Bioceramic/Poly (glycolic)-poly (lactic acid) composite induces mineralized barrier after direct capping of rat tooth pulp tissue

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Abstract: The aim of this study was to observe the histopathological pulp response following direct pulp capping of mechanically exposed teeth in rats with a composite of beta-tricalcium phosphate-hydroxyapatite bioceramic (BC) and poly (glycolic)-poly (lactic acid) (PLGA) material or a calcium hydroxide [$Ca(OH)_2$] material, compared to BC alone and a negative control of water. Pulp of the maxillary molars was exposed, followed by capping with the experimental material. The pulpal tissue response was assessed post-operatively at 1, 7, 14 and 30 d, followed by histological analysis. The $Ca(OH)_2$ group exhibited severe acute inflammatory cell infiltration at day 14. However after 30 d, a new hard tissue with macro porous obliteration of the pulp chamber and a characteristic necrotic area had appeared. BC and $Ca(OH)_2$ capping were associated with moderate inflammation and dentinal bridge similar. Meanwhile, in the BC/PLGA composite group, there was moderate inflammatory infiltrate and formation of a dense and complete dentinal bridge. In conclusion, the BC/PLGA composite material showed a large zone of tertiary dentin, and effectively reorganized the dentin-pulp complex.

Descriptors: Dental pulp capping; Polymers; Calcium hydroxide; Dentin.

Introduction

In vivo studies have confirmed the advantages of hydroxyapatite (HA)/calcium phosphate bioceramic (BC) materials with a porous structure for tissue growth,[1] however, the application of porous hydroxyapatite ceramics is limited, as their strength decreases exponentially with the pore volume ratio. Pulp capping provides adequate protection of vital pulp exposed to the oral environment. A biologically acceptable treatment was achieved with the advent of materials that promote pulp cells to stimulate deposition of a hard tissue bridge. Calcium hydroxide [$Ca(OH)_2$], dentin adhesives, hydroxyapatite, mineral trioxide aggregate, tricalcium phosphate, allogenic dentin matrix, and bone morphogenetic protein have been widely studied with regard to hard tissue barrier formation after pulp exposition to improve upon direct pulp capping.[2,3] The synthetic calcium phosphate (CaP) biomaterials, hydroxyapatite and tricalcium phosphate ceramic, are used in orthopaedic treatment and

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have been developed as pulp capping agents based on their biocompatibility and potential to stimulate osteogenesis. Studies have demonstrated that prefabricated PLGA/CaP cement loaded with 400 ng of TGF-β1 can trigger resident stem cells in the pulp to differentiate into odontoblast-like cells and induce the formation of tertiary dentin.[4] The cellular mechanism of the dissolution/precipitation process involved a formation of carbonate hydroxyapatite (CHA) on CaP surfaces *in vivo*. Acidity of the environment due to cellular activity (macrophages, osteoclasts) causes partial dissolution of CaP, leading to an increased saturation of the biologic or physiological fluid. This saturation results in precipitation of CHA with incorporated CO_3, other ions, and proteins.[5]

The BC micro-macro porous of β-tricalcium phosphate (βTCP) with hydroxyapatite (HA) has been studied and effectively used as biodegradable material for bone replacement.[6-8] The composite formed by BC and poly (glycolic)-poly (lactic acid) (PLGA) in the same natural bone inorganic/organic composition (75:25)[6-8] rendered a porous structure that served as a scaffold for cellular adhesion. We hypothesized that BC composite could serve well in the release of calcium ions and cellular adhesion through the stimulation of the mineralized dentin deposition. Thus, the aim of this study was to observe the histopathological response of pulp tissue after dental pulp-capping using a BC/PLGA composite material.

