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PRIMENA KALCIJUM-HIDROKSIAPATITA I FAKTORA RASTA U ENDODNTSKOJ TERAPIJI

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Running title:
Calcium hydroxyapatite in endodontic therapy
Abstract

**Background / Aim.** Hydroxypatite (HAp) is one of the most commonly used calcium phosphate bioceramics with osteoconductive properties. Growth factors (TGF-β) are capable of directly inducing morphological and functional differentiation of odontoblasts. The aim of this paper was to investigate the effectiveness of biomaterials in combination with growth factors in the creation of new dentine and obturation of the root canal apex in the teeth of our experimental model. **Methods.** Rodent (rabbit) teeth were used as the experimental animal model. After pulp removal with a pulp extirpator in vital pulpectomy, the biomaterial was applied using a Lentulo spiral in the apex portion at the level of the physiological *foramen apicale*. The experiment was performed in general anesthesia. Animals were kept alive for 3, 6 and 12 months. The extracted teeth were prepared for scanning electron microscopy (SEM) observation. **Results.** Using SEM, it was found that the number of teeth with newly created dentine and apex canal obturation was greater 12 months after the treatment. **Conclusion.** In our experimental groups, apex obturation of the dental root canal with newly created dentine took place.

**Key words:**

TGF-β, HAp/PLGA, endodontic therapy, apex root canal obturation, rabbits.
Abstrakt


Ključne reči: TGF-β, HAp/PLGA, endodonska terapija, apeksna opturacij kanala, kunići.

Introduction

A diagnosis of the degree of pulp inflammation is essential in the attempts to preserve pulp vitality by appropriate strategies whenever it is possible.

It is generally accepted that the prognosis of dental root canal treatment largely depends on the quality of root canal filling. The apical third of the canal deserves special attention in the mechanical preparation, since it is the most sensitive zone which communicates with vital tissue most important for the process of healing. A provision of biologically acceptable sealing of the apical portion of root canal before the definitive filling, has urged many authors to consider the issue of apical barrier formation during root canal treatment, which would prevent material crossing over during the obturation on one hand, and provide high quality, compact and airtight canal filling on the other. An apical
plug would prevent the occurrence of adverse effects associated with the material used for definitive canal filling in the periapical area.

Teodorović has combined hydroxyapatite powder with 35% of calcium sulphate and successfully used it for the formation of apical plugs in endodontically treated teeth with completed root growth.

There are few materials capable of inducing the creation of hard tissues, especially the cement tissue, and even able to stimulate bone reparation if large defects have occurred.

Over the years, various materials have been investigated as the potential therapeutic agents in vital pulpectomy. Calcium hydroxide has been most extensively studied and used; despite all of its positive properties, it is not an ideal biological material for apical sealing, and many authors think that its stimulative effects have not been sufficiently elucidated as yet.

Gollmer was the first author who created an apical plug out of dentine chips, believing that successful healing after pulpectomy could not be expected without an effective, biocompatible apical “sealant”, which would prevent an irritative contact of the used filling materials with periapical tissue.

Dianat et al. used dentine plugs to create an apical seal in their experiment on monkey teeth. The results of their study demonstrated solid tissue (osteodentine) creation at the interface of dentine powder and the remaining vital pulp stump after vital pulpectomy. The studies by Jacobsen et al. contested the positive results obtained by the use of dentine plugs on the account of significant apical „leakage“ after such an obturation. Recent studies in both medicine and dentistry have attempted to identify synthetic materials which would not act as antigens and at the same time would be able to successfully replace bone tissue, i.e. to have an osteoconductive effect. Ceramic biomaterials based on hydroxyapatite or three-calcium phosphate are most similar to inorganic bone tissue component by their chemical composition and structure. Some studies have investigated the use of biomaterials such as hydroxyapatite for pulp therapy within the techniques of direct pulp capping—, amputation of the coronal portion of the pulp— and for endodontic treatment of the teeth with completed root growth as a material for apical barrier formation. Hydroxyapatite (HAP) is one of the most commonly used calcium phosphate bioceramic
materials with osteoconductive properties. As a potentially good growth factor delivery vehicle (scaffold), calcium phosphate-based materials have been suggested, the porous structure of which enables gradual release and diffusion of growth factors.

