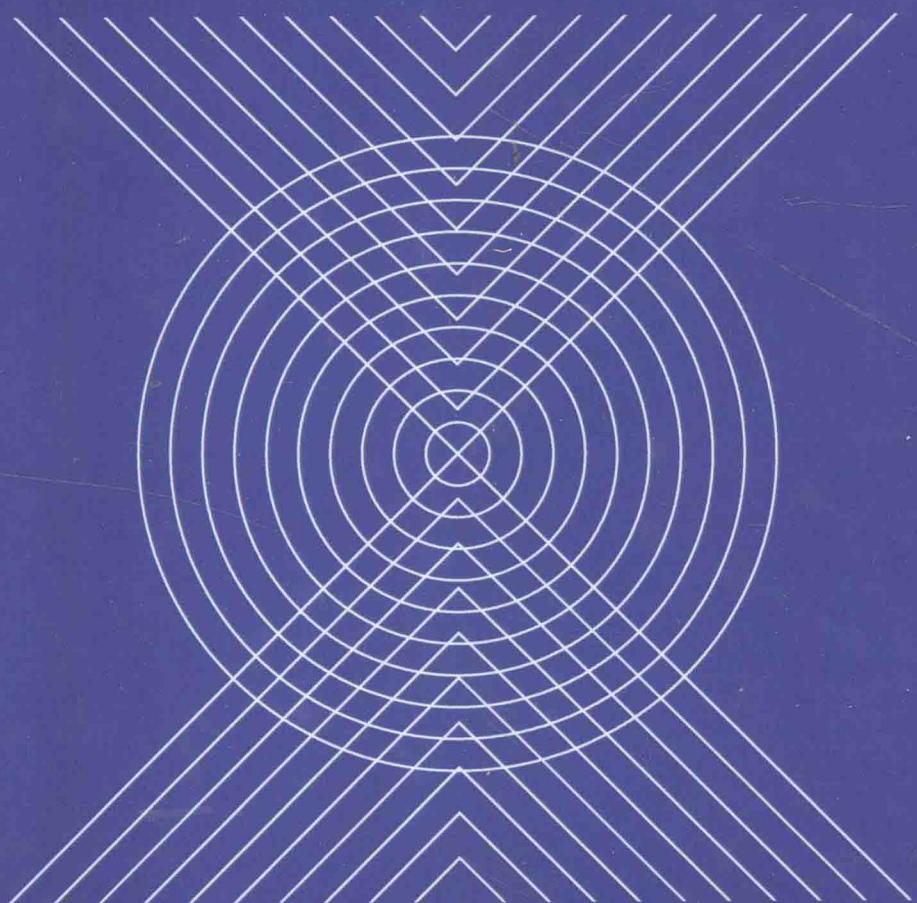
Advanced Materials, Mechanical and Structural Engineering

Editors: Seung Ho Hong, Junwon Seo & Kihoon Moon



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Advanced Materials, Mechanical and Structural Engineering

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Preface

AMMSE 2015 is an annual international conference on Advanced Material, Mechanics and Structural Engineering (AMMSE 2015) which took place in Jeju Island, South-Korea, on September 18–20, 2015. The purpose of the conference was to establish platforms for collaborative research projects in the field of advanced material, mechanics and structural engineering. In the conference, engineers from different industries and researchers from various academic institutions exchanged and shared their experiences, presented their research results, explored collaborations, and sparked new ideas with the aim of developing new projects and exploiting new technologies in the field.

This book is a collection of accepted papers. The papers presented in the proceedings were peer-reviewed by 2–3 expert referees. This volume contains four main subject areas: 1. Advanced material and application studies, 2. Management systems and civil engineering application, 3. Mechanical and structures engineering applications, and 4. Sensors, hydraulic and electric power engineering. The committee of AMMSE 2015 would like to express their sincere thanks to all authors for their high-quality research papers and careful presentations. Also, we would ike to thank the reviewers for their careful comments and advices. Finally, thanks are expressed to CRC Press/Balkema as well for producing this volume.