Materials and Methods

BC/PLGA composite preparation

A 75:25 molar ratio of BC/PLGA composite was prepared using a dual-phase mixing method; the β-Tricalcium phosphate (β-TCP) and (HA) BC (Einco Biomaterial Ltd.®, Belo Horizonte, Minas Gerais, Brazil) were dispersed in a polymeric biodegradable matrix made from a copolymer of lactic and glycolic acid (PLGA) (MW 60 kDa) (Birmingham Polymers, Inc., Durect Corporation, Pelham, Alabama, USA). The polymer was dissolved in an organic solvent, dichloromethane, and the respective portion of BC was added. In order to remove the maximum amount of solvent residue, the samples were subjected to a vacuum at 37°C for 24 h.[7-8] Samples were sterilized with ethylene oxide and stocked for one week before implantation in the dentin-pulp complex.[9]

Animals

This study was approved by the Ethics Committee for Animal Research of the Federal University of Minas Gerais (170/2007). The animal model used was chosen in accordance with the protocol for pulp capping for new material evaluation before use on humans to minimize atypical response.[10]

Fifty-two non-carious, upper first molars of healthy 4-week-old rats (*Rattus norvegicus*), weighing 250-300 g, were analyzed. The animals were randomly divided into 4 groups to receive a capping agent: $Ca(OH)_2$ powder mixed with distilled water; BC/PLGA; BC alone; and distilled water as a negative control. The animals were sedated with an intramuscular injection of 10% Ketamine (0.1 ml / 100 g of body weight) (Cetamin®, Syntec, São Paulo, São Paulo, Brazil) mixed with 10% Xylazine (Anasedam®, Vetbrands®, São Paulo, São Paulo, Brazil) at a 2:1 Ketamine to Xylazine v/v. A cavity was prepared on the occlusal phase of the maxillary first molars of each animal. Crown access was made with round carbide burs 1/4 (Maillefer®, Ballaigues, Orbe, Switzerland) to avoid leakage into the pulp tissue, to standardize cavity access, and to create a future histologic reference point. The procedure was carried out under isolation with a rubber dam and antiseptic treatment with 10% iodine povidone and 70% alcohol. The cavity was accessed using copious sterile water under low rotation with no additional retention. In order to expose the pulp, a final perforation was carefully performed with a steel probe and gently cleaned with cotton buds and distilled water for 2 min. A metal foil interface (stainless platinum, 2 mm diameter) was used to prevent detrimental discharge of ionic mercury contained in the final amalgam restorative material (Velvalloy, S.S. White, Ltd., Rio de Janeiro, Rio de Janeiro, Brazil).[11-12]

Histological procedures

At post-operative days 1, 7, 14 and 30, the animals were sedated with an intramuscular injection as described above, sacrificed by cervical traction,

and the maxillary molar specimens were dissected using a scalpel. The specimens were fixed in 10% formalin (Rioquímica®, São Paulo, São Paulo, Brazil) for 48 h at 4°C. Complete demineralization was obtained in 10% EDTA (Sinth®, São Paulo, São Paulo, Brazil) and renewed every 4 d until the corresponding end-point. The tissues were dehydrated in a gradual ethanol series followed by paraffin embedding. Five-micron-thick sections were obtained for Hematoxylin and Eosin (H&E) staining in order to assess the amount of dentin formation and to evaluate the qualitative and quantitative tissue response. Histological analyses were performed by the same single-blind examiner using a light microscope, to count the number of inflammatory cells in 8 different high power (200 X) fields of view according to the following standard histological gradation criteria: (i) Severe: ≥ 250 cells; (ii) Moderate: between 141 to 250 cells; (iii) Slight: between 31 to 140 cells; (iv) No significant or normal response: ≤ to 30 cells.

All statistics were performed using analyses of variance (ANOVAs) (Biostatistic®, Belém, Pará, Brazil). Differences of more than two groups were analyzed by Tukey-test. Statistical significance was defined as $p > 0.05$.

Table 1 - Inflammatory cell counts after pulp capping with tested materials observed at four experimental time points.

