AAPS PharmSciTech, Vol. 16, No. 1, February 2015 (© 2014)
DOI: 10.1208/s12249-014-0209-5

Research Article

Antibacterial Activity and Inhibition of Adherence of *Streptococcus mutans* by Propolis Electrospun Fibers

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Received 16 April 2014; accepted 25 August 2014; published online 23 September 2014

Abstract. Mouth-dissolving fibers with antibacterial activity for the oral cavity were prepared by an electrospinning technique. Propolis extract was used as an active ingredient and polyvinylpyrrolidone (PVP) K90 as the polymer matrix. The morphology and diameter of the fibers were characterized by scanning electron microscopy. Antibacterial activity against *Streptococcus mutans* and the inhibition of *S. mutans* adhesion on a smooth glass surface during the biofilm formation were tested. Propolis, 5% (*w*/*v*), was combined with a PVP K90 solution, 8% (*w*/*v*), with or without Tween 80 including flavor additives and electrospun with an applied voltage of 15 kV. Uniform and smooth fibers of propolis-PVP K90 were obtained. The results showed that electrospun fibers with propolis extract can dissolve and release the propolis in water. Propolis-PVP electrospun fibers showed better antibacterial activity by reduction of bacteria adhesion on a smooth glass surface when compared to some commercial mouthwash products. These results indicated the potential of electrospun fibers to be used as mouth-dissolving fibers for effective antibacterial activity in the oral cavity.

KEY WORDS: antibacterial activity; electrospun fibers; inhibition of adherence; propolis; *Streptococcus mutans*.

INTRODUCTION

Propolis is a resinous substance from beehives. It is a dark brown to yellow sticky resin collected from buds and bark of trees by honeybees (1,2). Many studies have shown that propolis has many good biological properties such as antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, and anticancer activities and can promote the immune system (3,4). The composition of propolis depends on its geographical and botanical origins. The most active constituents of propolis are aromatic acids and phenolic compounds, especially flavonoids and phenolic acids (5).

Dental caries is an infectious disease that is caused by pathogenic bacteria in the oral cavity. *Streptococcus mutans* is one of the most cariogenic microorganisms that is involved in the development of dental caries and dental plaque in humans. *S. mutans* can produce acid and synthesizes water-insoluble glucan by the action of glucosyltransferase (GTFase) which is the major source of dental plaque or biofilm. The biofilm encourages the aggregation of bacteria on the tooth surface and is the main factor that causes dental caries (6,7). The reduction of bacteria in the oral cavity and the prevention of biofilm formation or the removal of biofilm from tooth surfaces are important for the prevention of tooth decay. There are many products for the oral cavity such as toothpastes, gums, or mouthwashes that can reduce the risk of tooth decay. Many products contain chemical agents such as chlorhexidine, triclosan, or sodium fluoride that are the effective antibacterial and antiplaque agents in the oral cavity. Unfortunately, the daily use of chemical substances may cause local side effects or oral mucosa irritation. In recent years, many studies about natural products have been conducted, and they are recommended for caries control due to their antibacterial properties and limited side effects. Propolis is one of the natural materials that can be used in oral care products having anticaries and antiplaque properties (8). There are many products containing propolis extract in the market such as ethanol extracts, toothpastes, and mouthwashes.

Electrospinning is a process that uses electrostatic forces to form fibers. This process uses a high voltage supply to induce charge of a certain polarity into a polymer solution or polymer melt that is accelerated to a collector which is of opposite polarity (9). The obtained electrospun fibers have small diameters in the micrometer to nanometer range. The high surface area per unit mass and high porosity of electrospun fibers are advantageous for applications in many fields. Nowadays, electrospun fibers have potential for use in various applications such as filtration, protective clothing, and

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biomedical and tissue engineering scaffolds including drug delivery systems (10,11). The mouth-dissolved drug delivery system is one of the systems that dissolves or disintegrates quickly in the mouth when it is wet by water. To meet this requirement, the electrospinning technique is one of the effective methods for the production of soluble polymer fibers as a mouth-dissolved electrospun membrane that can be dissolved easily in the oral cavity (12,13).

In this study, we produced mouth-dissolving fibers containing propolis using the electrospinning technique. Water soluble polyvinylpyrrolidone polymer was selected as the polymer base for the electrospun fibers in which was incorporated propolis. The antibacterial activity of propolis electrospun fibers against *S. mutans* was studied as well as the inhibition of the adherence of *S. mutans* on smooth surfaces.

MATERIALS AND METHODS

Materials

Propolis was obtained from Chiangmai Healthy Product Co., Ltd. (Chiangmai, Thailand). Polyvinylpyrrolidone (PVP K90, Kollidon K90) was obtained from BASF Corp. (Ludwigshafen, Germany). Anhydrous ethanol of analytical grade was purchased from Merck (Germany). All other chemicals and solvents were of analytical grade and double-distilled water was used in this study.

Methods

Propolis Extraction

The extraction of propolis followed the procedure of Sanpa *et al.* (14). Before extraction, crude propolis was cooled with liquid nitrogen and ground. Thirty grams of ground propolis were mixed with 300 mL of 70% ethanol and extracted using an ultrasonic technique for 30 min and then filtered. The filtrate was evaporated using a rotary evaporator under reduced pressure at a temperature below 40°C. The residue was lyophilized and the dry powder of the propolis extract was kept in a closed container and protected from light.

Preparation of Propolis-PVP K90 Electrospun Fibers

For control polymer base electrospun fibers, 8% (*w*/*v*) PVP K90 was dissolved in absolute ethanol. For the case of active ingredient incorporation, 5% (*w*/*v*) propolis with or without small amounts of additives including 0.01% (*w*/*v*) menthol, 0.005% (*w*/*v*) thymol, 0.005% (*w*/*v*) methyl salicylate, and 0.001% (*w*/*v*) eucalyptus oil, which were used as flavoring agents. Tween 80, 1% (*w*/*v*), was used as wetting agent. All of the ingredients were dissolved in absolute ethanol to get clear solution before PVP K90 was dissolved in the solution. In the case of chlorhexidine electrospun fibers, chlorhexidine stock solution was added into 8% (*w*/*v*) PVP K90 polymer solution to make 4.8% (*w*/*v*) of chlorhexidine in the spinning solution. Mechanical stirring was applied for 2 h to achieve a clear solution. The homogeneous solution was then filled into a glass syringe equipped with a 20-gauge stainless steel needle (inner diameter, 0.66 mm). A power supply was used at a voltage of 15 kV, and the electrospun nanofibers were collected on aluminum foil at a distance of 15 cm. The feeding rate was controlled by a syringe pump at 2 mL per hour.