The Organizing Committee of AMMSE 2015
Committee Chair Prof. Seung Ho Hong,
West Virginia University

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Organization

CIVIL AND ENVIRONMENTAL ENGINEERING-WEST VIRGINIA UNIVERSITY

West Virginia University (WVU) is a public land-grant research university in Morgantown, West Virginia, United State. The total number of students and academic faculty members is 30,000 and 2,000 respectively. Based on the highest level of research activity, the Carnegie Foundation designated WVU as the Highest Research University.

Among the professional degree programs in 15 different colleges at WVU, the Civil and Environmental Engineering department is planning to be a global leader in designing, constructing, managing, and renewing built and natural environments, hereby contributing to a sustainable world, shaping public policy, and enhancing health, safety, and the quality of life. Through cross-disciplinary research, we are developing technological innovation that defines and solves complex problems at the interface of built, natural, and social systems, and engaging in service to the level of state, nation, and the world.

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Inhibitory effect of *Salvia officinalis* L. oil on Candida biofilm

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ABSTRACT: This study aims to determine the effect of *Salvia officinalis* L. (sage) essential oil on Candida biofilm formation in vitro. Sage oil (Hong-Huat Company, Thailand) was prepared in Tween-80 and diluted to concentrations of 0.5–100 mg/mL. Biofilms of *Candida albicans* (ATCC10231 and clinical strain) were grown in 96-well plates with Yeast Nitrogen Base medium (YNB) supplemented with 100 mM glucose in shaking incubator at 37°C for 24 h. After washing, each concentration of sage oil with YNB medium was added and further incubated for 24 h at 37°C. Evaluation of biofilm was assessed through the XTT reduction assay. A 0.2% chlorhexidine solution was used as positive control. It was found that 90% Candida biofilm reduction was demonstrated at concentrations of >5 mg/mL oil whereas chlorhexidine exhibited 89% biofilm reduction. In conclusion, our results indicate that sage oil may be a good alternative to current treatment for oral Candida infection.

1 INTRODUCTION

Fungal infection is one of the major health problems that frequently occur in the oral cavity. The consequences are exacerbated by a concomitant increase in resistance to traditional antifungal agents (Oberoi et al. 2012) due to the rise in the immunodeficient and immunocompromised populations globally. Among all causative agents, *Candida albicans* is the principal species and considered the most virulent. In this context, the identification of effective alternative therapies to the current antifungal agents is important.

The use of medicinal plants to improve oral health has been observed with increasing interest by researchers in the study of their biological properties and active ingredients responsible for their therapeutic effects. Salvia officinalis L. or sage is a member of the Lamiaceae or mint family which is widely used both in culinary and medicinal preparations. Sage leaves and its essential oil possess carminative, antispasmodic, antiseptic, anti-inflammatory, and mucolytic properties (Martins et al. 2015, Marchev et al. 2014). In dentistry, essential oil is applied for the treatment of inflammation and infections of the mucous membranes of the throat and mouth (stomatitis, gingivitis, and pharyngitis (Taheri et al. 2011). Previous studies have reported antimicrobial properties of sage oil against gram negative and gram positive bacteria, dermatophytes, and fungi (Abu-Darwish et al. 2013).

The aim of the present study was to assess the antifungal effect of sage oil against the biofilm of *C. albicans*.

2 MATERIALS AND METHODS

2.1 *Oil preparing*

Salvia officinalis L. or sage oil (Hong-Huat Company, Thailand) was prepared in Tween-80 and diluted to concentrations of 0.5–50 mg/mL.

2.2 *Candida*

Candida albicans ATCC 10231 and strain isolated from the oral lesion of patient were obtained from the culture collection of Oral Microbiology Department, Faculty of Dentistry, Mahidol University, Thailand. They were maintained on Yeast Nitrogen Base (YNB; Difco, USA) agar slant at 4°C. Few colonies from the agar were inoculated in YNB medium with 50 mM glucose and incubated at 30°C in shaking incubator, at 100 rpm for 24 h. Cells from this culture were harvested by centrifugation (3200 g, 5 min). The Cell pellet was washed twice with Phosphate Buffer Saline (PBS, pH 7.4). Candida suspensions were prepared in YNB supplemented with 100 mM glucose to yield a concentration of approximately 107 CFU/mL using McFarland standard.

