

Antibacterial activity of gypsum-based biomaterial compared to calcium hydroxide and glass ionomer cement on streptococcus sobrinus: an in vitro study

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Abstract

Direct pulp capping (DPC) is initiated to preserve the healthy state of the pulp tissue that has been compromised by trauma, restorative procedures, or caries. It involves the application of a suitable dental material to seal communications between the oral cavity and the exposed pulp. DPC is challenging due to bacterial infection.

This study aimed to investigate the antibacterial activity of gypsum-based biomaterial (Gyp-CHT) compared to Dycal (DYC) and Glass ionomer cement (GIC) on *Streptococcus sobrinus*. *S. sobrinus* were seeded on the surface of brain–heart infusion (BHI) agar. Gyp-CHT with various concentrations of chitosan (CHT), DYC, and GIC were then inserted in the wells of the agar. The diameter of the inhibition zones produced around the materials were measured after 48 hrs. Gyp-10%CHT has the highest antibacterial activity compared to DYC and GIC ($p < 0.05$). In addition, significantly decreased antibacterial activity ($p < 0.05$) was observed with Gypsum (Gyp) alone compared with 2.5%CHT, 5%CHT, and 10%CHT- containing Gyp.

Gyp-CHT has potent antibacterial activity against *S. sobrinus*. Gyp-CHT is a promising candidate for use as a pulp capping material.

Clinical Article (J Int Dent Med Res 2018; 11(2): pp. 477-481)

Keywords: Direct pulp capping, Gypsum, Chitosan, *Streptococcus sobrinus*.

Received date: 01 January 2018

Accept date: 29 January 2018

Introduction

Direct pulp capping is regarded as an efficient treatment method in today's endodontics, it is initiated to preserve and maintain the healthy state of the pulp tissue that has been compromised by trauma, restorative procedures, or caries. Its objective is to retain the tooth as a functional unit through the stimulation of the reparative dentin formation.

The presence of bacteria is the main cause of failure of the pulp capping.¹ Bacteria in deep caries can induce inflammatory reactions

and even necrosis of the pulp.² The pulp capping materials should exhibit antibacterial activity to sterilize the underlying dentin and residual caries in deep cavities, as well as to prevent the marginal infiltration around the restoration to restrict the bacterial toxins diffusion and soluble molecules into the dentin and pulp tissues.³ Thus, the application of antibacterial material before the final restoration is of great important.

Currently, the most common materials used for pulp capping include calcium hydroxide ($\text{Ca}(\text{OH})_2$) and mineral trioxide aggregate (MTA). $\text{Ca}(\text{OH})_2$ has antibacterial activity and induce dentin formation. However, the hard-setting $\text{Ca}(\text{OH})_2$ such as Life (Kerr, USA) and Dycal (Dentsply, USA) do not inhibit the microleakage completely because it has no adherence ability to dentin,⁴ and it may disappear within the time span. Moreover, the dentin bridge formed by $\text{Ca}(\text{OH})_2$ is porous and contain tunnel defects, thus, it is not an effective seal for long-term clinically.⁵

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Although MTA induces the mineralized tissue formation, it lacks good antibacterial activity,⁶⁻⁸ which is necessary to prevent the bacterial infection that leads to failure of the treatment of deep caries cases. Therefore, the investigation for alternative pulp capping materials with good antibacterial activity is highly desired.

Gypsum-based material that contain an excellent antibacterial component has been investigated for use as a direct pulp capping material with high biological characteristics.⁹ CHT was incorporated into calcium sulfate gypsum, both components have excellent biological properties particular to human tissue compatibility and regeneration process.

Gypsum is a highly biocompatible, nontoxic,¹⁰ easily moldable, and widely used as drugs carrier to treat bone defects.^{11,12} Chitosan is a biocompatible, nontoxic deacetylated derivative of chitin, found commonly in shells of crustaceans, marine and fungi cell walls.¹³ Due to its superior properties, CHT has been studied intensively in various aspects of dentistry, such as tissue engineering,¹⁴⁻¹⁷ production of antiplaque and antimicrobial toothpastes¹⁸⁻²⁰ and mouthrinse.²¹

CHT has demonstrated high antimicrobial activity against various pathogenic and spoilage microorganisms, including fungi and Gram-positive and Gram-negative bacteria.²² CHT can activate host defenses to prevent infection, accelerate wound healing, and promote tissue growth,^{22,23} which are considered the most desirable properties of pulp capping materials.

