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Research Report

# The increasing of odontoblast-like cell number on direct pulp capping of Rattus norvegicus using chitosan

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***ABSTRACT***

***Background:*** *Pulpal perforation care with direct pulp capping in the case of reversible pulpitis due to mechanical trauma was performed with chitosan which has the ability to facilitate migration, proliferation, and progenitor cell differentiation.* ***Purpose:*** *The purpose of this study was to determine the increasing number of odontoblast-like cells in direct pulp capping dental care of Rattus norvegicus using chitosan for seven and fourteen days.* ***Methods:*** *Samples were molars of male Rattus norvegicus strain wistar, aged between 8–16 weeks, divided into two treatment groups, namely group I given chitosan and group II as a control group given $Ca(OH)_2$. Those Rattus norvegicus' occlusal molar teeth were prepared with class I cavity, and then chitosan and $Ca(OH)_2$ were applied as the pulp capping materials. Afterwards, glasss ionomer cement type IX was used as a restoration material. Their teeth and jaw were then cut on the seventh day and the fourteenth day. Next, histopathological examination was carried out to observe the odontoblast like cells. All data were then analyzed by t test. Degree of confidence obtained, finally, was 95%.* ***Results:*** *The results obtained showed that the significant differences of odontoblast like cells on the seventh day observation was 0.001 ($p = 0.001$), and on the fourteenth day observation was 0.002 ($p = 0.002$).* ***Conclusion:*** *The number of odontoblast-like cells in direct pulp capping dental care of rattus norvegicus using chitosan is higher than the one using $Ca(OH)_2$ for seven and fourteen days.*

***Key words:*** *Chitosan, calcium hydroxide, direct pulp capping, odontoblast-like cells*

***ABSTRAK***

***Latar belakang:*** *Perawatan perforasi pulpa pada kasus pulpitis reversible karena trauma mekanis bur dilakukan direct pulp capping dengan cara pemberian bahan secara topikal pada daerah perforasi. Kitosan memiliki kemampuan untuk memfasilitasi migrasi, proliferasi dan diferensiasi sel progenitor pulpa.* ***Tujuan:*** *Tujuan penelitian ini adalah untuk menentukan jumlah peningkatan odontoblas-like cell pada perawatan direct pulp capping gigi Rattus norvegicus menggunakan kitosan selama 7 dan 14 hari.* ***Metode:*** *Sampel adalah gigi molar Rattus norvegicus jantan strain wistar, berusia antara 8–16 minggu, dibagi menjadi 2 kelompok perlakuan yaitu kelompok I yang diberi kitosan dan kelompok II sebagai kontrol yang diberi $Ca(OH)_2$. Oklusal gigi molar Rattus norvegicus dipreparasi kelas I kemudian kitosan dan $Ca(OH)_2$ diaplikasikan sebagai bahan pulp capping. Glass ionomer cement tipe IX digunakan sebagai bahan restorasi. Gigi beserta rahang tikus dipotong pada 7 dan 14 hari. Pemeriksaan histopatologi dilakukan untuk mengamati odontoblas-like cell. Semua data dianalisis dengan uji t. Tingkat kepercayaan = 95%.* ***Hasil:*** *Hasil penelitian menunjukkan perbedaan yang signifikan dalam odontoblas like cell pada pengamatan hari ke-7 ($p = 0,001$) dan pengamatan hari ke 14 ($p = 0,002$).* ***Kesimpulan:*** *Jumlah odontoblas like cell pada perawatan direct pulp capping gigi Rattus norvegicus menggunakan kitosan lebih tinggi dibandingkan dengan $Ca(OH)_2$ selama 7 dan 14 hari.*

