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RESEARCH ARTICLE

Antibacterial Efficacy of Dental Sealant Containing Phytoncide

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Background: Dental caries prevention is a key research focus in dentistry, requiring advancements in the formulation of dental sealants. This study investigated the physical and antibacterial attributes of dental sealant enriched with phytoncide.
Methods: Phytoncide was mixed with a commercially available dental sealant (Clinpro) at concentrations of 0 (control), 1.5%, 3%, and 4.5% by weight (wt%). The flexural strength, curing depth, and wettability of the dental sealant were measured. Antibacterial properties against *Streptococcus mutans* were evaluated through the enumeration of colony-forming units. Statistical analyses employed one-way variance analysis followed by Tukey's test ($p<0.05$).
Results: The dental sealant containing 3% phytoncide showed no significant difference in flexural strength and curing depth compared with that in the control group ($p>0.05$). The flexural strength and curing depth decreased with increasing phytoncide content and significantly differed in sealant containing 4.5 wt% phytoncide ($p<0.05$). Wettability did not differ between the experimental and control groups ($p>0.05$). The antibacterial properties of the sealant containing 1.5% phytoncide were the same as those of the control group ($p>0.05$). The bacterial viability was significantly reduced in groups containing 3% and 4.5% phytoncide compared with that in the control group ($p<0.05$).
Conclusion: Dental sealants incorporating phytoncide have a promising potential as antibacterial dental materials.

Key Words: Antibacterial efficacy, Dental sealant, Phytoncide, *Streptococcus mutans*

Introduction

1. Background

Dental caries is a highly prevalent oral disease globally[1)]. This susceptibility is intricately linked to the physical dimensions and unique morphology of depressions and cracks on the tooth surface that produce a conducive habitat for microorganisms and that render oral hygiene procedures in these areas more challenging and result in increased tooth staining[1,2)]. Such characteristics are recognized as significant risk factors because the accrual of bacterial biofilms significantly contributes to the onset of oral conditions, such as cavities and periodontitis[3,4)]. Applying a fissure sealant to these occlusal pits and fissures forms a tangible impediment against bacterial biofilms[5)].

Bacterial biofilms accumulate in these specific anatomical areas of the teeth[6)]. Applying sealants to vulnerable regions is a pivotal preventive measure to mitigate the risk of cavities. Sealing occlusal pits and fissures with resin-based sealants represents an established method for preventing occlusal caries[7)]. Recently, flowable composites have been proposed as fissure sealants owing to their advantageous attributes such as low viscosity and elastic modulus and ease of handling[8)]. However, the resin-based sealant remains susceptible to bacterial colonization despite its manifold benefits, potentially leading to secondary caries due to bacterial acid production[9)]. Despite increased interest and substantial progress in sealant applications,

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concerns persist regarding the occurrence of secondary caries resulting from plaque deposition and bacterial adhesion[7]. Therefore, a sealant that can suppress secondary caries is required because the presence of sealant around existing restorations is a primary cause of restoration failure[10]. The exploration of natural products has recently gained traction, driven by an enhanced understanding of their utility[11-14]. The notable advantages of natural products include low residual toxicity and side effects, although they can be limited because their composition or ingredients depend on manufacturing conditions[15,16]; additionally, in instances of materials with added antibacterial agents, the physical properties of the material may be reduced. Hence, antibacterial agents that are used within a range that does not compromise physical properties may have potential for clinical use[9,17]. However, a comprehensive analysis of existing studies reveals minimal research related to this. Research on natural organic substances, such as eucalyptus and phytoncide, which are characterized by their nontoxic nature and potent antibacterial properties, is actively underway[18], and an increasing awareness of phytoncide has garnered elevated expectations for its potential applications in dental care.

