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Use of calcium hydroxyapatite and growth factors in endodontic therapy

Primena kalcijum-hidroksiapatita i faktora rasta u endodontskoj terapiji

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Abstract

Background/Aim. Hydroxyapatite (HAp) is one of the most commonly used calcium phosphate bioceramics with osteoconductive properties. Growth factors are capable of directly inducing morphological and functional differentiation of neodontoblasts. The aim of this study was to investigate the effectiveness of HAp-based biomaterial in combination with transforming growth factor-β1 (TGF-β1) in the creation of new dentine and obturation of the root canal apex in the teeth of an experimental animal 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 after the treatment. The extracted teeth were analyzed by scanning electron microscopy (SEM). **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.** Apex obturation of the dental root canal with newly created dentine took place in our experimental groups treated by biomaterial with or without TGF-β1.

Key words:
odontoblasts; endodontics; dentin; biocompatible materials; transforming growth factors; tooth root; rabbits.

Apstrakt

Uvod/Cilj. Hidroksiapatit (HAp) je jedna od najčešće korišćenih kalcijum fosfatnih biokeramika koja ispoljava osteokonduktivna svojstva. Faktori rasta direktno indukuju morfološku i funkcionalnu diferencijaciju neodontoblasta. Cilj rada je bio ispitivanje efikasnosti biomaterijala na bazi HAp u kombinaciji sa faktorom rasta-β1 (TGF-β1) u stvaranju novog dentina i zatvaranju apeksa kanala korena na zubima eksperimentalnog modela. **Metode**. Kao eksperimentalni animalni model korišćeni su zubi glodara (zečeva). Nakon uklanjanja pulpe zuba pulpekstirpatorom kod vitalne pulpektomije, u apeksnom delu na nivou fiziološkog apikalnog foramena, lentulo spiralom aplikovan je biomaterijal. Eksperiment je obavljen u opštoj anesteziji. Životinje su održavane u životu 3, 6 i 12 meseci posle tretmana. Ekstrahirani zubi su analizirani pomoću *scanning* elektronske mikroskopije. **Rezultati**. *Scanning* elektronskom mikroskopijom dokazano je da je broj zuba sa novostvorenim dentinom i apeksnom opturacijom kanala veći 12 meseci nakon tretmana. **Zaključak**. Utvrđeno je da je u eksperimentalnim grupama tretiranim biomaterijalom, sa ili bez TGF-β1, došlo do apeksne opturacije kanala korena zuba novostvorenim dentinom.

Ključne reči:
odontoblasti; endodoncija; dentin; biokompatibilni materijali; faktori rasta, transformišući; zub, korenski kanal; zečevi.

Introduction

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

It is generally accepted that the prognosis of dental root canal treatment largely depends on the quality of the root canal filling [1]. The apical third of the canal deserves special attention in the mechanical preparation since it is the most sensitive zone that communicates with the vital tissue, which

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is most important for the healing process. A provision of biologically acceptable sealing of the apical portion of the root canal before the definitive filling has urged many authors to consider the issue of apical barrier formation during the root canal treatment. This would prevent material crossing over during the obturation on the one hand and provide high quality, compact, and airtight canal filling on the other [2]. An apical plug would prevent the occurrence of adverse effects associated with the material used for definitive canal filling in the periapical area [3].

Teodorović [1] 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 a 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 [4].

Over the years, various materials have been investigated as 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 yet [1–5].

Gollmer [6] 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. [7] used dentine plugs to create an apical seal in their experiment conducted 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. [8] contested the positive results obtained by the use of dentine plugs on account of significant apical "leakage" after such obturation. Recent studies in both medicine and dentistry have attempted to identify synthetic materials which would not act as antigens and which would, at the same time, be able to successfully replace the bone tissue, i.e. have an osteoconductive effect [9, 10]. Ceramic biomaterials based on hydroxyapatite (HAp) or three-calcium phosphate are most similar to inorganic bone tissue components by their chemical composition and structure [10]. Some studies have investigated the use of biomaterials such as hydroxyapatite for pulp therapy within the techniques of direct pulp capping [11–13], amputation of the coronal portion of the pulp [14], and for endodontic treatment of the teeth with completed root growth as a material for apical barrier formation [15]. Hydroxyapatite is one of the most commonly used calcium phosphate bioceramic materials with osteoconductive properties [14–17]. As a potentially good growth factor delivery vehicle (scaffold), calcium phosphate-based materials have been suggested, whose porous structure enables gradual release and diffusion of growth factors [18].

Based on the aforementioned studies, Pissiotis and Spangberg [19] concluded that due to tissue reaction, predictability, and stability of both HAp 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 HAp crystals into the apical third of the dental root canal warranted further extensive studies.

