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RESEARCH ARTICLE

The use of carbonate apatite bioglass cement as pulp capping material examined from reparative dentin

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ABSTRACT

Root canal infection potentially develops to periapical infection as a result of opening of pulp chamber as a pathway for irritants to periapical area. Proper pulp capping procedure can be done to prevent root canal infection by preserving the vitality of the pulp and inducing reparative dentin. Generally, calcium hydroxide ($Ca(OH_2)$) is used as pulp capping material although it still lacks mechanical properties and has high dissolution in oral environment. Carbonate apatite (CO_3Ap) and CO_3Ap bioglass cement are bioceramic materials proven for their ability to form new bone structure upon a research on bone tissue. These materials have better mechanical strength than $Ca(OH_2)$ and have the ability to set in humid environment similar to the body's physiological condition. This study was a preclinical trial of CO_3Ap and CO_3Ap bioglass cement as pulp capping material. The materials were applied on a perforation site of 24 Wistar rat teeth, divided into 4 groups of different treatments, negative control (no treatment), positive control ($Ca(OH_2)$), CO_3Ap, and CO_3Ap bioglass, respectively. Following the application of the pulp capping materials, the perforation sites were covered with light-cured glass ionomer cement. Evaluation of reparative dentin was performed after 21 days, where the rats were euthanized, and histopathological specimens were stained using hematoxylin and eosin. The results of the study revealed that dentinal bridge was formed on all the treatment and positive control groups (p=0.000) but not on the negative control. We concluded that CO_3Ap as well as CO_3Ap bioglass cement have the ability to induce the formation of reparative dentin, therefore both materials can be considered as pulp capping material for open pulp or thin dentin condition on pulp chamber roof.

Keywords: bioceramic cement; bioglass, carbonate apatite; dentinal bridge; pulp capping

INTRODUCTION

Healthy pulp is responsible for tooth viability, growth, defense, and its sensory function. Injury to the pulp by caries as well as trauma would affect its vitality. Pulp vitality is fundamental to the formation of reparative or tertiary dentin after injury or damage. Therefore, when a tooth injury has involved the dentin and the pulp, treatment should be performed not only to cover the pulp perforation with the formation of dentinal bridge, but also to maintain and stimulate the regeneration of pulp tissue, either it is infected or not.[1,2]

Pulp capping is a well-known method to manage vital pulp exposure. The purpose of pulp capping is to maintain the pulp vitality and develop dentinal bridge or tertiary dentin above the exposed pulp by odontoblastic activity in the pulp. Until now, $Ca(OH)_2$ is a material generally used for pulp capping treatment. Calcium hydroxide ($Ca(OH)_{2)}$ has a good antibacterial property and ability to establish dentinal bridge in exposed pulp. Many studies with long term observation showed satisfactory result after treatment, however this material has some drawbacks, such as persistent inflammatory reaction of the pulp especially in direct pulp capping, formation of porous and brittle dentinal bridge, and also moderately inadequate mechanical properties.[2,3] Nowadays we found that many conventional pulp capping treatments with $Ca(OH)_2$ cannot achieve their objectives such as pulp regeneration, particularly on exposed pulp due to deep caries. This is caused by the lack of

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pulp ability to regenerate innately after exposure. As a result, the pulp with irreversible inflammatory reaction often needs pulpectomy or even extraction.[1,2]