Phytoncide, derived from the Greek word for plant (phyton) and the Latin word for kill (cide), is a comprehensive term that encompasses both plant-derived volatile and nonvolatile compounds possessing sterilizing properties[18,19]. Phytoncide as a volatile aromatic component can be obtained by steam distillation of plants[18,20] and can comprise dozens to as many as 200 types of compounds. Phytoncide has been recognized as an essential oil and is acknowledged to have antibacterial and antifungal effects[21,22]. However, research aimed at improving the antibacterial efficacy of dental sealants by incorporating phytoncide remains insufficient.

2. Objectives

Therefore, this study formulated a sealant enriched with a phytoncide known for its antibacterial properties. The potential antibacterial effect against *Streptococcus mutans*, a microorganism closely associated with dental caries, was investigated to ensure that the physical properties of the sealant remained uncompromised.

Materials and Methods

1. Preparation of materials

Clinpro was used as a commercially available dental sealant product (3 M ESPE, St. Paul, MN, USA). The application followed the instructions of the manufacturer, and polymerization was accomplished using a light irradiator (650 mW/cm^2; 3 M Elipar Free Light 2, St. Paul, MN, USA). A dental sealant containing phytoncide was produced by adding varying concentrations (0%, 1.5%, 3%, and 4.5%) of phytoncide stock solution (PT) (Kim Min-jae Hinoki Cypress; Pyeonbaekcide Co. Ltd., Seoul, Korea), corresponding to control (0%), 1.5% PT, 3% PT, and 4.5% PT groups (Table 1)[21].

2. Measurement of polymerization depth

A cylindrical Teflon mold with a 10-mm length and 4-mm diameter was marginally overfilled with composite resin incorporating phytoncide at each specified concentration. Excess material was carefully removed by compression with a polyester film and a slide glass. The experimental procedure adhered to the test method outlined in ISO 4049. A plastic spatula was employed during this process to prevent air bubble formation. After confirming uniform material distribution, top-down light radiation lasting 60 seconds was administered, ensuring that the light irradiation surface was vertical. The material was then disengaged from the mold, any unpolymerized material was promptly removed using a plastic spatula, and the height of the polymerized material was measured using an electronic vernier caliper. This sequence was repeated five times to give an average value.

3. Flexural strength

The experiment was conducted according to the test

Table 1. Composition of Materials in the Control and Experimental Groups

Group	Group code	Sealant, wt%	Phytoncide (PT), wt%
1	Control	100	0
2	1.5% PT	98.5	1.5
3	3% PT	97	3
4	4.5% PT	95.5	4.5

method outlined in ISO 4049. Subsequently, each specimen was placed on a support with a 20-mm width, and a three-point bending test was performed using a universal testing machine (Rheometer Compac-100 II; Sin Scientific CO., LTD, Tokyo, Japan) at a crosshead speed of (0.75± 0.25) mm/min. The maximum load value was measured and recorded. This recorded value was then substituted into the following equation to calculate the flexural strength (MPa):

Flexural strength (δ)=3FL/2bh2,

where F represents the maximum load applied to the specimen (N), L denotes the distance between two supports (20 mm), b corresponds to the width of the specimen measured before the test (mm), and h denotes the thickness of the specimen measured immediately before the test (mm). Five specimens were manufactured, and repeated measurements were performed per experimental group. The upper and lower limit values in each experimental group were excluded from statistical processing.

4. Measurement of the wettability

The wettability of the samples was assessed by measuring the contact angle using a contact angle analyzer (Phoenix 300; Surface Electro Optics, Suwon, Korea) along with contact angle measurement software (Image XP version 5.9; Surface Electro Optics).

5. Bacterial strains and culture conditions

S. mutans (ATCC 25175) was cultured in brain heart infusion (Becton Dickinson and Co., MD, USA) and incubated at 37°C for 24 hours. The bacterial solution was adjusted to 1×10^8 colony-forming units (CFUs) for the experiment.