2.3 *Biofilm formation*

For initial adhesion to a solid phase, 100 µL of Candida suspension was added to each well of 96-well tissue culture plate and incubated in shaking incubator (75 rpm) at 37°C for 90 min. After that, the non-adhered cells were washed 3 times with PBS and further incubated in 200 µL of YNB

supplemented with 100 mM glucose for 24 h to allow biofilm formation.

2.4 *Effect of oil on biofilm*

After Candida biofilm was developed on the bottom of well, YNB medium supplemented with 100 mM glucose along with various concentrations of sage oil (0.5–100 mg/mL) was added to each well. The plate was incubated in shaking incubator for another 24 h at 37°C. Planktonic cells were removed by washing and biofilms were observed under an inverted microscope. Evaluation of biofilm was assessed through the XTT [2, 3-bis (2-methoxy-4-nitro-sulfophenyl)-2H-tetrazolium-5-carboxanilide] (Sigma-Aldrich, UK) reduction assay. XTT solution was prepared by mixing 1 mg/mL XTT salt in PBS and stored at –20°C. Prior use, menadione solution prepared in acetone was added to XTT to a final concentration of 4 µM. 100 µL of XTT-menadione solution was added to biofilm and incubated in shaking incubator in the dark at 37°C for 5 h. The color developed from the water soluble formazan product was measured at 492 nm. A 0.2% chlorhexidine gluconate solution and Tween-80 were used as positive and negative controls respectively.

All experiments were performed triplicate in three separate occasions.

3 RESULTS

The biofilm formation of standard and reference strains of *C. albicans* biofilm was demonstrated in Figure 1. It was clearly shown that all concentrations of sage oil used in the present study could affect *C. albicans* biofilm formation. 90% biofilm reduction was observed at concentration of >5 mg/mL sage oil compared to negative control using Tween-80 whereas 62% reduction was observed at 2.5 mg/mL (Table 1). For the positive

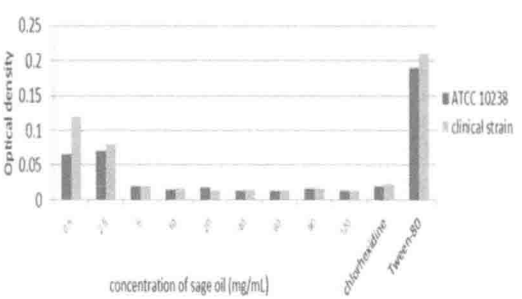

Figure 1. Effect of different concentrations of sage oil on *C. albicans* biofilm.

Table 1. Percentage of Candida biofilm reduction effect of *S. officinalis*.

	Candida biofilm reduction (%)	
S. officinalis (mg/mL)	ATCC 10238	Clinical strain
0.5	65.26	42.86
2.5	63.16	61.90
5.0	90.00	90.95
10.0	92.10	91.90
20.0	90.53	93.33
40.0	93.16	92.86
60.0	92.63	93.81
80.0	91.58	92.38
100.0	91.05	91.90
120.0	92.63	93.81
chlorhexidine	89.47	89.05

* Values are percentage of reduction compared to negative control, Tween–80.

control, chlorhexidine exhibited 89% biofilm reduction. There were no significant differences between the inhibitory effects on standard and clinical strain of C. albicans.

4 DISCUSSION

Salvia officinalis L. or sage is one of the oldest medicinal plants used in traditional medicine by different cultures to treat oral inflammation and digestive disorders (Raal et al. 2007). Essential oil extracted from the leaves has been demonstrated to have antimicrobial activities against many types of fungi including Candida (Abu-Darwish et al. 2013, Sookto et al. 2013, Pinto et al. 2007).

Candida species, especially *C. albicans*, are regarded as the most common opportunistic pathogens in oral cavity. One of the major factors contributing to the virulence of these fungi is their flexibility in adapting to a variety of different environments and attachment to the surface and growing in microbial communities known as biofilm. From the perspective of disease pathogenesis, the most important feature of biofilm development is the high resistance to antimicrobial agents that can be up to 1000-fold greater than that of planktonic cells. In the present study, sage oil exhibited inhibitory effects against biofilm of both strains of *C. albicans*. (90% biofilm reduction was demonstrated at the concentration of 5–120 mg/mL of the oil whereas 62% reduction was observed at 2.5 mg/mL (the concentration reported previously as MIC and MLC values of planktonic cells) (Sookto et al. 2013). However, the inhibition was not in a dose-dependent manner. The higher concentration of oil did not result in greater inhibition.