Bioceramic material is a substance developed largely in medical field as a substitute for bone damage. The ceramic material is used purposely as a substitution in the body, for example alumina, zirconia, bioactive glass, composite resin, hydroxyapatite, and carbonate apatite. Bioceramic material has biocompatible properties, capable to stimulate the formation of hard tissue and can be absorbed by body tissue, and also stimulate natural regeneration of those tissues. Carbonate apatite (CO_3Ap) and bioglass are both bioceramic materials with the ability to bind to bone structure and stimulate the development of bone tissue. Carbonate apatite (CO_3Ap) material is effective to develop hydroxyapatite layer similar to bone mineral. Good adaptation between bone tissue and CO_3Ap has also been proven in a study of bone defect implanted with CO_3Ap.[4-7] The bioactive properties of CO_3Ap encourage specific response to establish connection with mineralized bone hence functioning as a barrier against micro-leakage.[7,8] Silicacalcium phosphate nanocomposite bioglass as one of bioactive bioglass has showed good bone regeneration when implanted to alveolar bone socket following tooth extraction after 6 months. The bioglass is able to withdraw Ca^{2+}, stimulating the activity of osteoblasts to differentiate to mature bone cells.[9,10] Since the basic structure of bone and dentin is similar, which is derived from mesenchymal tissue, it brings up an analogy that CO_3Ap and bioglass also influence dentin and pulp tissue. Carbonate apatite cement has been applied to open pulp in an in vivo study and showed that the pulp remained vital and reparative dentin was formed in the perforation site.[11] This brings insight that in addition to its biocompatibility, this material is non-toxic and can stimulate tissue mineralization in the pulp.

Carbonate apatite bioglass cement is an improvement of previous CO_3Ap where the original cement was combined by bioglass to increase its bioactivity and physical properties. Our previous study had evaluated the best ratio of this combination by searching the best mechanical and chemical properties suitable for pulp capping application.[12-14] The purpose of this study was to identify whether CO_3Ap cement and CO_3Ap bioglass can stimulate the formation of reparative dentin.

MATERIAL AND METHODS

The samples for the positive control group consisted of commercial $Ca(OH)_2$ pulp capping material (Dycal, Dentsply, Konstanz, Germany) mixed according to the manufacture instructions in a glass slab. The tested pulp capping material groups comprised of CO_3Ap group and CO_3Ap bioglass groups. Cement was made from Dicalcium phospate anhydrous (DCPA) (60%) and vaterite (40%) powder, Na_3PO_4 0.2 M liquid, with L/P ratio of 0.5. Na_3PO_4 liquid would form CO_3Ap in the shortest time of 24 hours. When vaterite and DCPA were mixed with Na_3PO_4, vaterite and DCPA would dissolve and release calcium, carbonate, and phosphate. Afterwards the solution would pass saturation point and CO_3Ap would precipitate.[8] Carbonate apatite bioglass for the second test group was made by mixing CO_3Ap and bioglass with ratio as follows: DCPA 60%, vaterite 20%, and bioglass 20% mixed with Na_3PO_4 0.2 M liquid in ratio 0.5. The 60:20:20 ratio of DCPA:vaterite:bioglass acquired the most optimal mechanical strength.[10] Materials were prepared at the Faculty of Dentistry, Universitas Padjadjaran and Faculty of Medicine of Universitas Jenderal Achmad Yani.

Twenty four rats were divided into 4 groups, consisting of negative control, positive control with $Ca(OH)_2$, CO_3Ap treatment, and CO_3Ap bioglass treatment. The subjects were 32 mandibular incisors of *Rattus norvegicus* rats (at least 8 weeks old, healthy, active, male, weighed at least 300 g) to which pulp perforation was intentionally made using round bur.[11] Ethical clearance was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran Number 457/UN6/C.1.3.2/KEPK/PN/2016. Before pulp perforation procedure and pulp capping material application, the subjects in all the groups were given general anesthesia with ketamine 40-100 mg/

kg. Mandibular central incisor was prepared using a round no.1 diamond burr until pulpal chamber roof was perforated and subsequently the tooth was pressed with a cotton ball with a proper size until the bleeding stopped. Carbonate apatite and CO_3Ap bioglass cement were mixed subsequently on a glass slab before applied on each particular tooth that had been prepared, using a small cement stopper. Freshly prepared $Ca(OH)_2$ was also applied with the same procedure. The negative control group was only given a dab of normal saline using a cotton pellet after perforation. Afterwards, the teeth were covered and restored with light-cured glass ionomer cement (Fuji II LC, GC International Corp., Tokyo, Japan). The treatments to the rats were performed at the Animal Laboratory of Universitas Jenderal Achmad Yani, and the evaluation was performed after 21 days.[11]

Histopathology specimen of teeth was made by routine hematoxylin-eosin technique, which was previously decalcified using 10% EDTA solution for 15 days at the Pathology Anatomy Laboratory of Universitas Jenderal Achmad Yani and Universitas Padjadjaran. The histopatology samples were subsequently examined with light microscope in 10x and 40x objective magnification to ensure the presence of reparative dentin or dentinal bridge formation.