Morphological Characterization of Propolis-PVP K90 Electrospun Fibers

A scanning electron microscope (FIB Quanta 200 3D) was used to investigate the morphology and surface of the electrospun fibers. Samples were gold sputter-coated and pictures were taken. The diameters of more than 50 electrospun fibers were measured from scanning electron microscopy (SEM) images using Image J software. Average diameters and standard deviations were calculated.

Preparation of Bacterial Suspensions

A stock suspension of *S. mutans* ATCC 25175 was prepared in glycerol and kept at −20°C for further use. *S. mutans* was inoculated into brain heart infusion (BHI) broth and incubated for 18–24 h at 37°C in anaerobic condition. In this study, the bacterial suspension was adjusted to 1×10^8 colony forming unit (CFU)/mL by comparison to the turbidity of the standard 0.5 McFarland solution.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Propolis Extract on S. mutans

The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the extract that inhibited the growth of the microorganism. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of the extract that killed the bacteria by 99.9%. In this study, the twofold dilution method was used to determine the MIC and MBC. The microorganisms were treated with serial dilutions of propolis extracts that were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 300 mg/mL and compared to positive control of chlorhexidine mouthwash solution starting at a concentration of 1 mg/mL. Then, the culture was incubated for 24 h in anaerobic condition at 37°C. The maximum concentration of propolis extract that showed the turbidity of broth was determined as MIC. The MBC was determined by streaking plates with treated solutions, which did not show any growth after incubation on BHI agar and incubated for 24 h at the same condition. The MBC was determined as the lowest concentration of propolis extract which inhibited growth of the microorganism by 99.9%.

Antibacterial Activity of Dissolved Electrospun Fibers

The antibacterial activity of dissolved electrospun fibers against *S. mutans* was determined by the agar disk diffusion method. The bacterial culture was swabbed evenly on BHI agar. The electrospun fibers were dissolved in 1 mL of sterile distilled water or DMSO to final concentrations equivalent to 1, 2.5, 5, 7.5, 10, and 15 MIC using vortex mixer. The sterile filter paper disks (6 mm in diameter) were immersed into these solutions and then placed on the BHI agar. The plates were then incubated anaerobically at 37°C for 24 h. The

diameter of the inhibition zone was measured after the incubation. All tests were performed in triplicate.

Inhibition of Adherence of S. mutans to a Glass Surface

Bacterial adherence to a glass surface was based on a method that developed was by Rahim and Khan (15). The bacterial cultures (200 μL) was grown in 3 mL of BHI broth containing 1% sucrose (*w*/*v*) in a test tube which contained sub-MIC concentrations (0.015–1 MIC) of the propolis extract or propolis electrospun fibers. After an incubation period of 18 h at 37°C on a glass surface at an angle of 30°, the adhering bacteria in the test tubes were washed with normal saline solution and resuspended in normal saline solution using an ultrasonic probe. The amount of adherent bacteria was determined by measuring absorbance at OD_{550nm} and calculated as the percentage of adherence. Sterile distilled water used as the control represented 100% adhesion. In addition, samples of mouthwash solutions from the market were tested in the same manner. All tests were performed in triplicate. Results for this test were given as percentage of adherence calculated by the following formula:

$$\text{Percentage of adherence} = (\text{OD assay} / \text{OD control}) \times 100$$

We also determined the number of vital bacterial cells that adhered to the glass surface, suspended the adhered bacteria in sterile normal saline, and calculated the CFUs at the end of the incubation period.

Morphology of Adherent Bacteria and Biofilm Structure

In the test of inhibition of adherence of bacterial cells to a glass surface, a small piece of glass slides (1×1 cm) was placed into two tubes of treated samples. The slides were examined with light microscopy (LM) and SEM to determine the adherence of *S. mutans* and formation of biofilm on the glass surfaces. One of the glass slides was dried at room temperature and fixed with the heat. Crystal violet single staining was performed for 1 min and the glass was washed with water. Photographs were obtained using a light microscope (Olympus, Japan) with a digital camera. Another glass slide was prepared for scanning electron microscopy by immersion in a 0.1% glutaraldehyde solution for 5 min, then washed three times with normal saline solution, and immersed in an ethanol series (50, 60, 70, 90, 95, and 100%) during 20 min at room temperature. The glass slides were dried, and gold was coated with a sputtering coater technique. Micrographs were obtained with a scanning electron microscope (16).

Wetting and Disintegration/Dissolving Time of Electrospun Fibers

Two layers of absorbent paper were placed in the petri dish and wetted with distilled water. The electrospun fiber mats were place on the wet paper and observed during the time that electrospun fibers were completely wetted or dissolved (12,13). The wetting and disintegration/dissolving times were recorded with a digital camera video recorder (Nikon, Japan).

RESULTS

Morphology of Propolis-PVP K90 Electrospun Fibers

The morphology of electrospun fibers is shown in Fig. 1. The average diameter of 8% (*w*/*v*) PVP K90 electrospun nanofibers was 0.43±0.09 μm, and they were smooth and uniform fibers (Fig. 1a). After addition of 5% (*w*/*v*) propolis, the electrospun fiber average diameter increased to 1.20±0.34 μm (Fig. 1b). Some of the additives and wetting agent that were incorporated into propolis-PVP K90 electrospun fibers increased the electrospun fibers average diameter to 1.43±0.22 μm (Fig. 1c). Whereas, incorporated chlorhexidine as the active ingredient in electrospun fibers for antibacterial activity did not affect the morphology and diameter of electrospun fibers; the average diameter was 0.37± 0.09 μm (figure not shown)

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Propolis Extract and Electrospun Fibers with Propolis on *S. mutans*

For the preliminary study of inhibition of bacterial growth, antibacterial activity of the propolis extract against *S. mutans* was investigated by the agar disk diffusion technique. *S. mutans* was exposed to 37.5, 75, 150, 300, and 600 mg/ml of propolis extract that was completely dissolved in DMSO. The results showed that the antibacterial activity against *S. mutans* was dose-dependent and increased with increasing concentrations of propolis; the inhibition zone was 12.25±0.96 mm, 12.50±1.29 mm, 14.25±0.50 mm, 15.50± 1.29 mm, and 15.75±1.26 mm, respectively. The propolis extract showed an MIC of 1.172 mg/mL and MBC of 4.688 mg/mL. Chlorhexidine, which has the most powerful antibacterial activity against *S. mutans* and was used as the positive control in this study, showed MIC and MBC of 0.0039 mg/mL.