6. Counting of colony-forming units

A 1 ml volume of activated *S. mutans* (1×10^8/ml) was introduced to the sterilized specimen and cultured at 37°C. After 24 hours, the specimen was washed twice using a fresh BHI (brain heart infusion) liquid medium. Subsequently, the specimen was placed in 1 ml of BHI liquid medium and ultrasonically washed to physically detach the bacteria attached to the specimen. After that, 100 μl of the separated bacterial solution was dropped onto a BHI solid medium, smeared, and cultured for 24 hours. The number of CFU per ml was enumerated.

7. Statistical analysis

The significance of each experimental group was analyzed through one-way ANOVA using the IBM SPSS Statistics 20 program (International Business Machines Corp, New York, NY, USA). Subsequently, the Tukey test was applied at a significance level of 5%.

Results

1. Curing depth

The changes in curing depth of dental sealant with added phytoncide are shown in Fig. 1. When comparing the control

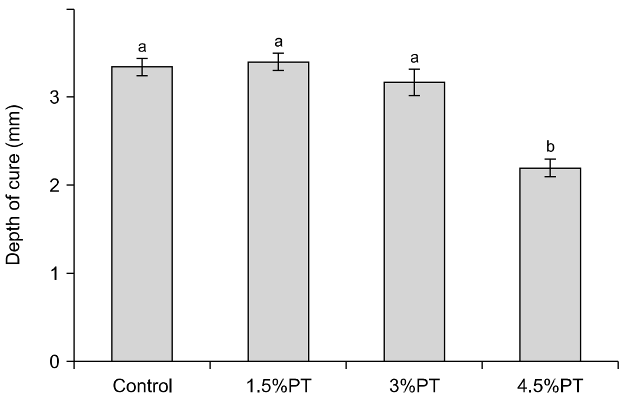

Fig. 1. Curing depth of dental sealant coating with different levels of phytoncide (PT). [a,b]The same lowercase letter indicates no difference in values between groups ($p>0.05$).

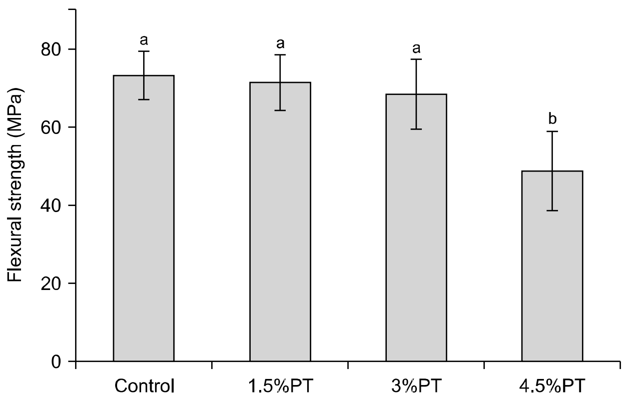

Fig. 2. Flexural strength of dental sealant coating with different levels of phytoncide (PT). [a,b]The same lowercase letter indicates no difference in values between groups ($p>0.05$).

without phytoncide with the 1.5% and 3% PT groups, no significant difference in polymerization depth >3 mm was observed ($p>0.05$). However, a significant reduction in polymerization depth to 3 mm was evident compared with that in the 4.5% PT group ($p<0.05$). No significant differences in the depth of cure were noted among the groups, indicating that phytoncide did not impact sealant curing.

2. Flexural strength

Fig. 2 shows that an increase in phytoncide content corresponds to a decrease in flexural strength. The control and the 1.5% and 3% PT samples (73.2±6.2, 71.4±7.1, and 68.4±8.9 MPa, respectively) did not significantly differ in flexural strength ($p>0.05$). However, the 4.5% PT (48.8±10.2 MPa) samples exhibited a significantly lower strength than that of the control group ($p<0.05$).

3. Wettability

No significant differences were observed in the wettability test results (Fig. 3) between the control and experimental groups with 1.5%, 3%, and 4.5% PT (68.06°±5.11°, 66.53°±4.2°, 63.54°±4.03°, and 65.7°±3.30°, respectively; $p>0.05$).