Pissiotis and Spangberg concluded, based on the above studies, that due to tissue reaction predictability and stability of both hydroxyapatite and its mixture with collagen, the „plug“ created by the compression of crystals of these materials could represent an optimal solution for apical plug formation. These authors also suggested that clinical problems associated with the manipulation and application of hydroxyapatite crystals into the apical third of dental root canal warranted further extensive studies.

According to Grossman, the ultimate goal of dental root canal therapy is airtight (hermetic) filling of the canal space.

Sagavara et al. have investigated in vivo on canine teeth the use of calcium phosphate ceramic as a material for definitive dental root canal filling. They proved that the material was compatible with periapical tissue and capable of binding and forming a solid mass in the presence of tissue fluids.

Mongiorgi, Prati et al. have studied new aloplastic bioceramic material formulations, but with a new cement composition for definitive dental root canal filling – Proendo, Vebas, Italy. The authors concluded, based on the obtained results, that the material was biocompatible, osteoconductive, non-toxic, with good adhesive properties and that it provided good apical sealing which prevented percolation and transit of both bacteria and their products along the endodontically processed/prepared and definitively filled dental root canals.

There have been attempts to use hydroxyapatite and growth factors for the same purpose, although with very low success rates. These studies are still very attractive, though.

The results of the above studies have shown that the use of some of the growth factors, especially TGF-β, can stimulate odontoblast differentiation and induces the release of endogenous growth factors contained in the organic dentine matrix, which additionally stimulates dentinogenesis. Recent insights into the role of growth factors in dental tissue reparation, whether it is reactive or reparative dentinogenesis, could represents the basis for a different approach to pulp treatment. Of course, nowadays, therapeutic procedures
involving teeth with incomplete root growth are being rationalized, and the time required for a therapeutic procedure is being increasingly shorter.

Some clinical studies have shown that the use of Platelet-Rich Plasma (PRP) has beneficial effects on the reparation processes, while other studies do not report such an effect. These conflicting data can be perhaps explained by different methods of preparation and, consequentially, different PRP concentrations. In fact, the issue of PRP concentration which is optimal for tissue reparation and regeneration is still unresolved.

**Aim of the paper**

Our aim in this study was to investigate the effectiveness of calcium hydroxyapatite and growth factors as medicaments on the creation of new dentine and apical dental root canal obturation in vital pulp extirpation on the teeth of our experimental model.

**Methods**

The study was performed at the Institute for Biomedical Research, Faculty of Medicine in Niš, and at the Faculty of Medicine in Priština – seated in Kosovska Mitrovica, with the approval of the Ethics Committee of the Faculty of Medicine no. 05-603/1, obtained in 2011.

Three 6-month old chinchilla rabbits, 3-4 kg BW each, were included in the experiment. The animals were anesthetized by intramuscular administration of Zoletil 100 (Virbac S.A. ere avenue-2065 m-L.I.D. 06516 Carros, France) at a dose of 10 mg/kg BW and Ketalar (1-4.5 mg/kg BW). After pulp space trepanation, in samples for vital extirpation, the pulp was removed using a pulp extirpator, and the biomaterial was applied with Lentulo spiral up to the level of physiological apical foramen. Flexofile (Mallefer) endodontic files were used for biochemical canal processing. Definitive canal obturation was performed with Lentulo spiral again, with an AH plus root canal sealer and gutta-percha points. All the cavities were definitively capped with a glass ionomer cement (GIC) and dental amalgam.

In this study we used calcium hydroxyapatite/poly(lactide-co-glycolide) HAp/PLGA and recombinant human growth factor TGF-β.

The teeth were divided into three groups:
1. Experimental group (n=15), composed of lower jaw teeth on the left side (incisors, premolars, molars), into which calcium hydroxyapatite/poly(lactide-co-glycolide) (HAp/PLGA) biomaterial was applied.

2. Experimental group (n=15), composed of upper jaw teeth on the right side (incisors, premolars, molars), into which calcium hydroxyapatite/poly(lactide-co-glycolide) (HAp/PLGA) biomaterial was applied, combined with TGF-β growth factor. Calcium hydroxyapatite/poly(lactide-co-glycolide) (HAp/PLGA) biomaterial served as the delivery vehicle, 80:20 (0.5 g) (product of ITN SANU, Belgrade).