Antibacterial Activity of Propolis-PVP K90 Electrospun Fibers

In the present study, the antibacterial activity of propolis-PVP K90 electrospun fibers against *S. mutans* was investigated by the paper disk method. The propolis-PVP K90 electrospun fibers were weighed in amounts equivalent to 1, 2.5, 5, 7.5, 10, and 15 MIC when dissolved in the solvent (1 MIC of propolis extract=1.172 mg/mL).

At low concentrations of propolis extract, electrospun fibers did not show bacterial inhibition zones on BHI nutrient agar. The inhibition zones occurred when the concentration of propolis extract was ≥10 MIC higher. For further study, the antibacterial activity of propolis extract and electrospun fibers that incorporated propolis at 10 and 15 MIC were compared. DMSO and sterile distilled water were used to dissolve propolis extract and electrospun fibers with propolis. Inhibition zones of propolis extract and electrospun fibers with propolis at the amount of 10 and 15 MIC are shown in Table I and Fig. 2. Solvents that were used to dissolve the samples and 0.12% chlorhexidine mouthwash (product A) were used as the negative and standard treatment, respectively. Besides 0.12% chlorhexidine mouthwash (product A), five different mouthwash products from the market (product B, C, D, E, and F) were tested for antibacterial activity against *S. mutans*,

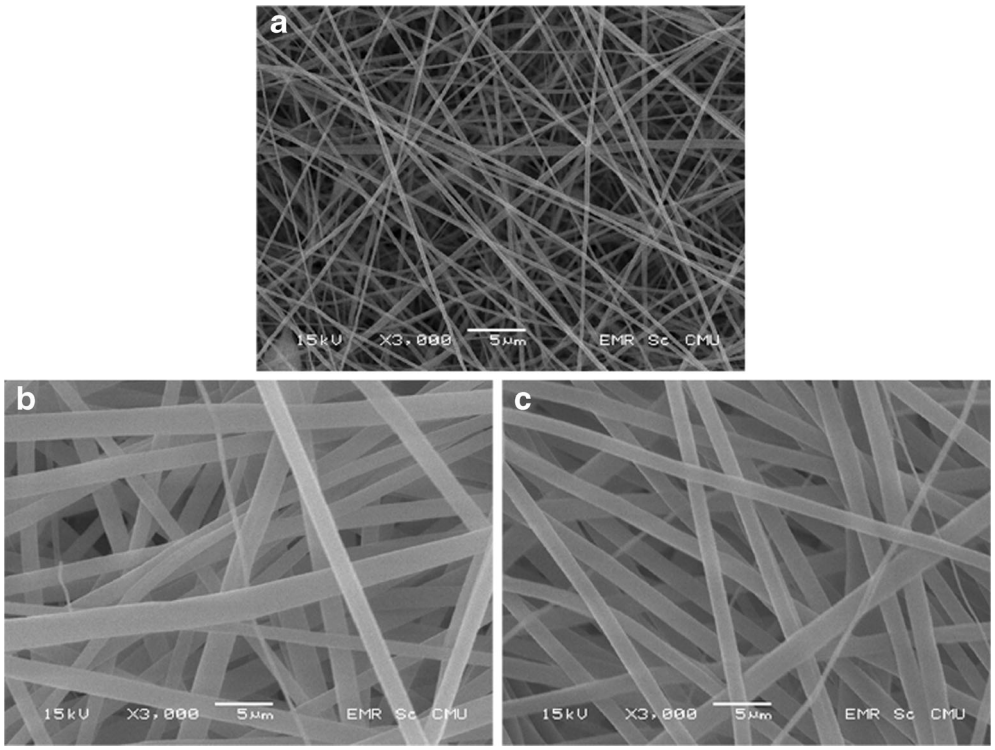

Fig. 1. SEM images of electrospun fibers **a** 8% (*w*/*v*) PVP K90, **b** 8% (*w*/*v*) PVP K90 with 5% (*w*/*v*) propolis, and **c** 8% (*w*/*v*) PVP K90, 5% (*w*/*v*) propolis with 1% (*w*/*v*) Tween 80 and additives

and the results are shown in Table II. It was found that electrospun fibers with propolis were not dissolved completely in water. Therefore, 1% (*w*/*v*) of Tween 80 was used as a wetting agent and added in the polymer solution to improve wettability of the electrospun fibers when the fibers were dissolved in a small amount of water. Electrospun fibers that incorporated propolis at 10 MIC with Tween 80 and additives as flavoring agents were chosen for testing antibacterial activity and compared to other mouthwash products. The results are also shown in Table II.

Inhibition of Adherence of *S. mutans* to a Glass Surface

The effect of propolis extract and propolis electrospun fibers loaded with propolis at sub-MIC (0.015, 0.03, 0.04, 0.06, 0.15, 0.30, 0.60, and 1.0 MIC) on the adhesion of *S. mutans* to the glass surface is shown in Fig. 3. It was found that propolis extract dissolved in DMSO at 0.15 and 0.3 MIC showed a stronger inhibitory effect than propolis electrospun fibers dissolved in DMSO at those concentrations. Propolis electrospun fibers dissolved in DMSO was more effective at 0.3–1.0 MIC than propolis electrospun fibers in water alone at the same concentrations. The inhibitory activity increased when the concentration of propolis extract was increased regardless of the solvent used

Because mouth-dissolved fibers had a limited ability to dissolve in small amounts of liquid in the oral cavity, propolis electrospun fibers dissolved in sterile distilled water were studied. The propolis electrospun fibers were used at concentrations that were equivalent to sub-MIC at 0.6 MIC and 1 MIC from the study of inhibition of adherence of *S. mutans* to a glass surface

Table I. The Antibacterial Activity of Propolis Extract, Propolis Electrospun Fibers, and Chlorhexidine Fibers on *S. mutans*

Samples	Inhibition zone (mm)	
	DMSO (solvent)	Sterile distilled water (solvent)
Control	0	0
Propolis extract at 10 MIC	9.40±0.36	0
Propolis electrospun fibers at 10 MIC	8.53±0.42[a]	7.67±0.29[a]
Propolis extract at 15 MIC	12.17±0.29	0
Propolis electrospun fibers at 15 MIC	9.33±0.29[a]	8.40±0.17[a]
Chlorhexidine mouthwash solution (product A)	N/A	24.67±0.29
Chlorhexidine electrospun fibers	N/A	23.83±0.29[b]

N/A not studied, *MIC* minimum inhibitory concentration, *DMSO* dimethyl sulfoxide

[a] Significant different from each other ($p<0.05$), paired *t* test; compared between propolis extract and propolis electrospun fibers at the same concentration of propolis in each solvent

[b] Significant different from each other ($p<0.05$), paired *t* test; compared between chlorhexidine mouthwash solution and chlorhexidine electrospun fibers at the same concentration