This may be due to the penetration limitation of oil into biofilm matrix to reach the target cells or the occurrence of some resistant microbial cells.

Many investigations have confirmed the pharmaceutical importance of sage due to its diverse bioactivities. The pharmaceutical important compounds of sage oil are sterols, phenolics (caffeic acid, rosmarinic acid, luteolin), and terpenoids (1,8-cineole, cis- and trans-thujone, camphor, oleanolic acid, ursolic acid, carnosic acid) (Martins et al. 2015, Abu-Darwish et al. 2013). However, environmental conditions such as growing conditions (soil, climate, light, altitude), harvesting, and processing are factors that can directly influence quantitative compositions of the oil (Farhat et al. 2009).

Since essential oils are complex mixtures of several compounds, it is difficult to attribute their biological activity to a particular constituent. Usually, major compounds are the ones responsible for the antifungal activity of the essential oils. However, some studies show that minor components may have a crucial role in the biological activity of the oils (Koroch et al. 2007). In the case of sage oil, cisthujone, 1,8-cineole, and camphor are suggested to be the main components responsible for its antifungal activity against Candida and other filamentous fungi (Pinto et al. 2007). However, there are very limited numbers of papers dealing with the mechanisms of antifungal property of sage oil. It has been proposed that the interaction of essential oil with lipid components in the cellular structure of yeast cells, thereby increasing membrane permeability and electrolyte imbalance impairs initial adhesion of yeast cells and then affects biofilm formation (Braga et al. 2008).

Considering the toxicity of sage oil, it is Generally Recognized As Safe (GRAS) by the Food and Drug Administration in USA (2012) and drugs and tinctures prepared from these plants are listed in several official Pharmacopoeias (Marchev et al. 2014). Previous study in cell cultures stated that bioactive concentrations of sage oil did not affect mammalian macrophages and keratinocytes viability, making them suitable to be incorporated in skin care formulations for cosmetic and pharmaceutical purposes (Abu-Darwish et al. 2013).

5 CONCLUSION

In this part results from this study demonstrated that *S. officinalis* oil at concentrations of 5–120 mg/mL could inhibit (90% of *C. albicans* biofilm formation. The effect was similar on both standard strain and strain isolated from the patient's lesion. It may be a good alternative to current treatment for oral Candida infection. However, further studies are required to evaluate the effect on other yeast strains or cytotoxicity before considering for use in patients.

ACKNOWLEDGEMENT

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Adhesion and biofilm formation of *Streptococcus mutans* on dental sealant incorporated with silver-nanoparticle

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ABSTRACT: This study aims to investigate the adhesion and biofilm formation of *Streptococcus mutans* on dental sealant incorporated with silver-nanoparticle. Dental sealants containing 0.5%, 1.0% and 1.5% $AgZrPO_4$ nanoparticle were prepared. Mixture of bacterial suspension (2×10^6 cells/mL) and pooled saliva was added onto dental sealant. For adhesion assay, the plate was incubated in shaking incubator at 37°C for 3 h. Biofilm formation was done as the adhesion assay and further incubated for 24 h. The amount of adhering bacteria and biofilm formation was determined by crystal violet technique. Significant reduction of *S. mutans* biofilm formation was observed in the groups of 0.5%, 1% and 1.5% silver-nanoparticle compared with control even though no inhibitory effect was observed on initial adhesion. The incorporation of low concentrations of silver-nanoparticle to dental sealant clearly exhibited the suppressive effect on *S. mutans* biofilm formation. The use of these sealants could have a potential for caries prevention.

1 INTRODUCTION

Dental caries is one of the serious public health problems affecting children and adults worldwide. The prevalence of the disease is high in children. It is the most important oral disease and is of medical, social and economic importance. The disease is the result of a dental decay process in which mainly acidogenic and aciduric bacteria residing in a complex biofilm degrade tooth structure, leading to demineralization and cavitation. Unlike classical infectious diseases, which are caused by microbial pathogens, dental caries is caused by resident oral microflora. *Streptococcus mutans* appears to play an important role in the initiation of dental caries since its activities lead to colonization of the tooth surface, dental plaque or oral biofilm formation and demineralization of tooth enamel by acids (Loesche. 1986).