Groups	Time point			
	1 d	7 d	14 d	30 d
$Ca(OH)_2$	95	175*	76*	32
BC/PLGA	125	169	75*	25
BC alone	168*	185	12	18
Negative control	202	398	442	347

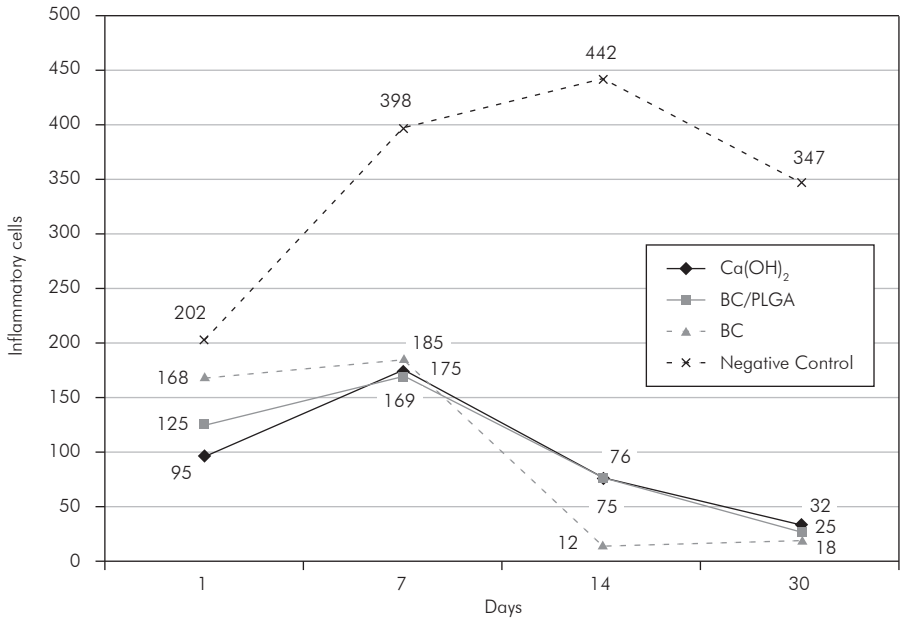
*$p > 0.05$ between the $Ca(OH)_2$ and BC/PLGA groups.

Results

The Inflammatory cells count analysis after pulp capping in each group after 1, 7, 14 and 30 days are shown in Graph 1.

$Ca(OH)_2$ group

On the first day after pulp capping with $Ca(OH)_2$, the cavity floor was densely filled by scattered granular structures overlying a proliferation of the normal cellular zone of the pulp (Table 1). A large number of neutrophils, enlarged capillaries, and some macrophages were observed adjacent to the superficial necrotic tissue. The predominant acute infiltrate was slight and degenerative, and necrotic or debris cells were scattered in direct contact with the exposed area. On the 7th day, the inflammatory infiltrate became moderate and was mixed with macrophages, polymorphonuclear cells, fibroblast-like cells, and predominantly enlarged capillaries especially close



Graph 1 - Inflammatory cell count analysis after pulp capping in each group after 1, 7, 14 and 30 d.

to the $Ca(OH)_2$ treatment site. There was a significant difference between the more intensive pulp response of teeth treated with $Ca(OH)_2$ than those treated with the BC/PLGA composite at post-operative day 7 ($p > 0.05$). On the 14th day, the pulp tissue showed a slight inflammatory infiltrate with lymphocytes, plasma cells, and enlarged capillaries. The exposed area was obstructed by fibrous and mineralized tissue, and in some cases, there was also porosity formation. On the 30th day, there was only a slight presence of inflammatory infiltrate. Dentin formation sufficient to cause complete obstruction after pulp exposition occurred in only 30% of rats in this group. Tertiary dentin, tunnels, fibrous formation and plasma cells were observed adjacent to the exposed site (Figure 1).