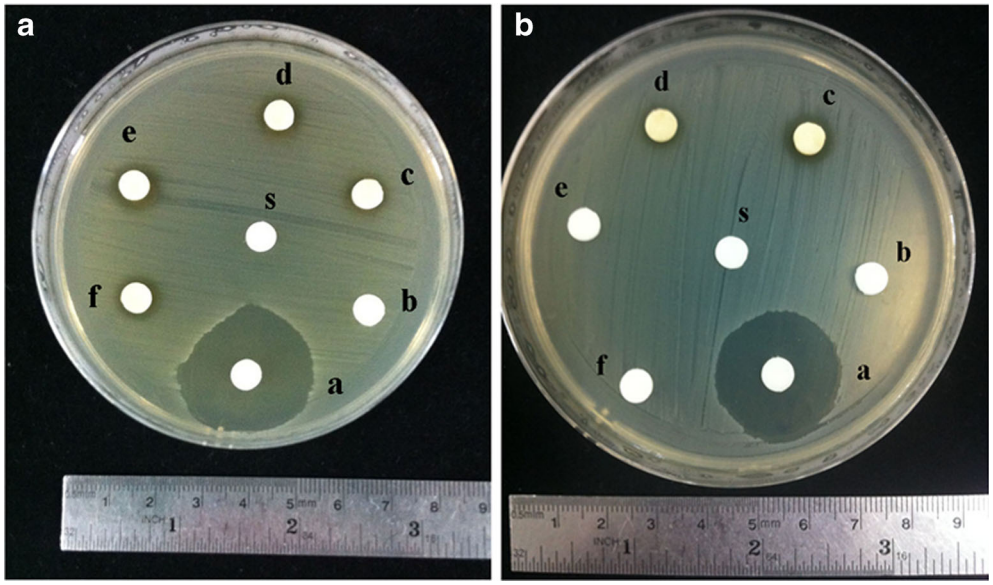

Fig. 2. Antibacterial activity (zone of inhibition) of propolis extract and propolis-PVP K90 electrospun fibers on *S. mutans* when dissolved in **a** DMSO and **b** sterile distilled water (note: *a* chlorhexidine solution, *b* mouthwash solution (product C), *c* 8% (*w*/*v*) PVP K90 with 5% (*w*/*v*) propolis electrospun fibers at 15 MIC, *d* 8% (*w*/*v*) PVP K90 with 5% (*w*/*v*) propolis electrospun fibers at 10 MIC, *e* propolis extract at 10 MIC, *f* propolis extract at 15 MIC, *s* solvent; DMSO or sterile distilled water)

and compared to different mouthwash solutions. The results in Fig. 4 show that both of electrospun fibers with propolis at concentrations 0.6 and 1 MIC were more effective than four kinds of mouthwash solutions (products C–F). The 0.6 MIC fibers with Tween 80 and additives showed not significantly different inhibition of *S. mutans* adherence to a glass surface when compared with 0.6 MIC fibers without Tween 80 and additives. Small amounts of flavoring agents also gave a good odor to the fibers. However, propolis electrospun fibers at these concentrations were less effective than chlorhexidine mouthwash solutions (product A) and a mouthwash solution that contained chemical substances (product B).

The examination of bacterial adherence to the glass surface showed that the amount of *S. mutans* from 0.6 MIC fibers of propolis-PVP electrospun fibers with and without Tween 80 and additives was around 4.5–7.1×10^5 CFU/mL. Various formulations of mouthwash solutions from the market had different abilities to inhibit the growth of *S. mutans*. A chlorhexidine mouthwash solution (product A) that was used as a standard treatment in this study and a mouthwash solution that incorporated the chemical substances as o-Cymen-5-ol and dipotassium glycerrhizate (product B) reduced the *S. mutans* by nearly 100%. It was found that the amount of *S. mutans* from product C, D, E, and F were around 1.0–2.0×10^7 CFU/mL, whereas the amount of *S. mutans* which were treated with sterile water as control was around 2.0×10^{12} CFU/mL after 18 h of incubation time.

Table II. The Antibacterial Activity of Various Mouthwash Products and Propolis Electrospun Fibers on *S. mutans*

Mouthwash products	Inhibition zone (mm)
Control (sterile distilled water)	0
Product A (chlorhexidine mouthwash)	24.67±0.29
Product B	14.16±0.29
Product C	0
Product D	0
Product E	0
Product F	0
10 MIC of propolis electrospun fibers with Tween 80 and additives	7.00±0.00

MIC minimum inhibitory concentration

Morphology of Adherent Cells and Biofilm Structure

Photographs of *S. mutans* attached on the glass surface are shown in Figs. 5 and 6. From optical micrographs, the morphology and the arrangement of *S. mutans* on a glass surface without treatment with an antibacterial substance

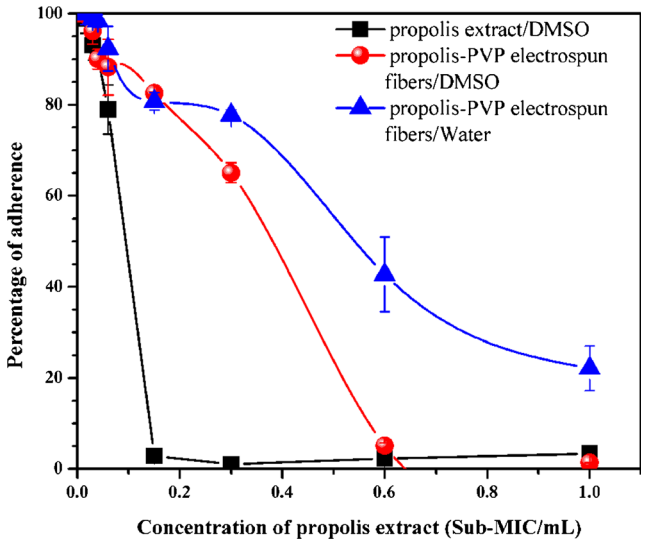

Fig. 3. Effect of propolis from propolis extract and propolis electrospun fibers at sub-MIC on the adhesion of *S. mutans* to the glass surface