Kruskal-Wallis test was used for semiquantitative data on the four groups and considered significant if $p<0.05$. Assessment was based on the presence of reparative dentin found in histopathological examination. Post Hoc Mann-Whitney test was performed subsequently to analyze the difference between the two groups.[15]

RESULTS

Twenty-one days after the treatments in the treatment groups, the clinical state of the rats and their teeth were good, and there were not any inflammatory signs on the gingiva. No tooth color change was examined in all the control and treatment groups.

Examination of 24 teeth sample obtained from the experimental animal showed that all the samples from the positive control with $Ca(OH)_2$ and treatment groups with CO_3Ap and CO_3Ap bioglass had formed reparative dentin in 21 days after cement application. In contrast, all the samples from the negative control group did not show any formation of reparative dentin. Figure 1 showed dentin reparative condition that had been examined microscopically on each group that underwent development of reparative dentin. Compared to primary and secondary dentin, reparative dentin was more atubular, did not represent dentinal tubule yet, and less organized.

Kruskal-Wallis test to all the four groups showed a significance between the groups ($p=0.000$). However, based on the Mann-Whitney post hoc test results, it was found that significant differences were found only if the positive control and treatment groups were compared to the negative control group ($p=0.002$), since dentinal bridge or reparative dentin was seen on all the treatment and positive control groups (Table 1).

Table 1. Semiquantitative observation of reparative dentin formation

	Group				
	Negative control	$Ca(OH)_2$	Carbonate apatite	Carbonate apatite bioglass	p-value
Formed	0	6	6	6	0.000*
Not formed	6	0	0	0	

*Kruskal-Wallis test ($p<0.05$) is considered significant)

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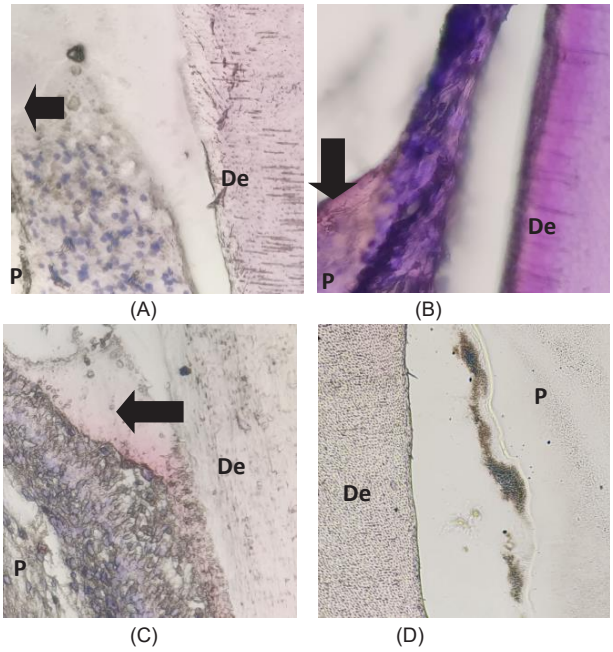

Figure 1. Histological section of rat teeth stained with Hematoxylin-Eosin. Reparative dentin is shown by black arrow sign (40x objective magnification): (A) Positive control group with $Ca(OH)_2$; (B) Treatment group with CO_3Ap; (C) Treatment group with CO_3Ap bioglass; (D) Negative control group shows no formation of reparative dentin; (P) Pulp; (De) dentin.