***Kata kunci:*** *Kitosan, kalsium hidroksid, direct pulp capping, odontoblast-like cells*

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## INTRODUCTION

Pulpal perforation care in the case of reversible pulpitis due to mechanical trauma or deep caries cleaning can be conducted by direct pulp capping using topical materials applied in the perforation area. The application of wound covering materials on the opened pulp is for bacterial prevention and to iniate soft tissue healing as well as to repair the dentin tissue of the opened area, therefore the wound will not progress to pulpitis irreversible which can eventually cause pulpal death. Calcium hydroxide ($Ca(OH)_2$) is considered as the standard material used for direct pulp capping care until now. Nevertheless, this material has some disadvantages, for example, it can cause necrosis in the superficial layers of pulp since $Ca(OH)_2$ can be ionized into $Ca^{++}$ and $OH^-$ which form strong alkali. This alkaline character can trigger the risk of pulp and apical lesion abnormalities.[2] The lack of $Ca(OH)_2$, can be solved by developing chitosan biomaterial that is safe (not toxic), biocompatible, and biodegradable, since it can accelerate wound healing activity, adsorption, and anti-infection.[3]

Chitosan used in this study was derived from white shrimp shell with a 88.96% degree of deacetylation. Chitosan is actually polycationic complex carbohydrate that is able to facilitate the migration and proliferation of progenitor cells.[4] Biocompatibility test of chitosan derived from shrimp shell was conducted in this study by using a skin patch test which cannot cause allergic reaction in individuals with a history of allergies or seafood allergies.[5] Chitosan test with deacetylation degree of 88% towards antimicrobial also showed lower growth of *Streptococcus mutans* and *Candida albicans*.[6] Thus, this study was finally aimed to determine the ability of chitosan in increasing the number of odontoblast-like cells on direct pulp capping of *Rattus norvegicus.*

## MATERIALS AND METHODS

The type of this study was an experimental research with completely randomized design using samples of molars of male *Rattus norvegicus strain* wistar weighting 200–250 g and aged between 8–16 weeks. The samples of this study were divided into two treatment groups, namely group I given chitosan and group II as a control group given the $Ca(OH)_2$. Each treatment group, were divided in two samples of observations, seven day observation and fourteen day observation, with 8 samples in each group. Next, the occlusal part of the *Rattus norvegicus*' molar was prepared with class I cavity by using low-speed tapered round diamond bur, and then was perforated with the tip of explorer.

The pulp capping materials, chitosan and Ca $(OH)_2$, were applied as much as 0.01 grams into the cavity, and then the cavity was covered with type IX glass ionomer cement as a restoration material. The teeth and jaw of rats were cut on the seventh and fourteenth day. All samples were stored in fixation solution for 48 hours, and then were decalsificated until soft. After that, it was embedded in paraffin blocks to be prepared for 4–5 µ cutting, and then was continued with HE staining in order to observe the odontoblast-like cells. The odontoblast-like cells on both group on the seventh day can be seen on figure 1. The calculation was finally conducted by using light microscope with magnification view of 400×/, to obviously the number of odontoblast-like cell in the left and right edges of the perforation.

## RESULTS

Analysis result of statistical calculations showed that the mean and standard deviation of odontoblast-like cells number in the chitosan group was higher than that in the $Ca(OH)_2$ group (Table 1). The result of t test showed that in comparison between the chitosan group and the $Ca(OH)_2$ group in the seventh and fourteenth day observations, there was a significant difference in the seventh day observation at $p = 0.001$ ($p < 0.05$). Meanwhile, the result of t test in the comparison between the chitosan group and the $Ca(OH)_2$ group in the fourteenth day observation showed that there was significant difference of odontoblast-like cell number at $p = 0.002$ ($p < 0.05$).

## DISCUSSION

Based on table 1, the results of this study could indicate that the mean number of odontoblast-like cells in the chitosan group was higher than in the $Ca(OH)_2$ group. The comparison of chitosan and $Ca(OH)_2$ towards odontoblast-like cells on the seventh day and the fourteenth day after the treatment could also indicate that there were significant differences among them (Table 2). It is because chitosan as direct pulp capping material has a 88.957% degree of deacetylation which has a high percentage of acetyl groups that are more active so that chitosan could stimulate the differentiation of odontoblast-like cells. The degree of deacetylation of chitosan, could also affect the biological character of chitosan, including biodegradation of chitosan caused by lysozyme enzyme produced by inflammatory cells and macrophages neutrophil.[7] Actually, chitosan is a source of the active N-acetyl-D-glucosamine dimer, therefore if it is applied to the wound area, it will make inflammatory cells release lysozyme.[3] Neutrophils produced by inflammatory cells, furthermore, will migrate into the wound area several hours after the injury and will reach a maximum concentration in about 24 hours. Similarly, macrophages, the dominant cells, will migrate into the wound area after 24 hours and for about five days,[8] and the inflammatory cells will decline after seven days.[9]