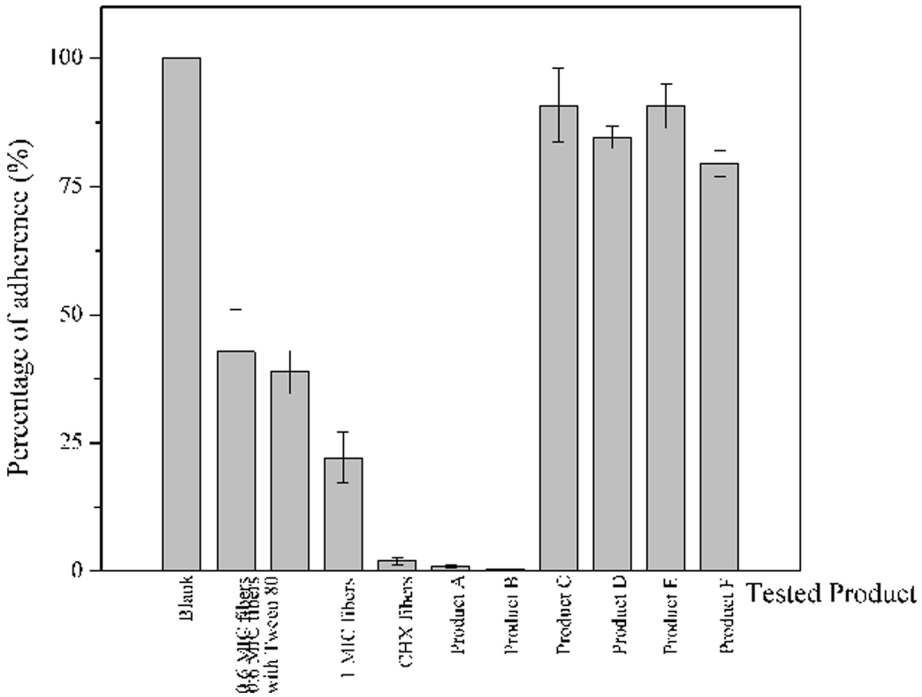

Fig. 4. Effect of propolis electrospun fibers compared to marketed mouthwash products on cell adhesion of *S. mutans* to the glass surface

(control) is shown in Fig. 5a. *S. mutans* which was treated with propolis from electrospun fibers at concentration of propolis of 0.6 MIC showed decreasing adherence of *S. mutans* to the glass surface as well as dead bacterial cells (Fig. 5b). SEM photographs comparing the morphology of adhering control bacteria with those treated with propolis electrospun fibers at 0.6 MIC are shown in Fig. 6. Bacteria in the control (Fig. 6a) are more numerous and conglomerate to form biofilms.

Wetting and Disintegration/Dissolving Time of Electrospun Fibers

The PVP K90 electrospun fibers mats and PVP K90 with chlorhexidine electrospun fiber mats were wetted, dissolved rapidly, and disappeared on wet paper within 1 s as showed in Fig. 7a, b. Propolis in PVP K90 electrospun fibers retarded the wetting time; the propolis-PVP electrospun fiber mats were completely wetted in 45.40±1.51 s (Fig. 7c). Adding 1% (*w*/*v*) Tween 80 in the electrospun fiber mat as wetting agent with small amount of flavoring agents in the formulation reduced the wetting time to 1.67±0.33 s (Fig. 7d). Figure 8a shows the release of propolis from electrospun fibers without Tween 80 after testing their wetting and dissolution time. It was found that a yellow gel-like structure penetrated the wetted paper, showing the release of propolis from the electrospun fibers. Greater lateral movement of the yellow gel-like structure was observed from the propolis-PVP K90 electrospun fiber mat when 1% (*w*/*v*) Tween 80 was added as a wetting agent (Fig. 8b).

DISCUSSION

PVP K90 was suitable for use as the polymer base for mouth-dissolved fibers produced via an electrospinning process because of its solubility in water and its spinnability (13). An 8%

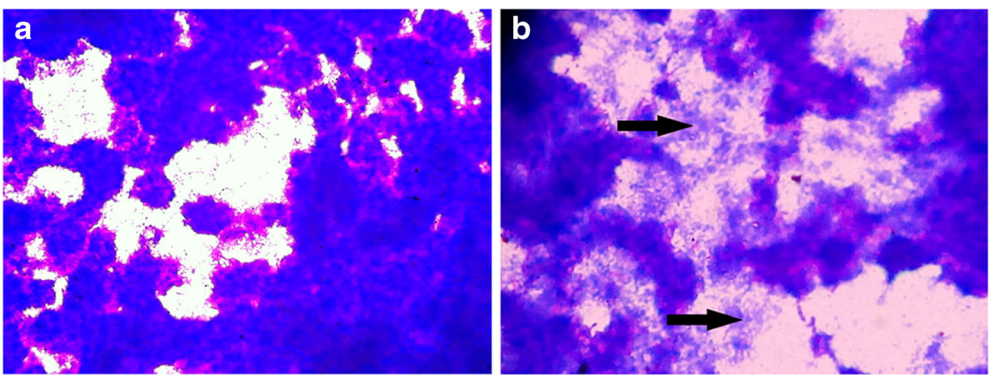

Fig. 5. Photographs from optical microscopy of the adhesion of *S. mutans* cells on the smooth glass surface compared **a** control, sterile distilled water, and **b** 0.6 MIC of propolis from propolis-PVP electrospun fibers (*arrow* shows dead cells of *S. mutans*)

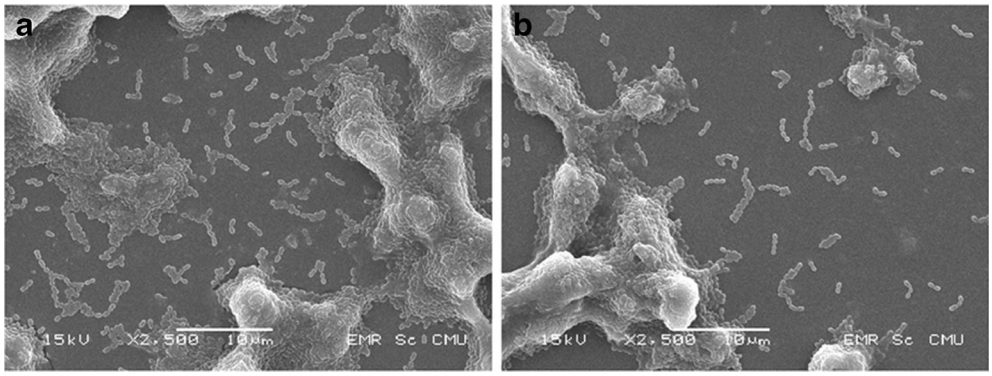

Fig. 6. SEM photographs of the adhesion of *S. mutans* cells on the smooth glass surface compared **a** control, sterile distilled water, and **b** 0.6 MIC of propolis from propolis-PVP electrospun fibers

(*w*/*v*) PVP K90 can be used to produce fibers with a suitable morphology. Propolis is resinous and has poor solubility in water. Therefore, using PVP K90 as the polymer base and the structure of electrospun fibers that have high surface area per unit mass including the formation of a high porosity nonwoven fiber mat enhances the solubility and provides a mouth-