BC/PLGA group

Results for days 1, 7, and 14 in the BC/PLGA group were similar to those in the $Ca(OH)_2$ group. However, on the 30th day, formation of mineralized tissue (dentinal bridge or osteodentin) was observed with complete filling of the exposed sites in the BC/PLGA group specimens (Figure 2). The inflammatory process was completely resolved by post-operative day 30.

BC group

In the BC group, a large number of neutrophils, fibroblast-like cells, enlarged capillaries, as well as macrophages were observed adjacent to the exposure zone at day 1. The infiltrate presence was generally moderate. After 7 d, pulp tissue was mainly infiltrated by polymorphonuclear cells surrounding the biomaterial. On the 14th day, fibrosis filled the total extension of the pulp chamber, but no infiltrate was observed and there was statistical difference compared to the $Ca(OH)_2$ group ($p > 0.05$). After 30 d, osteodentin formation was found, but with discontinuous obstruction of the pulp exposure (Figure 3).

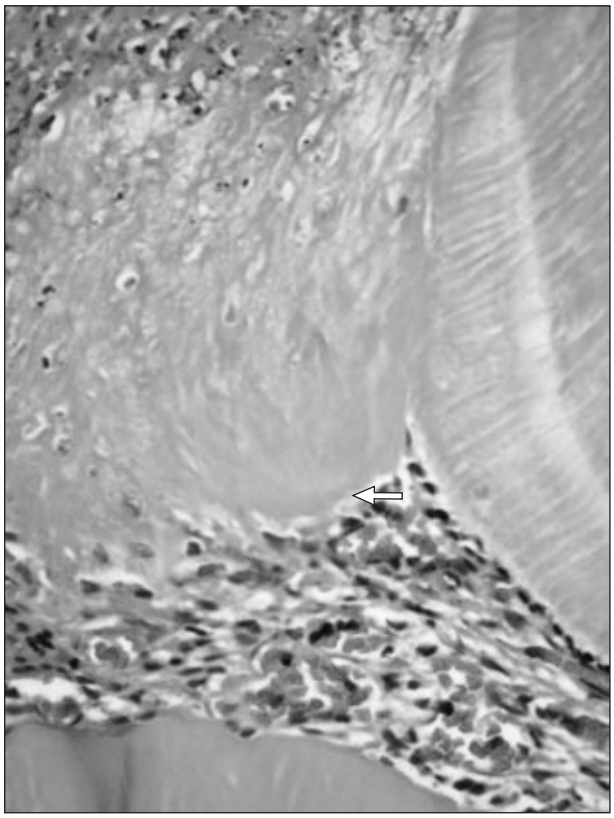


Figure 1 - Hematoxiline & Eosine-stained section of tissue collected 30 d after pulp capping with $Ca(OH)_2$. A complete calcified bridge was observed (arrow)(40 X).