4. Bacterial viability

The number of CFUs of *S. mutans* attached to the specimen is shown in Fig. 4. The number of CFUs in the 1.5% PT group did not significantly differ compared with that in the control group ($p>0.05$). However, the number of CFUs in the 3% PT group was significantly lower than that in the control group. Furthermore, in the 4.5% PT group, the number of colonies decreased by >50% compared with that in the control group ($p<0.05$). A significant difference was confirmed between the groups with and without phytoncide ($p<0.05$).

Discussion

1. Interpretation

The use of pit and fissure sealants has been acknowledged as an effective method for preventing early occlusal caries[10]. Consequently, efforts have been directed toward enhancing their clinical performance through the addition of antibacterial properties[8,10], and the development of a sealant with antibacterial attributes has been the subject of several studies[23]. However, the incorporation of antibacterial agents may compromise mechanical strength, and the release of antibacterial substances from restorative materials can induce changes in material properties[9,17]. Therefore, a balanced consideration of both physical properties and antibacterial effects is required to produce a dental sealant.

2. Key results and comparison

The depth of cure, important for class 2 sealants relying on external energy sources for polymerization (ISO 6874: 2005), was evaluated[23]. Our results indicated that the addition of phytoncide did not significantly impact the

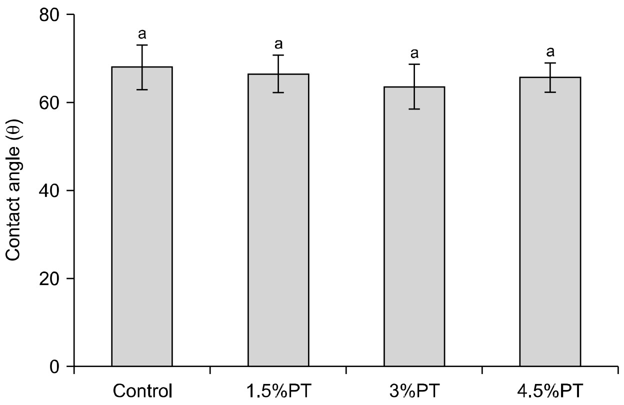

Fig. 3. Contact angle of dental sealant coating with different levels of phytoncide (PT). [a,b]The same lowercase letter indicates no difference in values between groups ($p>0.05$).

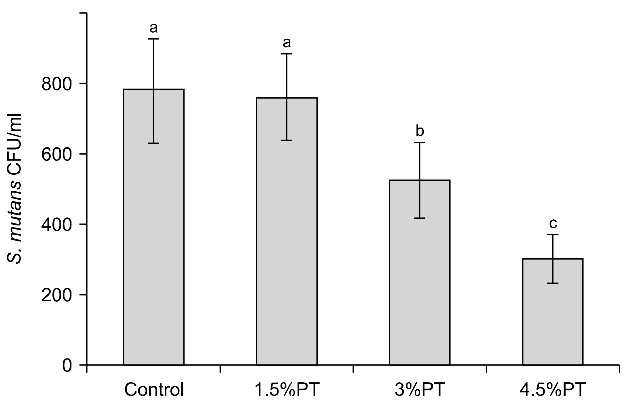

Fig. 4. Colony-forming unit counts of *Streptococcus mutans* attached on the surfaces of sealant coating with different levels of phytoncide (PT). [a,b,c]The same lowercase letter indicates no difference in values between groups ($p>0.05$).