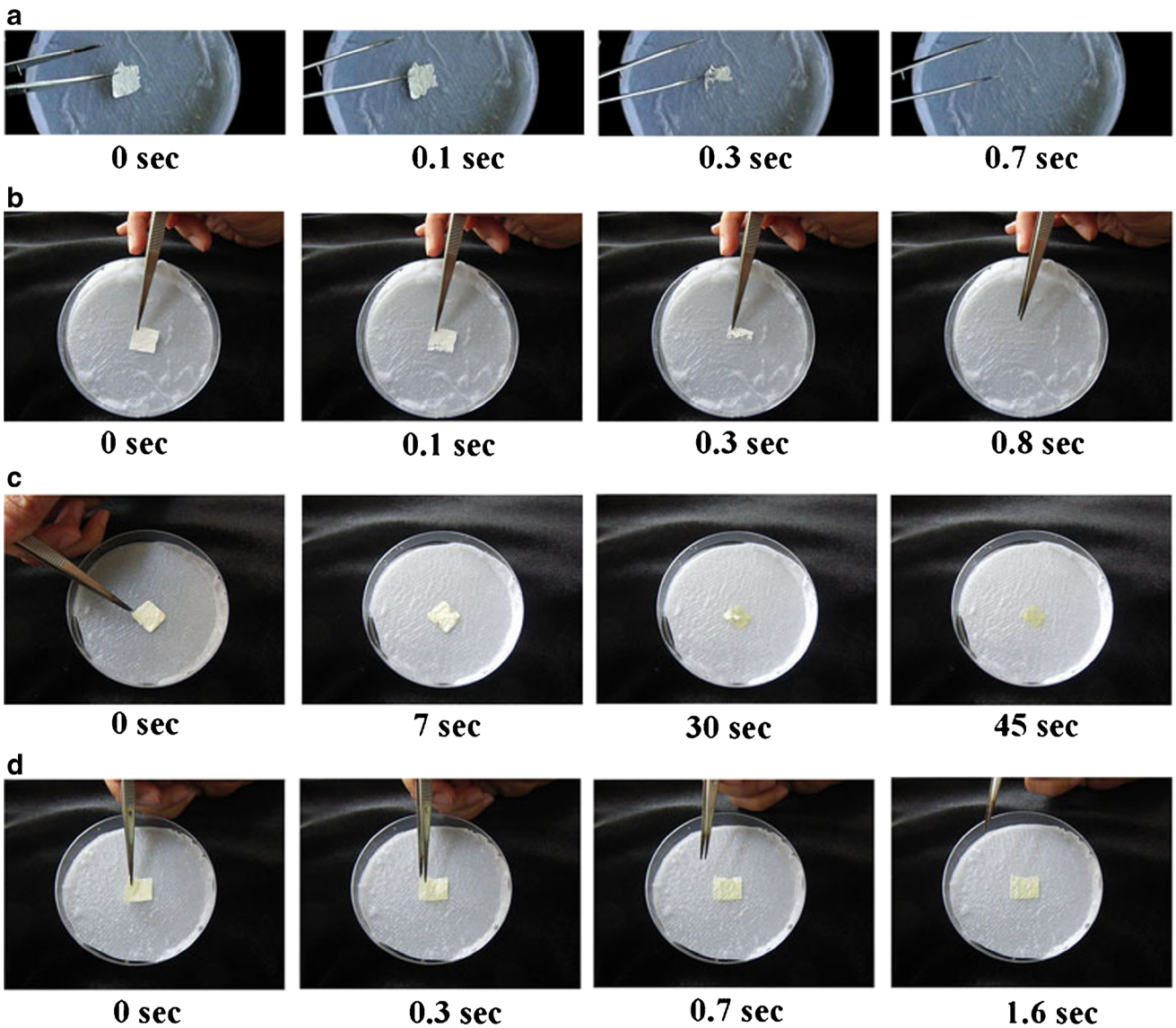

Fig. 7. The dissolution time of **a** PVP K90 electrospun fibers, **b** chlorhexidine-PVP electrospun fibers, and the wetting time of **c** propolis-PVP electrospun fibers and **d** propolis-PVP electrospun fibers with 1% (*w*/*v*) Tween 80 and additives

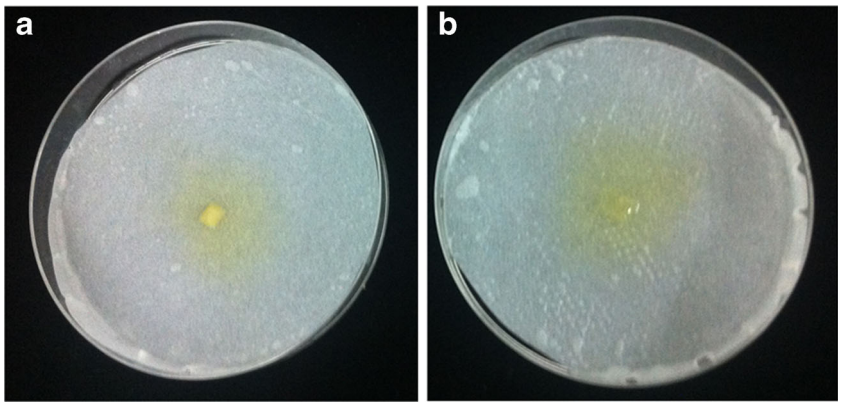

Fig. 8. The release of propolis from electrospun fiber mats of 8% (*w*/*v*) PVP K90 with 5% (*w*/*v*) propolis **a** without Tween 80 and **b** with 1% (*w*/*v*) Tween 80

dissolving dosage form. Incorporation of 5% (*w*/*v*) propolis into PVP K90 8% (*w*/*v*) ethanolic solution enabled fiber fabrication through the electrospinning technique and produced fibers with a suitable morphology, although the diameter of nanospun fibers with propolis increased when compared to the pure PVP K90 nanospun fibers. This might be due to the influence of both higher viscosity and repulsive force of the components in the propolis extract. On the other hand, the addition of chlorhexidine into the PVP K90 8% (*w*/*v*) solution did not affect the size of electrospun fibers.

The MIC and MBC values of propolis extract were found to be 1.172 and 4.688 mg/mL, respectively. Chemical components in propolis extract differ significantly according to its geographical and botanical origins, and this variability may affect the antibacterial efficiencies of propolis extracts from various sources (17,18). Propolis antibacterial property has been attributed to phenolic compounds, especially flavonoids, phenolic acids, and their esters (19). Much research has indicated the presence of different flavonoids and the inhibitory effects of propolis on cariogenic bacteria (20,21).