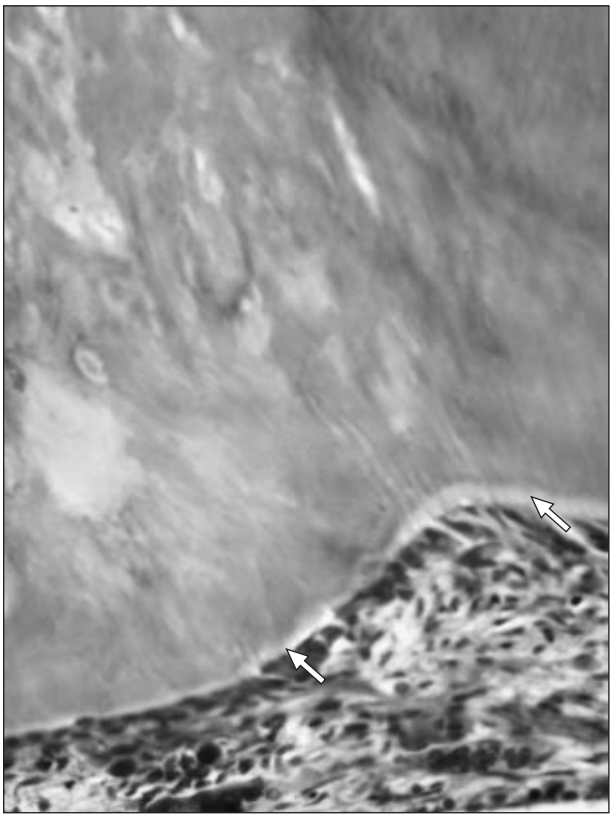


Figure 2 - Hematoxiline & Eosine-stained section of tissue collected 30 d after pulp capping with BC/PLGA composite. A complete calcification bridge was observed (arrows)(40 X).

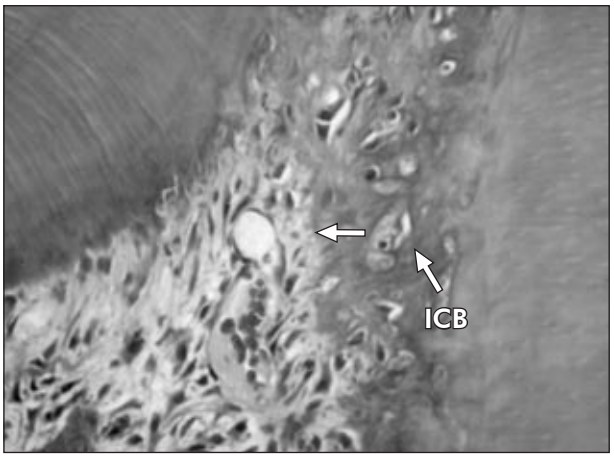


Figure 3 - Hematoxiline & Eosine-stained section of tissue collected 30 d after pulp capping with BC alone. Dense connective tissue (arrow) and incomplete calcified bridge (ICB) were observed (40 X).

The dentin bridge in the BC group was similar to that in the $Ca(OH)_2$ group.

Negative control group

The inflammatory infiltrate varied from slight to absent in this post-operative period. Post-operative day 1 showed the cavity floor of the negative control group was densely filled with granular structures in which plasma cells were scattered. Superficial necrotic tissue was presented adjacent to the exposed site, and a large number of neutrophils and enlarged capillaries proliferated adjacent to the necrotic tissue. The infiltrate extent was predominantly severe; the necrotic pulp cells and cellular debris were scattered only in the superficial zone of the pulp tissue. On the 7th day, the intense inflammatory infiltration persisted and enlarged capillaries were predominant. Polymorphonuclear cells were found in large numbers, and macrophages and giant cells were also observed. On the 14th day, the inflammatory infiltrate continued severely, with a mixed infiltrate of lymphocytes, plasma cells, and enlarged capillaries. Furthermore, necrosis of the connective tissue was observed in the exposure site. On the 30th day, inflammatory infiltration composed of mononuclear cells remained severe, (Figure 4) nevertheless, there were fewer inflammatory cells than at the earlier time points. While fibrous tissue was observed in two specimens, complete obstruction of the exposed site was not detected.