3. Control group (n=15), composed of intact teeth in the left upper jaw and right lower jaw (incisors, premolars, molars) from the same sacrificed animals.

After this, initial part of the study, the animals were kept alive for 3, 6 and 12 months, and after that sacrificed with a lethal dose of Ketalar. Jaw bones were disarticulated and each tooth was extracted separately. Material preparation involved the storage of teeth in sterile saline at 40°C, without any fixing agents.

All the samples were processed by a single operator. Occlusal surfaces 2-3 mm thick (dental crowns) were circularly cut with the finest fissure diamond burs. Dental roots were incised longitudinally with dental separator discs in order to provide adequate separation into the oral and vestibular halves. Each half of the sample was mounted onto an appropriate stand, and the samples thus fixated were gold evaporated in a vacuum evaporator and observed under a scanning electron microscope JEOL-JCM-5300.

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

The differences between the parameters of interest among the groups, as well as within the groups, were established using the Mantel-Haenszel chi-square test or Fisher’s test of exact probability of the null hypothesis (when some of the expected frequencies was below 5).

**Results**

Statistical significance of the obtained results was presented in Tables 1 and 2. In total, 45 teeth from 3 sacrificed experimental animals (chinchilla rabbits) were included in the investigation. Vital pulp extirpation (VPE) was performed in both experimental groups
on the same number of teeth (15 teeth from each group). In control group, there were no teeth with dental root canal obturation. Comparing the groups with TGF-β1+HAp/PLGA and HAp/PLGA after 12 months, the greatest difference in the number of teeth with obturated canals was found, but the difference did not reach the level of statistical significance (p=0.3).

The results obtained after the application of TGF-β and HAp/PLGA – calcium hydroxyapatite/poly(lactide-co-glycolide) – obtained after 3, 6 and 12 months by SEM microscopy for vital pulp extirpation (VPE) are shown in Figures 1-8.

Discussion

The results of the study showed that apical obturation of dental root canal occurred in our experimental groups.

In order to evaluate objectively and interpret clinically in a valid way these results, we should review the methodology employed in the study. Rodent (rabbit) teeth were used as an animal model, although frequently regarded as inappropriate for such experiments due to their specificity reflected in constant growth and wear (which refers especially to frontal teeth). Nevertheless, many authors dispute such an attitude, emphasizing that the rodent pulp-dentine complex has a significant potential for the study of many aspects of reactive dentinogenesis, as well as in the observation of pulp reactions to bioactive molecules.

The interaction between the material and injured pulp tissue, as well as the pathways of initiation and progression of healing and regeneration processes, are still insufficiently understood. There are numerous hypotheses about that, but the latest studies have paid significant attention to growth factors and their roles in angiogenesis, progenitor cell mobilization, differentiation and, finally, biomaterial-supported mineralization.

In all the samples of the study, in the period of 12 months, the application of hydroxyapatite and growth factors produced complete apical dental root canal obturation with newly formed dentine.

Many authors have also noticed the difficulties in clinical manipulation, application and retention of material at the application site, especially with high pulp amputations and deeper material placements into the root canal. These authors therefore recommended a
collagen-HAp combination, with satisfactory results in laboratory animals and in pre-prosthetic preparation of the alveolar process.

Calcium phosphate vehicles/scaffolds for growth factors have also been suggested as potentially good (which agrees with our study), which enable gradual release and diffusion of growth factors due to their porous structure. In our study, HAp was a good growth factor delivery vehicle.

Our results also corroborate other authors’ findings in studies with dogs, which demonstrated stability and osteoconduction using calcium phosphate ceramic as a definitive filling material.

Petrović et al., using synthetic hydroxyapatite in their study (with average particle size of 100 µm) on laboratory animals – dogs, applied the material on the pulp of young teeth with incomplete root growth. In one part of the study, in addition to the tested material (hydroxyapatite), they also applied an autogenic growth factor originating from platelet-rich plasma in amputations and high amputations of the pulp. All the samples were radiographically controlled and compared to contralateral untreated teeth. Based on the analysis of dental x-rays, it was found that root apex formation continued in all the studied samples.