DMSO enabled the propolis extract and propolis electrospun fibers to dissolve completely as indicated by inhibition of *S. mutans*. Our research indicates that electrospun fibers enable the dissolution of the water-insoluble propolis extract. The electrospinning process produced fibers with small diameter, very high porosity and also promoted high solubility. PVP is a polymer that has hygroscopic and hydrophilic properties so that the polymer chain of electrospun fibers can absorb water and dissolve rapidly (13,22). Propolis electrospun fibers which were loaded with propolis at 10 and 15 MIC could not dissolve completely in water. This might be due to the components of propolis that usually include resins (50–55%) and waxes (30%) that are hydrophobic (2). The microstructure of propolis-loaded electrospun fibers, using PVP K90 as the polymer base, enabled the dissolution and release of propolis from the electrospun fibers in water as indicated by inhibition zones of 7.67±0.29 and 8.40±0.17 mm at concentrations of 10 and 15 MIC, respectively. Propolis electrospun fibers had smaller inhibition zones when compared to the inhibition zones of propolis extract dissolved with DMSO at the same concentration of propolis. The high viscosity of the polymer solution retarded its diffusion and prevented contact with the bacteria. The same result was revealed when inhibition zone of 0.12% chlorhexidine solution was compared with chlorhexidine electrospun fibers at the same concentration. The Noyes-Whitney equation (23,24) defines dissolution rate (dX/dt) as follows:

$$dX/dt = A \times D/\delta \times (C_0 - X/V)$$

where X is the amount of drug in solution, t is time, A is surface area, D is the diffusion coefficient of the drug, δ is the effective diffusion boundary layer, C_0 is the saturation solubility of the drug, and V is the volume of dissolution medium. Even though the structure of fiber mats had a high surface area and porosity that increased the dissolution rate, and PVP K90 adsorbed water and dissolved in a small amount of water, a thick viscous layer formed which retarded diffusion. When this layer became more viscous, it interrupted the dissolution and diffusion of propolis molecules to medium. The propolis electrospun fibers were wetted and disintegrated rapidly in small amounts of water as mouth-dissolved fibers mats. Due to the hydrophobicity of propolis and the viscous layer of soluble polymer, the dissolution of the fibers was retarded as indicated by the yellow gel-like structure that formed on the wetted paper in the test for wetting time of electrospun fiber mats.

Product A, which contains a 0.12% chlorhexidine mouthwash solution, and Product B, containing o-Cymen-5-ol and dipotassium glycerrhizate as active ingredients, showed the highest antibacterial activity against *S. mutans*. Product B showed a smaller inhibition zone when compared with product A. Other products of mouthwash solutions (products C–E) which contained volatile oils with or without sodium fluoride or natural extracts did not show the inhibition zone. Many reports have indicated that chlorhexidine was the most effective antimicrobial agent used in the oral cavity (25,26). Volatile antiseptic oils such as eucalyptol, methyl salicylate, thymol, and menthol had low antibacterial activity when compared with the conventional chemical substances; these mouthwash products did not show inhibitory effect because they were formulated at lower than the MIC against *S. mutans* (27). Propolis electrospun fibers at 10 MIC with Tween 80 as the wetting agent and small amount of volatile oils as flavoring agent showed better solubility in water that improved the potential acceptance of the consumer but did not affect the antibacterial activity. No statistically significant differences were observed when the inhibition zones between propolis-PVP electrospun fibers with Tween 80 and volatile oils and propolis-PVP electrospun fibers without Tween 80 and volatile oils as additives were compared.

It was observed that the adherence of *S. mutans* to the glass surface was affected by the amount of propolis. It has been reported that propolis extract inhibited both the growth of *S. mutans* and glucosyltransferase activity (8,21). Therefore, even though the propolis extract was used at sub-MIC concentrations, it still reduced the adherence of *S. mutans* cells to a glass surface, possibly through metabolic interference with glucosyltransferase activity. Both electrospun fibers with propolis dissolved in DMSO or in sterile distilled water showed the ability to inhibit *S. mutans* adherence to the glass surface but were less effective than propolis extract dissolved in DMSO. Propolis electrospun fibers with sub-MIC of propolis (0.6 MIC and 1 MIC), dissolved in sterile water to simulate the condition in the mouth, reduced the adherence of *S. mutans* by more than 50%. There were no statistically significant differences in the percentage of adherence of *S. mutans* on the glass surface between the formulation of propolis-PVP electrospun fibers at 0.6 MIC with Tween 80 as wetting agents and flavoring agents compared with the formulation without Tween 80 and flavoring agents. Other mouthwash solutions from the market that incorporated some conventional chemical substances as antiseptic agents showed significant reductions of the adherence of *S. mutans* to a glass surface; the percentage of adherence was less than 1% due to their antibacterial activity. Even though other mouthwash solutions in this study did not cause an inhibition zone, they still reduced the adherence of *S. mutans* growing on a glass surface by about 10–20%. Therefore, the current research showed that a propolis extract in electrospun fibers at 0.6 and 1 MIC was more effective than mouthwash solutions containing natural essential oils or natural extract as active ingredients. Propolis electrospun fibers at 0.6 MIC with Tween 80 as the wetting agent and small amounts of volatile oils as flavoring agents also showed the same result when compared with propolis electrospun fibers at 0.6 MIC. The ability of propolis to inhibit the growth of *S. mutans* and reduce biofilm formation was also investigated by light microscopy and SEM. Propolis caused morphological changes in bacterial cells and smaller biofilms, indicating that bacteria cells were destroyed or changed. Many dead bacteria were observed following treatment of *S. mutans* with propolis. The reduction in the number of surviving *S. mutans* cells in plaque that adhered to the smooth glass surface from propolis-PVP electrospun fibers was confirmed. These results indicated that propolis electrospun fibers demonstrated antibacterial activity against *S. mutans* and an inhibitory effect on the formation of biofilm by the bacterium.

PVP K90 electrospun fibers showed a very fast wetting and disintegration/dissolving time. Incorporating chlorhexidine as an active ingredient in electrospun fibers did not affect the dissolution property of the electrospun fibers. However, incorporation of the propolis extract into electrospun fibers retarded the wetting and dissolution time of the fibers. Due to the components within propolis, resins or waxes, the electrospun fibers absorbed water and formed a gel-like structure before the dissolution and release of the propolis. Adding 1% (*w*/*v*) Tween 80 in the formulation improved wettability of the electrospun fibers. The electrospun fibers absorbed water faster to form a gel-like structure. PVP K90 dissolved rapidly and increased the dissolution and release of propolis from electrospun fibers.