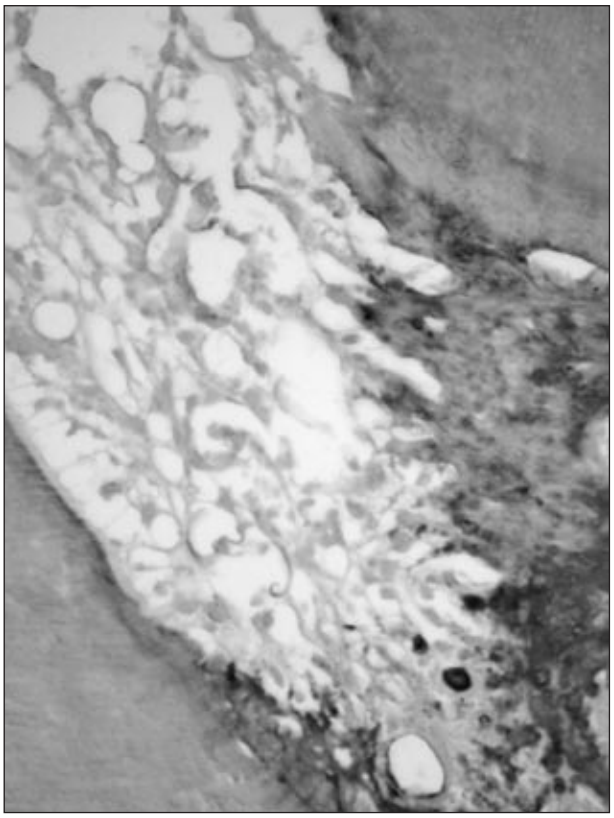


Figure 4 - Hematoxiline & Eosine-stained of tissue collected 30 d after negative control pulp capping. Pulp necrosis was evident (40 X).

Discussion

Many studies have shown that CaP BCs are highly biocompatible with cells and tissues[13] and they are therefore recommended for use in the dentin pulp complex.[14] The BC/PLGA has mainly been used as a scaffold, we believe this is most likely due to the biodegradation process of PLGA. The polymer degradation of the most soluble phase of this BC favored growth of pulp tissue adjacent to the exposure site. BC biphasic composition may have acted as a physiological stimulus and anchor for tissue neoformation.[15-16] In the present study, smaller residues in the pulp chamber filled with BC/PLGA were observed relative to that seen with free BC capping 30 d after treatment. These results could be due to the simultaneous hydrolyses of a polymer/ceramic that may have provided a better physiological environment for odontoblasts than polymer or BC.

The crucial effects of HA can be seen in its ability to enhance bone growth, both across a gap and around an implant, in stable and unstable mechanical conditions, additionally, it can facilitate the conversion of the induced fibrous membrane into a bony anchorage.[17] In the BC/PLGA composite group, a fibrous layer formed (after 30 d) which was later supplanted by a newly formed dentin-like tissue. Likewise, this immediate tissue formation can be favored by the properties of these materials, such as cellular adhesion, strong cohesion, and phase stability.[18] Usually after 5-8 weeks in contact with other materials used for direct pulp capping, tissue shows signs of dentin deposition and inflammation reduction.[19-21] In addition to the presence of some specific inflammatory cells, hydrolytic enzymes also participate in a micro-atmosphere, decreasing the tissue pH, which is important to the process of bone neoformation.[1] Various extents of inflammation were observed within each group in our study.

Since the use of $Ca(OH)_2$ for direct pulp capping was first introduced, it has been used to improve the inner capability of repairing exposed pulp.[22] However, the efficacy of $Ca(OH)_2$ is limited, especially due to the superficial necrosis and the structure quality of the tertiary dentin.[23-25] Histological studies show that highly mineralized layers of tubular dentin (osteodentin) comprise the reparative dentin. During the differentiation process, the cells show characteristics similar to the fibroblasts of the pulp parenchyma and may be responsible for calcified tissue synthesis.[26-28] Experiments examining $Ca(OH)_2$ in animals have resulted in conflicting observations. Some researchers have reported that $Ca(OH)_2$ was comparable to other materials in terms of its efficacy in supporting pulp healing and repair of dentin bridging.[29] Some of the effects of $Ca(OH)_2$ treatment may include pulp necrosis, apical lesions, and excessive reparative dentin formation,[22,23,28] whereas others have reported severe inflammation and poor quality, irregular dentin repair.[30]

Conclusion

The BC/PLGA composite was capable of stimulating pulp tissue reorganization and barrier formation of a mineralized tissue which successfully filled the pulp exposition. The inflammatory infiltrate observed was compatible with a tissue repair process.

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