According to Grossman [20], the ultimate goal of dental root canal therapy is an airtight (hermetic) filling of the canal space.

Sugawara et al. [21] have investigated *in vivo* the use of calcium phosphate ceramic as a material for the definitive dental root canal filling on canine teeth. 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 et al. [22] have studied the new alloplastic bioceramic material formulations, but with a new cement composition for the definitive dental root canal filling (Proendo, Vebas, Italy). Based on the obtained results, the authors concluded that the material was biocompatible, osteoconductive, non-toxic, with good adhesive properties, and that it provided good apical sealing. The sealing 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 of using 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 using some of the growth factors, especially transforming growth factor-beta (TGF-β), can stimulate odontoblast differentiation and induce the release of endogenous growth factors contained in the organic dentine matrix, which additionally stimulates dentinogenesis [23]. Recent insights into the role of growth factors in dental tissue reparation, whether it is reactive or reparative dentinogenesis, could represent the basis for a different approach to pulp treatment. Naturally, nowadays, therapeutic procedures involving teeth with incomplete root growth are being rationalized, and the time required for a therapeutic procedure is getting increasingly shorter [24].

Some clinical studies have shown that the use of platelet-rich plasma 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, consequently, different PRP concentrations. In fact, the issue of PRP concentration, which is optimal for tissue reparation and regeneration, is still unresolved [25].

The aim of this study was to investigate the effectiveness of calcium HAp 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 the Faculty of Medicine in Kosovska Mitrovica, with the approval of the Ethics Committee of the Faculty of Medicine in Niš.

Three 6-month-old chinchilla rabbits, 3–4 kg of body weight (BW) each, were included in the experiment. The animals were anesthetized by intramuscular administration of combination of toletamine and zolazepam (Zoletil 100®, Virbac S.A., France) at a dose of 10 mg/kg BW and ketamine hydrochloride (Ketlar®, Pfizer, UK) at a dose of 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 the physiological apical foramen. FlexoFile® endodontic files (Maillefer, Switzerland) were used for biochemical canal processing. Definitive canal obturation was performed with Lentulo spiral again, with an AH Plus® (combination of calcium tungstate and zirconium oxide) 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 TGF-β1.

The teeth were divided into three groups. The first experimental group (n = 15), composed of lower jaw teeth on the left side (incisors, premolars, molars), into which calcium HAp/PLGA biomaterial was applied. The second experimental group (n = 15), composed of upper jaw teeth on the right side (incisors, premolars, molars), into which calcium HAp/PLGA biomaterial was applied, combined with TGF-β. Calcium HAp/PLGA biomaterial served as the delivery vehicle, 80 : 20 (0.5 g) (product of the Institute of Technical Sciences of the Serbian Academy of Sciences and Arts, Belgrade). The third group (n = 15) composed of intact teeth in the left upper jaw and right lower jaw (incisors, premolars, molars) from the same sacrificed animals (control group).

After this initial part of the study, the animals were kept alive for 3, 6, and 12 months, and after that, they were sacrificed with a lethal dose of Ketalar®. Jawbones 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 2–3 mm thick surfaces (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 thus the samples fixated were gold evaporated in a vacuum evaporator and observed under a scanning electron microscope (SEM) JEOL-JCM-5300.

Data entry and tabular data representation were done using the MS Office Excel software, and calculations were made using the 2007 SPSS, version 15.0.

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

Results

Obtained results are 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 the 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 ($p = 0.30$).

Table 1

Total number of treated teeth in experimental groups and the control group

Animal number	Number of treated teeth		
	Control	TGF-β1+HAp/PLGA	HAp/PLGA
I	5	5	5
II	5	5	5
III	5	5	5
Total number of teeth	15	15	15

TGF-β1– transforming growth factor- β;
HAp/PLGA – hydroxyapatite/poly (lactide-co-glycolide)

Table 2

Dental root canal capping during vital pulp extirpation in experimental groups and the control group

Group	n	Period		
		3 months	6 months	12 months
		n (%)	n (%)	n (%)
(TGF-β + HAp/PLGA)	15	3 (20.0)	3 (20.0)	4 (26.7)
(HAp/PLGA)	15	0 (0)	1 (6.7)	1 (6.7)
(Control)	15	0 (0)	0 (0)	0 (0)

For abbreviations see under Table 1.

The results obtained 3, 6, and 12 months after applying TGF-β1 + HAp/PLGA and HAp/PLGA by SEM microscopy for vital pulp extirpation (VPE) are shown in Figures 1–8.