CONCLUSION

Mouth-dissolving fibers loaded with propolis could be formulated with PVP K90 as the polymer base by the electrospinning technique. The propolis-PVP K90 electrospun fibers had a good morphology with a narrow size distribution. Propolis that was incorporated in the electrospun fibers released when the electrospun fibers were dissolved in water and showed antibacterial activity against *S. mutans* including greater inhibition of *S. mutans* adherence to a smooth glass surface than the commercial mouthwash solutions that were formulated with natural essential oils as active ingredients. These results confirmed the potential use of propolis electrospun fibers as a mouth-dissolving dosage form and as an anticariogenic agent in the oral cavity.

ACKNOWLEDGMENTS

This work was supported by the National Research University Project under Thailand's Office of the Higher Education Commission, Thailand. The authors would also like to thank the Department of Physics and Materials Science, Department of Biology, Faculty of Science, and Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University for their support.

REFERENCES

1. Sforcin JM, Bankowa V. Propolis: is there a potential for the development of new drug? J Ethnopharmacol. 2011;133:253–60.
2. Kumazawa S, Hayashi K, Kajiya K, Ishii T, Hamasaka T, Nakayama T. Studiesof the constituents of Uruguayan propolis. J Agric Food Chem. 2002;50(17):4777–80.
3. Mirzoeva OK, Grishanin RN, Calder PC. Antimicrobial action of propolis and some of its components: the effects on growth, membrane potential and motility of bacteria. Microbiol Res. 1997;152:239–46.
4. Grange JM, Davey RW. Antibacterial properties of propolis (bee glue). J R Soc Med. 1990;83:159–60.
5. Popova MP, Bankova VS, Bogdana S, Tsvetkova I, Naydenski C, Marcazzan GL, *et al*. Chemical characteristics of poplar type propolis of different geographic origins. Apidologie. 2007;38:306–11.
6. dos Santos Nobre M, dos Santos Melo L, Francisco SB, Cury JA. Relationship among dental plaque composition, daily sugar exposure and caries in the primary dentition. Caries Res. 2002;36:347–52.
7. Wiater A, Choma A, Szczodrak J. Insoluble glucans synthesized by cariogenic streptococci: a structural study. J Basic Microbiol. 1999;39:265–73.
8. Hayacibara MF, Koo H, Rosalen PL, Duarte S, Franco EM, Bowen WH, *et al*. In vitro and in vivo effects of isolated fractions of Brazilian propolis on caries development. J Ethnopharmacol. 2005;101:110–5.
9. Bhardwaj N, Kundu SC. Electrospinning: a fascinating fiber fabrication technique. Biotechnol Adv. 2010;28:325–47.
10. Sill SC, von Recum HA. Electrospinning: application in drug delivery and tissue engineering. Biomaterials. 2008;29:1089–2006.
11. Chakraborty S, Liao IC, Adler A, Leong KW. Electrohydrodynamics: a facile technique to fabricate drug delivery system. Adv Drug Deliv Rev. 2009;61:1043–54.
12. Li X, Kanjwal MA, Lin L, Chronakis IS. Electrospun polyvinyl-alcohol nanofibers as oral fast dissolving delivery system of caffeine and riboflavin. Colloids Surf B: Biointerfaces. 2013;103:182–8.
13. Quan J, Yang Y, Brandford-White C, Williams GR, Yu DG, Nie W, *et al*. Preparation of ultrafine fast-dissolving feruloyl-oleyl-

glycerol-loaded polyvinylpyrrolidone fiber mats via electrospinning. Colloids Surf B: Biointerfaces. 2011;88:304–9.
14. Sanpa S, Sutjarittangtham K, Tunkasiri T, Eitssayeam S, Chantawannakul P. Antimicrobial effect of brazillian propolis/polycaprolactone polymer on some human pathogenic bacteria. Adv Mater Res. 2012;506:537–40.
15. Rahim ZHA, Khan HBSG. Comparative studies on the effect of crude aqueous (CA) and solvent (CM) extracts of clove on the cariogenic properties of *Streptococcus mutans*. J Oral Sci. 2006;48(3):117–23.
16. Espinosa-Cristobal LF, Martinez-Castanon GA, Tellez-Dector EJ, Nino-Martinez N, Zavala-Alonso NV, Loyola-Rodriguez JP. Adherence inhibition of *Streptococcus mutans* on dental enamel surface using silver nanoparticles. Mater Sci Eng C. 2013;33:2197–202.
17. Seidal V, Peyfoon E, Watson DG, Fearnley J. Comparative study of the antibacterial activity of propolis from different geographical and climatic zones. Phytother Res. 2008;22(9):1256–63.
18. Ahn MR, Kumazawa S, Hamasaka T, Bang KS, Nagayama T. Antioxidant activity and constituents of propolis collected in various areas of Korea. J Agric Food Chem. 2004;52:7286–92.
19. Bankova V, Christov R, Kujumgiev A, Marcucci MC, Popov S. Chemical composition and antibacterial activity of Brazilian propolis. J Biosci. 1995;50(3–4):167–72.
20. Park YK, Koo MH, Abreu JAS, Ikegaki M, Cury JA, Rosalen PL. Antimicrobial activity of propolis on oral microorganisms. Curr Microbiol. 1998;36:24–8.
21. Duarte S, Rosalen PL, Hayachibara MF, Cury JA, Bowen WH, Marquis RE, *et al*. The influence of a novel propolis on mutans *Streptococci* biofilms and caries development in rats. Arch Oral Biol. 2006;51:15–22.
22. Yu DG, Shen XX, Branford-White C, White K, Zhu LM, Bligh SWA. Oral fast-dissolving drug delivery membranes prepared from electrospun PVP ultrafine fibers. Nanotechnology. 2009;20:055104.
23. Noyes AA, Whitney WR. The rate of solution of solid substances in their own solutions. J Am Chem Soc. 1897;19:930–4.
24. Wurster DE, Taylor PW. Dissolution rates. J Pharm Sci. 1965;54:169–75.
25. Mandel ID. Antimicrobial mouthrinses: overview and update. J Am Dent Assoc. 1994;125 suppl 2:2S–10.
26. Ribeiro LGM, Hashizume LN, Maltz M. The effect of different formulations of chlorhexidine in reducing levels of mutans streptococci in the oral cavity: a systematic review of the literature. J Dent. 2007;35:359–70.
27. Chung JY, Choo JH, Lee MH, Hwang JK. Anticariogenic activity of macelignan isolated from *Myristica fragrans* (nutmeg) against *Streptococcus mutans*. Phytomedicine. 2006;13:261–6.