The aim of this study was to evaluate the antibacterial activity of Gyp-CHT with various concentrations of CHT compared to DYC and GIC against *streptococcus sobrinus*.

Methodology

Preparation of the biomaterials

PROTASAN UP CL 113 is a well-characterized water-soluble chitosan chloride referred to as CHT in this study (NovaMatrix, Norway; 75%–90% degree of deacetylation; molecular weight of 50,000–150,000 g/mol). Calcium sulfate dihydrate (Sigma-Aldrich, India) was heated at 110°C for 3 h in an electric oven (Universal Oven Memmert Life 600, Schwabach, Germany) for conversion into a hemihydrate form

(CaSO₄•½ H₂O). CHT powder was added to sterile distilled water to prepare CHT solution with 4 different concentrations of 10%, 5%, 2.5%, and 0% w/v CHT solutions (10g, 5g, 2.5g, and 0g of CHT was dissolved in 100 ml of distilled water, respectively) and dissolved completely with a continuous stirring. The CHT solutions were then mixed with Gyp with L/P ratio of 0.6 using a sterile dental spatula on a sterile glass slab to obtain a paste with a consistency similar to that of dental cements. The above L/P ratio was chosen based on a pilot study which obtains the best consistency of the material. Dycal (DENTSPLY Caulk, USA) and GC Fuji Lining LC glass ionomer cement (GC, Japan) were prepared in accordance with the manufacturers' instructions and used to compare the antibacterial properties.

Agar Diffusion Test

The antibacterial activity of the experimental biomaterial was tested by agar diffusion method against *S. sobrinus* (ATCC 33478), and compared with DYC and GIC. *S. sobrinus* was cultured on blood agar at 37 °C for 48 h under anaerobic conditions and the colonies were harvested and suspended in BHI broth (R&M Chemicals, UK) overnight. The concentration was then adjusted to obtain a 0.5 McFarland turbidity standard. After that, the bacterial suspension was poured and spread onto the BHI agar surface (R&M Chemicals, UK) and the excess was extracted. Uniform wells (5 mm diameter × 5 mm height) were prepared at equidistant points in BHI agar with a sterile copper coil and immediately filled with freshly manipulated test materials using a sterile amalgam carrier and condensed gently. Three samples for each material were tested. The plates with and without inoculums incubated under identical conditions and time period were used as positive and negative controls. The test materials were prediffused for 2 h at room temperature. Then, the plates were incubated in anaerobic condition at 37°C and evaluated at 48 hours. All assays were performed under aseptic conditions.

Bacterial inhibition zones were then measured with a precision ruler to the closest 0.5 mm and determined by measuring the mean of two perpendicular diameters of inhibition zone through the sample centre. The results were

expressed as the mean and standard deviations of 3 independent experiments.

Statistical analysis

The results were expressed as the mean and standard deviation. Data were analysed statistically by Kruskal-Wallis 1-way ANOVA and multiple comparison test to determine statistical

differences among groups ($p < 0.05$), using SPSS software 22.0 (IBM, Armonk, NY, USA).

Results

The results of mean inhibition zone against *S. sobrinus* are shown in Table 1. Bacterial growth was evident in positive control, whereas no bacterial growth was observed in negative control.

Table 1. The antibacterial activity of Gyp-CHT, DYC and GIC

	Gyp-0%CHT	Gyp-2.5%CHT	Gyp-5%CHT	Gyp-10%CHT	DYC	GIC
Mean	7.08	8.83	8.67	9.53	7.33	7.92
SD	0.97	1.68	1.04	0.72	0.79	1.04
Gyp-0%CHT	-					
Gyp-2.5%CHT	0.025*	-				
Gyp-5%CHT	0.013*	0.816	-			
Gyp-10%CHT	0.00*	0.141	0.215	-		
DYC	0.758	0.053	0.030*	0.001*	-	
GIC	0.253	0.272	0.183	0.010*	0.404	-

Values are expressed as mean \pm SD of diameter of inhibition zones in mm (n=9). Multiple comparison of the antibacterial activity of the test materials at 48 hours * $p < 0.05$ against *S. sobrinus*.