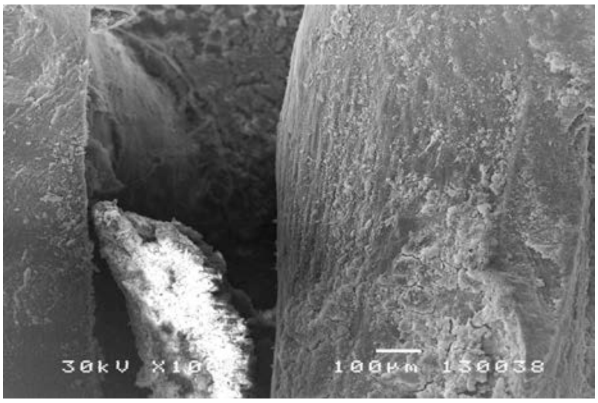


Fig. 1 – TGF-β1 (scanning electron microscopy): Incomplete apical sealing with newly formed dentine (3-month observation period). For abbreviations see under Table 1.

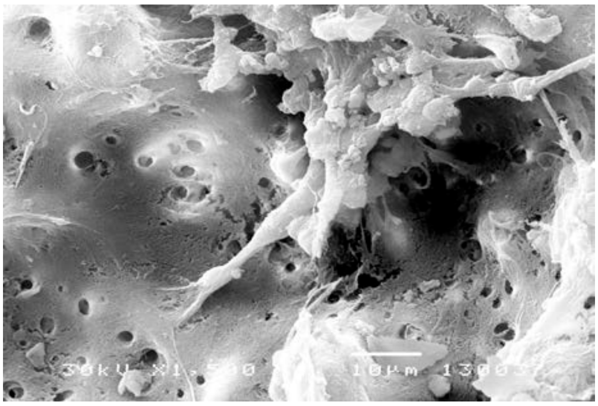


Fig. 2 – HAp/PLGA (scanning electron microscopy): Incomplete apical sealing with newly formed dentine (3-month observation period). For abbreviations see under Table 1.

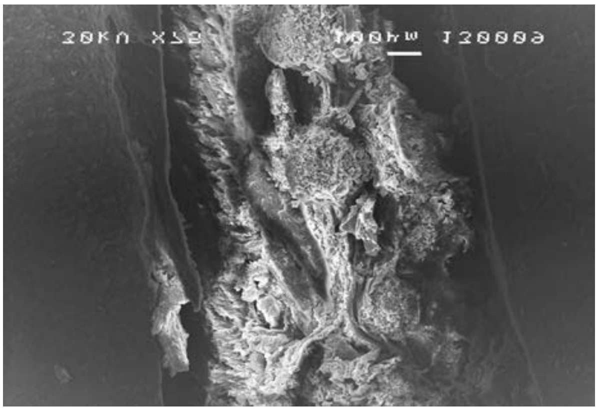


Fig. 3 – TGF-β1+ HAp/PLGA (scanning electron microscopy): Complete root canal obturation with newly formed dentine (6-month observation period). For abbreviations see under Table 1.

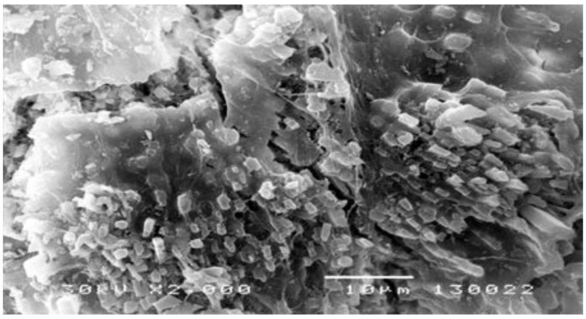


Fig. 4 – HAp/PLGA (scanning electron microscopy): Irregular dentine (6-month observation period). For abbreviations see under Table 1.

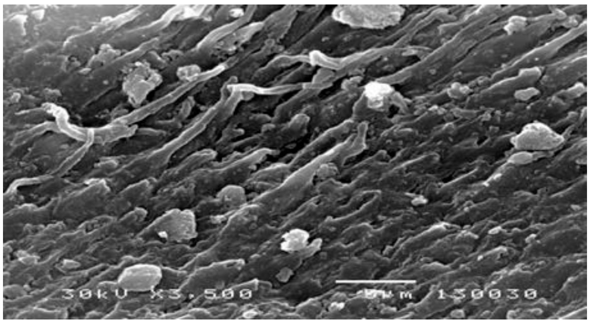


Fig. 5 – TGF-β1+HAp/PLGA (scanning electron microscopy): Complete apical obturation with newly formed dentine (12-month observation period). For abbreviations see under Table 1.