Therefore, if chitosan containing the active N-acetyl-D-glucosamine dimer experiences biodegradation, it will form cross-linked with glycosaminoglycan and

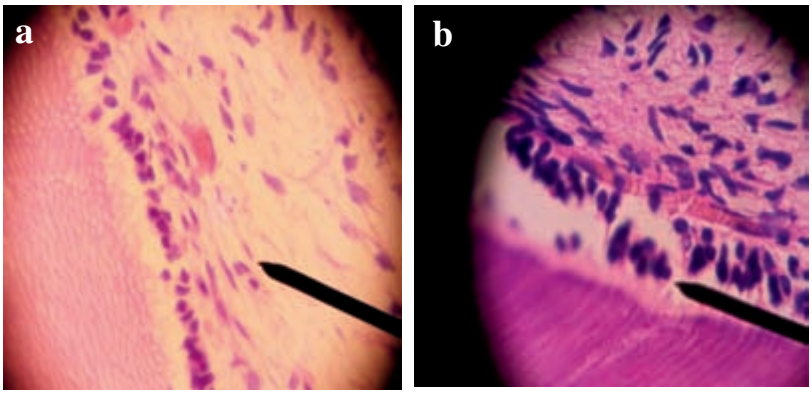


**Figure 1.** a) Odontoblast-like cell in the chitosan group on the seventh day, b) Odontoblast-like cell in the $Ca(OH)_2$ group on the seventh day.

glycoprotein which plays a role in biological processes, including both the cell and matrix interactions and the activation of growth factors.[7] Growth factors, such as bone morphogenetic protein-2 (BMP-2) which is a superfamily of transforming growth factor $\beta$ (TGF-$\beta$) then will stimulate the differentiation of osteoblastic-cell.[10] Thus, chitosan is able to accelerate wound healing process through fibrinogenic mediators, such as growth factors. The increasing of the expression of growth factors will also be able to increase the activity of fibroblasts. This is because of the ability of chitosan in forming polyelectrolyte complex by using polyanion heparin to improve and extend the half-life of growth factors in stimulating cell differentiation. Through in vitro studies, it is also known that mesenchymal cells exposed with chitosan showed a higher differentiation than control, indicating that chitosan could stimulate the differentiation of osteoprogenitor cell and bone formation.[11]

**Table 1.** The mean and standard deviation of the number of odontoblast-like cell in the chitosan group and the $Ca(OH)_2$ group for seven and fourteen days

| Materials | Seven days | | Fourteen days | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Chitosan | 17.75 | 1.28 | 19.00 | 1.30 |
| $Ca(OH)_2$ | 13.63 | 2.32 | 14.88 | 2.85 |

**Table 2.** The significance rate of odontoblast like cell in the comparison of the chitosan group and the $Ca(OH)_2$ group on the seventh day and the fourteenth day

| Variable | p Score chitosan– $Ca(OH)_2$ | |
|---|---|---|
| | The seventh day | The fourteenth day |
| Odontoblast like cell | 0.001* | 0.002* |

Note: * = significant difference

Chitosan is a natural cation that plays a role in electrostatic interactions with anionic glycosaminoglycan and proteoglycan that will improve the effectiveness of growth factors.[10] Osteoblast cell cultures stimulated by chitosan, as a result, can cause the increasing of the expression of both alkaline phosphatase (ALP) mRNA after 3 days and BMP-2 mRNA after seven days.[9] It is also because chitosan is able to directly stimulate the differentiation of multipotent mesenchymal progenitor cells into osteogenic cells. Another evidence even stated that chitosan implantation of absorbable collgen sponge on rat calvarials can increase the formation of new bone, greater than just giving absorbable collagen sponge only, after eight weeks of treatment. It means that chitosan is a potential material used to accelerate the regeneration of bone since chitosan can trigger the differentiation into osteogenic cells.[4]

Finally, it may be concluded that the number of odontoblast-like cells on direct pulp capping of *Rattus norvegicus* using chitosan is higher than using $Ca(OH)_2$ for seventh and fourteenth days.

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