5–28.
32. *Tziafas D, Pantelidou O, Alvanou A, Belibasakis G, Papadimitriou S*. The dentinogenic effect of mineral trioxide aggregate (MTA) in short term capping experiments. Int Endod J 2002; 35(3): 245–54.
33. *Chang KC, Chang CC, Chen WT, Hsu CK, Lin FH, Lin CP*. Development of calcium phosphate/sulfate biphasic cement for vital pulp therapy. Dent Mater 2014; 30(12): e362–70.
34. *Swarup SJ, Rao A, Boaz K, Srikant N, Shenoy R*. Pulpal response to nano hydroxyapatite, mineral trioxide aggregate and calcium hydroxide when used as a direct pulp capping agent: an in vivo study. J Clin Pediatr Dent 2014; 38(3): 201–6.
35. *Popović Bajić M, Danilović V, Prokić B, Milošević V, Živković S*. Histologocal effects of Emdogain Gel on exposed dental 10 pulp. 20th Congress of the Balkan Stomatological Society (BaSS); Bucharest; 2015. (oral presentation)
36. *Hebling J, Giro EM, Costa CA*. Biocompatibility of an adhesive system applied to exposed human dental pulp. J Endod 1999; 25(10): 676–82.
37. *Mjör IA*. Pulp-dentin biology in restorative dentistry. Part 7: The exposed pulp. Quintessence Int 2002; 33(2): 113–35.
38. *Shahravan A, Jalali SP, Torabi M, Haghdoost AA, Gorjestani H*. Ahistological study of pulp reaction to various water/powder ratios of white mineral trioxide aggregate as pulp-capping material in human teeth: a double-blinded, randomized controlled trial. Int Endod J 2011; 44(11): 1029–33.
39. *Accorinte Mde L, Holland R, Reis A, Bortoluzzi MC, Murata SS, Dezan E Jr,* et al. Evaluation of mineral trioxide aggregate and calcium hydroxide cement as pulp-capping agents in human teeth. J Endod 2008; 34(1): 1–6.
40. *Chen CL, Huang TH, Ding SJ, Shie MY, Kao CT*. Comparison of calcium and silicate cement and mineral trioxide aggregate biologic effects and bone markers expression in MG63 cells. J Endod 2009; 35(5): 682–5.
41. *Bègue-Kirn C, Smith AJ, Ruch JV, Wozney JM, Purchio A, Hartmann D,* et al. Effects of dentin proteins, transforming growth factor beta 1 (TGF beta 1) and bone morphogenetic protein 2 (BMP2) on the differentiation of odontoblast in vitro. Int J Dev Biol 1992; 36(4): 491–503.
42. *Piattelli A, Rubini C, Fioroni M, Tripodi D, Strocchi R*. Transforming growth factor-beta 1 (TGF-beta 1) expression in normal healthy pulps and in those with irreversible pulpitis. Int Endod J 2004; 37(2): 114–9.
43. *Jabbarifar E, Razavi SM, Ahmadi N*. Histopathologic responses of dog's dental pulp to mineral trioxide aggregate, bio active glass, formocresol, hydroxyapatite. Dent Res J (Isfahan) 2007; 4: 83–7.
44. *Popović Bajić M, Jokanović V, Danolović V, Živković S*. Histological Evaluation of Direct Pulp Capping with Novel Nanostructural Materials based on Active Silicate Cements on Pulp Tissue. 47th Meeting of the Continental European Division of the International Association for Dental Research (CED-IADR). Belek, Antalya; 2015. (poster presentations)
45. *Nowicka A, Wilk G, Lipski M, Kołecki J, Buczkowska-Radlińska J*. Tomographic Evaluation of Reparative Dentin Formation after Direct Pulp Capping with Ca(OH)2, MTA, Biodentine, and Dentin Bonding System in Human Teeth. J Endod 2015; 41(8): 1234–40.

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