curing depth, eliminating concerns about adverse effects[24]. Similarly, the cure depth of the modified sealant was evaluated in adherence to ISO guidelines[23]. The similarity between the curing depth of samples containing phytoncide and that of control samples means that the phytoncide did not have an adverse effect on curing depth. Similarly, the flexural strength measurements aligned with the trends observed in curing depth measurements. Flexural strength, a parameter defined in ISO 4049[4,23], an international standard applied to dental sealant for aesthetic restoration, is expressed in MPa. No significant difference was noted in all groups except for that of 4.5% phytoncide. Hence, dental sealant with 3% or less of phytoncide was deemed suitable for clinical use. Surface wettability, as evidenced by the contact angle, exhibited no significant difference between the control and the experimental groups, supporting the assumption that the surface wettability remains unaffected[23]. The antibacterial activity of the phytoncide was measured using *S. mutans* as this is a primary causative bacterium in dental caries development[25,26]. The number of *S. mutans* CFUs was found to be significantly decreased in groups containing $>$3% phytoncide. This underscores the heightened inhibitory effect on the growth of *S. mutans* with increasing phytoncide concentration.

Although phytoncides do not exert a direct influence on the growth and differentiation of plants, they are reported to have excellent antioxidant, anti-inflammatory, sterilizing, and nerve stabilizing effects[18,19,27]. Phytoncide is recognized as an antibacterial and plant-based sterilizing substance[22] and plays a pivotal role in safeguarding trees against external attacks, including by microorganisms[20]. Analysis involving plating on BHI agar medium revealed that both *S. mutans* and *Streptococcus sobrinus* succumbed to the bactericidal effects of phytoncide, extending its antibacterial effect across a broad spectrum of microorganisms[28]. Moreover, the efficacy of phytoncide in combating periodontal disease has been demonstrated[22].

3. Suggestion

The reported antibacterial effect of phytoncide has spurred the development of their incorporation into various household products. Given the escalating prevalence of antibiotic-resistant pathogens, the potential use of natural substances, such as phytoncide, in oral hygiene products or dental clinical trials is highly desirable. While the precise mechanism of phytoncide remains elusive, damage to cell membranes and their subsequent dissolution may be triggered by the bactericidal action to inhibit vital processes in bacterial cells[18,19,27]. Distinguishing between the dissolution phenomenon due to bacterial death and the phenomenon of death after direct damage to the cell membrane, leading to dissolution as the cytoplasmic contents are released, is challenging. Hence, comprehensive research on the antibacterial mechanism effect of phytoncide warrants future exploration[20,21].

4. Limitations

This study sought to evaluate the potential clinical performance of sealants with antibacterial activity through the incorporation of phytoncide. However, the applied methodology had several limitations, primarily associated with the reproducibility of the physiological and biological conditions within the oral cavity. Consequently, findings from in vitro studies cannot be extrapolated to in vivo clinical scenarios, where the effectiveness of biomaterials may differ. Consequently, more laboratory tests are required to fully characterize the materials involved. Furthermore, only the short-term antibacterial properties of sealants containing phytoncide against cariogenic bacteria were assessed. Future studies should therefore focus on demonstrating the long-term efficacy of these substances in reducing cariogenic bacteria when used as sealants containing phytoncide. The research presented herein presents preliminary results from the early stages of biomaterial development, necessitating continued supplementation in the future.

5. Conclusion

Dental sealants containing phytoncide at concentrations of up to 3% demonstrated no adverse effects on properties such as curing depth, flexural strength, and wettability compared with dental sealants lacking phytoncide. This novel material exhibited notable antibacterial properties, affirming its potential as a promising candidate for a caries-inhibiting dental material with appropriate physical properties.

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

Not applicable.

Author contributions

Conceptualization: Myung-Jin Lee. Data acquisition: Song-Yi Yang. Formal analysis: Song-Yi Yang. Funding: Myung-Jin Lee. Supervision: Myung-Jin Lee. Writing-original draft: Song-Yi Yang and Myung-Jin Lee. Writing-review & editing: Song-Yi Yang and Myung-Jin Lee.

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Data availability

Data supporting the results of this study are available from the corresponding author or the Korean Society of Dental Hygiene Science upon reasonable request.

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