Teodorović combined hydroxyapatite powder with 35% of calcium sulphate and used it with success as a paste for the formation of apical plugs in endodontically treated teeth with completed root growth. In addition to biocompatibility, the studies have shown that HAp is a stable and osteoconductive material. The results of the histological analysis showed adequate stability, evidenced by the presence of HAp in the period of 24 experimental weeks without any signs of resorption. Furthermore, other authors’ results agree with these results, demonstrating stability and osteoconduction in their experiments on dogs, using calcium phosphate ceramic as a definitive root canal filling material. The studies have shown that all the reactions between hard tissues (dentine, cement) and HAp take place at their interface (contact surfaces).

As some studies have demonstrated, HAp is applicable in clinical practice in the formation of apical plugs, but care should be taken regarding the type of material for definitive root canal filling which covers the placed biological plug. At the end of the 12-month observation period, the results were identical for both the samples treated with HAp.
and those treated with HAp and platelet-rich plasma, suggesting that growth factors influenced more rapid healing, i.e. dentine bridge creation and complete apical dental root canal obturation with newly formed dentine-, which agreed with our findings.

In recent years, much attention has been paid to growth factors and their role in the initiation of reparation processes in pulp damage, which constituted a part of our study as well. These bioactive molecules promote proliferation and differentiation of cells, matrix synthesis and angiogenesis. Very attractive are also the studies, both preclinical and clinical, the results of which indicate that the use of growth factors is able to provide favorable prognosis regarding bone, periodontium, and cement regeneration.

The results by Tziafas et al. have shown that the use of some of the growth factors, especially TGF-β, is able to stimulate odontoblast differentiation and leads to the release of endogenous growth factors contained in the dentine organic matrix, which additionally stimulates dentinogenesis.

A strictly applied contemporary conception of root canal treatment enables and facilitates healing processes in the apical periodontium, which is the principal goal of a successful endodontic treatment. Apical barrier formation during the treatment is especially important in specific clinical situations. The barrier, i.e. apical „plug“, plays multiple roles, opposing toxic actions at the interface of the definitive filling material and vital periapical tissue and enabling high quality and complete, airtight dental root canal filling. Apical plug formation and „micro leakage“ problems have not been solved by the attempts with calcium hydroxide, nor with the use of dentine chips.

Sugawara et al. and Teodorović et al. have reported that ceramic biomaterials are capable of binding and forming a solid mass in the presence of tissue fluids following a definitive root canal filling.

In the era of regenerative endodontics, the introduction of new procedures and materials is expected to take place as both a biological treatment and for the purpose of tooth revitalization.

**Conclusion**
Based on the above facts, a conclusion may be drawn that in the experimental groups new dentine was indeed created and apical root canal closure occurred. HAp/PLGA was shown to be a good growth factor delivery vehicle.

**Literature**

3. Teodorović N. Research in adhesive performances of three canals sealers – SEM study. 7th Congress of the Balcan Stomatological Society (BaSS); 2002; Kushadası, Turkie.
Table 1 – Total number of treated teeth in experimental groups and in control group

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Number of treated teeth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>I</td>
<td>5</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total number of teeth:</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>

Table 2 – Dental root canal capping during VPE in experimental groups and in control group (SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>(TGF-β + HAp/PLGA)</td>
<td>15</td>
<td>3 (20.0)</td>
<td>3 (20.0)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>(HAp/PLGA)</td>
<td>15</td>
<td>0 (0)</td>
<td>1 (6.7)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>(Control)</td>
<td>15</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Figure 1 - TGF-β1 Incomplete apical sealing with newly formed dentine (3-month observation period)

Figure 2 - HAp/PLGA - Incomplete apical sealing with newly formed dentine (3-month observation period)

Figure 3 - TGF-β1+ HAp/PLGA Complete root canal obturation with newly formed dentine (6-month observation period)
Figure 4 - HAp/PLGA- Irregular dentine (6-month observation period)

Figure 5 - TGF-β1 +HAp/PLGA- Complete apical obturation with newly formed dentine (12-month observation period)

Figure 6 - HAp/PLGA- Apical obturation with newly formed dentine (12-month observation period)
Figure 7 - TGF-β1 +HAp/PLGA- Complete apical obturation with newly formed dentine (12-month observation period)

Figure 8 – Control group – SEM intact tooth. a) open dental root canal apex, b) regular dentine