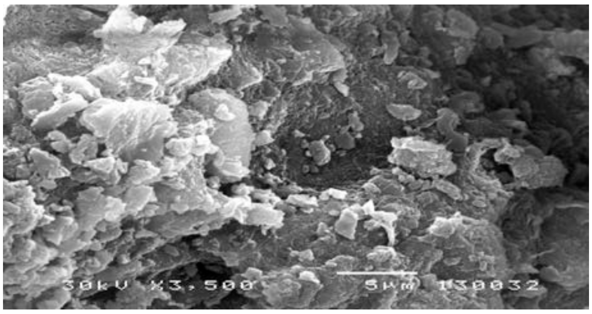


Fig. 6 – HAp/PLGA (scanning electron microscopy): Apical obturation with newly formed dentine (12-month observation period). For abbreviations see under Table 1.

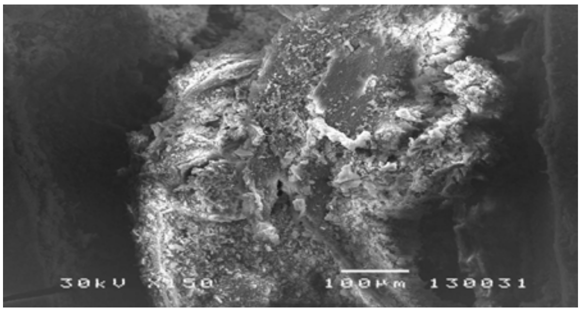


Fig. 7 – TGF-β1 +HAp/PLGA (scanning electron microscopy): Complete apical obturation with newly formed dentine (12-month observation period). For abbreviations see under Table 1.

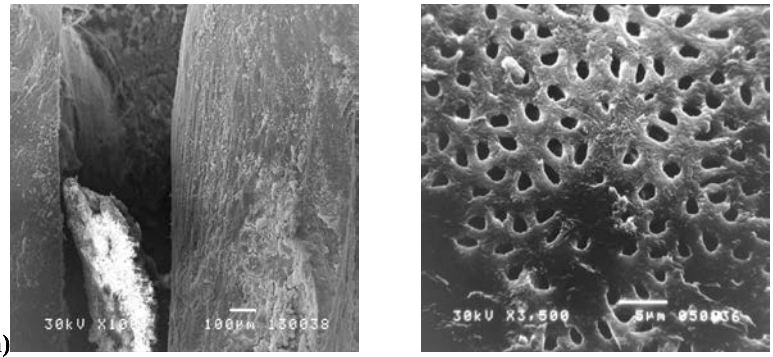
a)  b)

Fig. 8 – Control group ─ intact tooth (scanning electron microscopy): a) open dental root canal apex; b) regular dentine.

Discussion

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

In order to objectively evaluate and interpret these results clinically in a valid way, 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 front teeth) [25]. Nevertheless, many authors dispute such an attitude, emphasizing that the rodent pulp-dentine complex has a significant potential for studying many aspects of reactive dentinogenesis [26], as well as for observing pulp reactions to bioactive molecules [27].

The interaction between the material and the 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 [28].

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

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 pre-prosthetic preparation of the alveolar process [19].

Calcium phosphate vehicles/scaffolds for growth factors have also been suggested as potentially good (which agrees with our study). They 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 [29].

Petrović et al. [4], using synthetic HAp in their study (with an 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 (HAp), 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 and Martinović [30] combined HAp powder with 35% of calcium sulphate and used it successfully 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 samples, those treated with HAp and those treated with HAp and platelet-rich plasma. This suggests that growth factors influenced more rapid healing, i.e. dentine bridge creation and complete apical dental root canal obturation with newly formed dentine [31], 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, whose results indicate that the use of growth factors can provide a favorable prognosis regarding bone, periodontium, and cement regeneration [7].

The results by Tziafa et al. [26] have shown that the use of some of the growth factors, especially TGF-β, is able to stimulate odontoblast differentiation and lead to the release of endogenous growth factors contained in the dentine organic matrix, which additionally stimulates dentinogenesis.

A strictly applied contemporary conception of the root canal treatment enables and facilitates healing processes in the apical periodontium, which is the principal goal of a successful endodontic treatment [29]. Apical barrier formation during the treatment is especially important in specific clinical situations. The barrier, i.e. the 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 [29]. Apical plug formation and "microleakage" problems have not been solved by the attempts with calcium hydroxide, nor with the use of dentine chips [15].

Sugawara et al. [21], Teodorović [1, 15], and Teodorović and Martinović [30] 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 biological treatment and for the purpose of tooth revitalization [31].

Conclusion

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

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