The diameter of the inhibition zone was measured in millimeter (mm) which was significantly greater in Gyp-10%CHT than both DYC ($p=0.001$) and GIC ($p=0.01$). Gyp-5%CHT showed significantly higher antibacterial activity than DYC ($p=0.03$) but not against GIC ($p=0.18$). Significantly decreased antibacterial activity was observed with Gyp alone compared with 2.5%CHT ($p=0.02$), 5%CHT ($p=0.01$), and 10%CHT ($p=0.000$)- containing Gyp.

Discussion

This antibacterial study aimed to evaluate the effect of Gyp-CHT with different concentrations of chitosan on *S. sobrinus* and compare with the DYC and GIC. Agar diffusion test is largely used in microbiology to evaluate the antibacterial activities of dental materials.²⁴⁻²⁷

GIC was included in this study due to its cariostatic behavior compared to the other dental restorative materials and its effects to suppress caries formation,^{28,29} thus it is considered as a suitable material for comparison along with DYC.

Oral streptococci make up a high proportion of oral normal flora, however, they are involved in formation of dental caries.^{30,31} The

cariogenic bacteria have 3 characteristics, including the ability to adhere to the tooth surface, resist in the acidic environment and produce acid.³² Mutans streptococci are highly acidogenic; they can produce short-chain acids that can dissolve tooth hard tissues. Mutans streptococci have the ability to metabolize the sucrose to synthesize insoluble extracellular polysaccharides that encourage their adherence and enhance formation of the biofilm.³³ *S. sobrinus* along with *S. mutans* are the most mutans streptococci isolated from the dental caries, and responsible for the initiation of dental caries.³⁴ *S. sobrinus* are involved in development of dental caries particularly in instances where the development of caries appears to be independent of *S. mutans*. It showed higher acid production and acid tolerance compared to *S. mutans*.^{31,35-37}

Pulp capping material inhibits bacterial growth through the antibacterial effect of the material itself and/or change of the environmental conditions.³⁸ Gyp served as a resorbable scaffold used to deliver antibiotics and growth factors.^{39,40} In this study, it was applied as a resorbable material to release the antimicrobial components of CHT. Recently, the attention has been directed

toward the utilization of CHT in numerous dental applications.⁴¹⁻⁴³ The inclusion of chitosan in pulp capping material can be very promising because of its high antibacterial properties.

The results found that Gyp-CHT has potent antibacterial activity against *S. sobrinus* and the means of inhibition zone significantly increase when the CHT was added. Gyp-10%CHT showed higher antibacterial activity than DYC and GIC. Significantly decreased antibacterial activity was observed with Gyp-2.5%CHT and Gyp-5%CHT compared to Gyp-10%CHT.

Several studies reported that the chitosan has a considerable antibacterial effects against a broad spectrum of bacteria.^{44,45} One of the most acceptable proposed mechanisms of the antimicrobial activity of CHT is that the CHT has positive charged molecules, it can interact with the negatively charged cell wall of the microbes leading to the leakage of the intracellular contents. This leakage results in binding between DNA and CHT, then, the synthesis of mRNA is inhibited by penetration of CHT into the microorganisms nuclei, hence resulting in interference with the proteins synthesis in microorganism.⁴⁶

The antibacterial activity was significantly higher in Gyp-10%CHT compared to Gyp-5%CHT and Gyp-2.5%CHT, that may be related to the effect of $-NH_3^+$ amount,⁴⁷ which is directly represented by CHT concentration, this finding is comparable with other study.⁴⁸

DYC showed high antibacterial activity as reported in previous studies.^{38,49} Its antibacterial activity originates from the release of hydroxyl ions, which are highly oxidant and show extreme reactivity with several biomolecules.⁵⁰ In addition, the antibacterial activity of GIC is also indicated as reported previously,^{51,52} this antibacterial activity is related to the low pH and fluoride release,^{53,54} which has inhibitory effect on numerous enzymes and fermentative activities and viability of the bacteria.⁵⁵

Conclusion

Gyp-CHT has potent antibacterial activity against *S. sobrinus*. Gyp-10%CHT has the highest antibacterial effectiveness when compared with DYC and GIC. This observation suggests that Gyp-CHT is a promising candidate for use as a pulp capping material. However,

further *in vivo* studies are required to investigate its antibacterial activity in clinical applications.

Acknowledgement

This study was supported by 'short term grants, No. 304/PPSG/61313006' of Universiti Sains Malaysia. The authors would like to thank the staffs of Craniofacial Science Laboratory, School of Dental Sciences, Universiti Sains Malaysia for their technical supports.

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