DISCUSSION

Carbonate apatite and CO_3Ap bioglass cement are examples of bioceramic material that nowadays has been studied as a substance for bone tissue and dentinal repair. Previous study on CO_3Ap material implant in bone defect showed that this substance has the ability to adapt with bone tissue, evident by the fact that there is no gap between CO_3Ap layer and examined bone tissue. It was also presented that CO_3Ap would eventually be replaced with exact bone tissue.[8] Promising results are also shown by the application of bioglass granules in bone, showing that these bioactive materials help the regeneration of new vital bone in extracted tooth sockets and enhance bone integration to maxillofacial implants coated with bioglass with minimal inflammatory responses.[9,10] The precipitation of CO_3Ap crystal from the drip of $CaCO_3$ and DCPA mixed with Na_2HPO_4 solution as PO_4^{3-} donor that also dissociates Ca^{2+}, CO_3^{2-} ions from vaterite might therefore relate to the formation of mineralized tissue to form a dentinal bridge. Subsequently, DCPA and vaterite will also interact with PO_4^{3-}, existed blood, pulp tissue, and the remaining intact dentin following the transformation of cement to CO_3Ap, that will enhance the mineralization.[11,12]

The present study showed that the formation of reparative dentin took place on the perforation pulp site of the animal model and that the tooth capped with the tested material remained vital. This study also showed that the rat's dental tissue can represent the ability of pulp capping materials to the pulp tissue and evaluate its reaction to pulp capping material as also reported by other studies.[16-18] Histologically, signs of severe inflammatory conditions were also absent. The structure of primary and secondary dentin were more organized than the reparative dentin which had a more dense and atubular form of dentin with a darker colour seen in the specimen because it tended to absorb more stains. Reparative dentin was also formed in the control group capped with $Ca(OH)_2$. This is in line with histological studies on pulp response capped by $Ca(OH)_2$.[18,19] However one of the limitations of this study was produced a thinner histological specimen due to difficulties in cutting the hard tissue of the tooth although decalcification had been performed.

In general, light pulp irritation from pulp exposure would form reparative dentin of which the dentinal tubule and mineralization pattern are similar to those of primary dentin. On the other hand, severe pulp irritation will create atubular, irregular, and less mineralized reparative dentin. Dentinal structure formed is influenced by many factors such as rate of inflammation, cellular damage, and the condition of pulp-dentin complex by the time of odontoblast differentiation.[1,2] The formation of reparative dentin takes place if odontoblasts stimulated by irritant activate them to differentiate and subsequently form mineralized dental tissue. Unlike reactionary dentin which is formed from previously existing odontoblasts, dentinogenesis process on reparative dentin caused by exposed pulp involves dentin stem cells or odontoblast-like cells. Because of that, the structure developed also has fewer dentinal tubules than primary dentin, and the quality of hard tissue is somewhat lesser.[20-22]

Optimal direct pulp capping material is expected to be a foundation of recruitment and differentiation of stem cells and undifferentiated mesenchymal cells to form reparative dentin. The most important properties of direct pulp capping material are its high pH but still on physiological degree, antibacterial characteristic, and the ability to release calcium ions required for mineralization. Just as in bone tissue, calcium ions also induce odontogenic differentiation from the mesenchyme. Carbonate apatite and bioglass are able to adapt on bone structure, support bone regeneration, and can be absorbed by bone tissue with alkaline pH, and sustain calcium ion release. It also has more favorable setting time, better mechanical strength and higher resistance to dissolution with more compact microstructure compared to conventional $Ca(OH)_{2.}$ However its antimicrobial properties and more in vivo studies may need to be confirmed.[12-14]

CONCLUSION

Carbonate apatite and CO_3Ap bioglass cement can be considered as a new material for pulp capping treatment for its property to form dentinal bridge in a similar way to calcium hydroxide as a standard material for pulp capping treatment. Further study is important to measure pulp and dentin condition specifically related to cells and other components in the pulp.

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