Dental sealant which is a material introduced into the occlusal pits and fissures of the teeth as a protective layer has been widely used to prevent dental caries. According to American Dental Association expert panel's conclusion, the reduction of dental caries incidence in children and adolescents after placement of pit and fissure sealant was reported from 60–86% (Beauchamp et al. 2008). In addition, application of dental sealant could delay the progression of carious lesions in adolescents and adults (Beauchamp et al. 2008). On the other hand, the miroleakage and microbial effect on food residue on sealant is also reported as the major cause of secondary caries on these tooth surfaces (Simonsen & Neal. 2011). The incorporation of the antimicrobial agent to sealant materials could have the potential benefit of additional caries protection. Today, the silver-nanoparticle is going favoured as an antimicrobial agent due to its broad spectrum, low toxicity and lack of bacterial resistance (Peng et al. 2012). Therefore, the aim of the present study was to investigate the adhesion and biofilm formation of *S. mutans* on dental sealant incorporated with silver-nanoparticle.

2 MATERIALS AND METHODS

2.1 *Microorganisms*

Streptococcus mutans KPSK2 was obtained from a culture collection of Oral Microbiology Department, Faculty of Dentistry, Mahidol University, Thailand. The bacteria were grown in 50 mL of brain heart infusion broth at 37°C in 5% CO_2 atmosphere for 24 h. After that, they were centrifuged at 5000 rpm for 15 min and the pellet was resuspended in phosphate buffer saline solution to 2×10^6 cells/mL.

2.2 *Saliva*

Paraffin stimulated whole saliva was collected from four healthy volunteers who had good oral hygiene, no history of systemic disease and not taken any antibiotics or other drugs during and two weeks prior to the experiment. The saliva samples were pooled, centrifuged at 10,000 rpm for 20 min to remove cell debris and then filtered through the Millipore membrane (0.22 μm).

2.3 *Dental sealant*

Dental sealant used in this study was Helioseal fissure sealant (Ivoclar Vivadent, USA). The sealant composition was: bisphenol A glycidyl methacrylate (Bis-GMA), triethyleneglycol dimethacrylate (TEGDMA) and titanium dioxide. Silver zirconium phosphate ($AgZrPO_4$) nanoparticles (National Direct Network Company, Thailand) were added to dental sealant at the concentrations of 0.5%, 1.0% and 1.5% w/w. Dental sealant samples were prepared on flat bottoms of 96-well plate.

2.4 *Adhesion assay*

One hundred µL of bacterial suspension and 100 µL of pooled saliva were mixed together and added onto the prepared dental sealant with different concentrations of silver nanoparticle on 96-well plates. The plates were incubated in shaking incubator at 37°C, 100 rpm for 3 h. After washing to remove the non-adhered bacterial cells, the adhered cells were fixed by 99% methanol and stained with 0.5% crystal violet for 20 min. Two hundred µL of 33% acetic acid was added and the optical density was determined at 575 nm.

2.5 *Biofilm assay*

The experiment was done as in the adhesion assay. Two hundred µL of brain heart infusion broth supplemented with 50 mM glucose was added to the adhered bacterial cells on dental sealant and further incubated in shaking incubator at 37°C, 100 rpm for 24 h. For biofilm quantification, the plates were immersed in deionized water and slowly shaken to remove remaining planktonic or loosely bound bacterial cells. The amount of biofilm formation was evaluated by staining with crystal violet and measuring the optical density at 575 nm.

Dental sealant without a silver nanoparticle was used as a control.

2.6 *Statistical analysis*

Experiments were done triplicate in three separate occasions. Comparison of S. mutans adhesion on dental sealants was analyzed by one-way analysis of variance (ANOVA) and Dunnett's test to determine significant differences. In the evaluation of biofilm formation, Kruskal Wallis rank analysis of variance was applied to assess any differences among groups. Pairwise comparisons were performed by Mann-Whitney U tests. Data were considered statistically significant at p-value <0.05M.

Table 1. *S. mutans* adhesion on dental sealants containing various concentrations of silver-nanoparticle.

Dental sealant with $AgZrPO_4$	Optical density (mean ± SD)	p-value
0.5%	0.622 ± 0.003	0.54
1.0%	0.607 ± 0.004	0.85
1.5%	0.621 ± 0.008	0.61
Control	0.612 ± 0.005	

Table 2. *S. mutans* biofilm formation on dental sealants containing various concentrations of silver-nanoparticle.

Dental sealant with $AgZrPO_4$	Optical density (mean rank)	p-value
0.5%	5.33	0.001
1.0%	5.00	0.000
1.5%	5.00	0.000
Control	13.67	

3 RESULT

No significant difference of *S. mutans* adhesion was observed among any groups of dental sealant incorporated with silver-nanoparticle or control group, silver-nanoparticle free sealant (Table 1). However, significant inhibition of *S. mutans* biofilm development was demonstrated in the groups of sealant containing 0.5%, 1% and 1.5% silver-nanoparticle compared with control (Table 2). The reduction was not different among each concentration of silver nanoparticle.

4 DISCUSSION

The adhesion of cariogenic bacteria such as *S. mutans* to tooth surfaces is the primary and essential prerequisite for the development of biofilm related to dental caries. It was found that secondary caries under and around restorative filling materials (Sarrett. 2007, Friedl et al. 1995). Therefore, materials with a low susceptibility to bacterial adhering or biofilm developing are preferable.

The antimicrobial properties of silver have long been recognized and used extensively in applications for disinfecting medical devices and home appliances for water treatment (Bosetti et al. 2002, Li et al. 2008). Several kinds of inorganic materials containing silver using different carriers such as zeolite, activated carbon and phosphate were developed to extend lifetime of silver. The prolonged constant dissociation of silver ions into the surrounding environment provides a higher clinical efficacy of these silver containing materials. In addition, these materials do not cause toxic effect on humans or disturb the color of the products

(Sekhon & Kambojet. 2010 a,b). In dentistry, silver particles have been developed and investigated for a range of possible applications, for example, incorporated into denture base materials (Bajracharya et al. 2014), dental implants (Almaguer-Flores et al. 2010) and filling materials (Burgers et al. 2009). In our study, the incorporation of 0.5–1.5% silver-nanoparticles to dental sealant did not show any inhibitory effect on bacterial initial adhesion. This was contrary to the result of previous study (Burgers et al. 2009) which demonstrated that the addition of microparticulate silver to resin composite could reduce the number of adhering bacteria. The difference may be from the artificial saliva employed in their initial adhesion assay and whole saliva used in the present study since multiple proteins in whole saliva mediate the adhesion of bacteria to the surfaces (Rudney. 1999).

Considering the prolonged outcome of bacterial biofilm development, dental sealant containing silver-nanoparticles clearly exhibited the suppressive effect compared with control without silver particle (Table 2). However, no significant difference was observed among each concentration of silver. Our data seem consistent with other previous studies that have reported antibacterial efficacy of dental resin incorporating silver-nanoparticles on cariogenic biofilm (Cheng et al. 2012, Akhavan et al. 2013). In addition, the activity has been shown to be time-dependent, such that extending the length of silver-nanoparticles exposure to 24 h enhanced the inhibitory effects on microorganisms (Bahador et al. 2013). Several mechanisms have been proposed for the antimicrobial property of silver-nanoparticles: (1) adhesion of particles to the surface altering membrane properties. Silver-nanoparticles have been reported to degrade lipopolysaccharide molecules, accumulate inside the membrane forming pits and increase membrane permeability (Sondi & Salopek-Sondi. 2004); (2) particles penetrating inside the bacterial cell, resulting in DNA damage; (3) interaction of particles with thiol and carbonyl groups in proteins, resulting in inactivation of respiratory chains of bacteria, ATP production and growth inhibition (Chen et al. 2011, Li et al. 2011).

Regarding adverse effect on the materials, it has been shown that the low amount of silver-nanoparticles (0.5%) incorporated to dental resin did not change color or mechanical properties of the polymer (Bajracharya et al. 2014, Monteiro et al. 2012).

Results from our investigation suggest potential anti-biofilm property of dental sealant containing low concentrations of silver-nanoparticle (0.5–1.5%). However, it should be noted that the activities of materials were influenced by silver particles dispersing as well as the homogeneous incorporation into the resin.

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