Asia Journal of Public Health
Journal homepage:http://www.ASIAPH.org

Original Articles

Effect of Oil-Pulling on Oral Microorganisms in Biofilm Models

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ARTICLE INFO

Article history :
Received December 2010
Received in revised form June 2010
Accepted July 2011
Available online August 2011

Keywords:
Oil-pulling
Oral microorganisms
Biofilm

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Asia J Public Health 2011; 2(2): 62-66

ABSTRACT

Objective: To investigate the effect of oil-pulling against oral microorganisms in biofilm models. **Materials and Methods:** The effect of oil-pulling using coconut oil, corn oil, rice bran oil, palm oil, sesame oil, sunflower oil and soy bean oil was evaluated on biofilm models formed by *Streptococcus mutans* KPSK2, *Lactobacillus casei* ATCC 6363 and *Candida albicans* ATCC 13803 on salivary-coated microtiter plates. Saline solution and 0.2% chlorhexidine gluconate solution were used as negative and positive controls, respectively. **Results:** It was found that coconut oil exhibited antimicrobial activity against *S. mutans* and *C. albicans*. Sesame oil had antibacterial activity against *S. mutans* whereas sunflower oil had antifungal activity against *C. albicans*. However, *L. casei* was found to be resistant to all tested oils. **Conclusion:** Results from the present study could be scientific evidence to demonstrate that oil-pulling therapy with some edible oils could probably be used as a preventive home therapy to maintain oral hygiene against dental caries especially in developing countries.

INTRODUCTION

Dental caries are an infection associated with microorganisms embedded in a matrix of polymers of bacterial and salivary origin. The occurrence of dental caries is directly linked to the ability of microorganisms to colonize the tooth surface and form biofilm or dental plaque. Formation of biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists facilitate the arrival of others by providing adhesion sites and the matrix that holds the biofilm together. *Streptococcus mutans*, *Lactobacilli* spp. and *Candida albicans* are the predominant microorganisms found in dental plaque associated with a caries lesion[1]. *Streptococcus mutans*, *Lactobacilli* spp. are considered crucial for the initiation and progression of dental caries as they have shown by their more acidogenic and acidophilic properties than those of other oral bacteria [2]. They can convert dietary carbohydrates into acid, which lowers the pH of the environment, and solubilizes the calcium phosphate of the enamel to produce a caries lesion. Conditions of low pH created by these bacteria shift the balance of plaque

microflora and favor the proliferation of *Candida* spp.

Oil-pulling or oil-swishing is a procedure in which the practitioners swish or rinse oil in their mouth for oral health benefit to prevent dental caries, oral malodor, bleeding gums, dryness of the throat and cracked lips [3]. In addition, this simple method is claimed to cure many diseases ranging from thrombosis, eczema, intestinal infection, and diabetes, to bronchitis and asthma [3]. This procedure is typically performed daily by sipping or sucking oil and pulling it between the teeth for 1-10 min before brushing. The oil will turn thin and milky white. This should be followed by brushing the teeth and is preferably done on an empty stomach in the morning. Scientific evidence shows that oil-pulling therapy could reduce the plaque index, modify gingival scores and the total oral bacteria count in gingivitis patients [4-5]. Moreover, it can convert caries susceptibility from marked susceptibility to slight or moderate susceptibility [6].

Therefore, the aim of the present study was to investigate the effect of oil-pulling against oral microorganisms in biofilm models.

MATERIALS AND METHODS

Oil

All of the oils used in the present study were Thai products. They included: coconut oil (Grandmom Herbal House), corn oil (Thai Vegetable Oil Public Company), rice bran oil (Thai Edible Oil Company), palm oil (Thai Vegetable Oil Public Company), sesame oil (Grandmom Herbal House), sunflower oil (Thanakorn Vegetable Oil), and soy bean oil (Thai Vegetable Oil Public Company).

Microorganisms

The tested microorganisms were obtained from the culture collections of the Department of Microbiology, Faculty of Dentistry, Mahidol University, Thailand. Organisms were as follows: *Streptococcus mutan*s KPSK2, *Lactobacillus casei* ATCC 6363 and *Candida albicans* ATCC 13803. They were maintained on Brain Heart Infusion (BHI) agar (BBL, USA). Overnight cultures were prepared by inoculating approximately 2 ml of Mueller Hinton broth (Difco, USA) with 2-3 colonies of each organism taken from BHI agar. The broth was incubated overnight at 37 °C. Inocula were prepared by diluting overnight cultures with saline to approximately 10^8 CFU/ml for bacteria and 10^7 CFU/ml for yeast. These suspensions were further used for antimicrobial determination.

Effect on biofilm models

The effect of the oils was evaluated on biofilm models formed by *S. mutans* KPSK2, *L. casei* ATCC 6363 and *C. albicans* ATCC 13803 on the bottom of microtiter plates. Biofilm assays were done using the protocol modified from Loo *et al* [7] and Guggenheim *et al*[8]. Briefly, all microorganisms were cultivated in a fluid thioglycolate medium (Difco, USA) supplemented with 0.3% glucose for seven hours. Each microbial suspension (10^6 CFU/ml) was combined and placed at cold temperature for six hours. Approximately 60 ml of unstimulated saliva were collected from three volunteers two hours after eating, drinking or having a hygiene procedure. The pooled saliva was centrifuged at 20,000 g for thirty minutes at 4°C. The supernatant was filtered through a 0.45 µm-pore membrane (Sartorius, Germany). Absence of any viable microorganisms in the saliva was checked by culturing on blood agar. Salivary components were absorbed onto ninety-six polystyrene, flat-bottomed well microtiter plates for four hours at room temperature to form an acquired pellicle. After removal of excess saliva, the coated microtiter plates were inoculated with a mixture of saliva, thioglycolate medium supplemented with 3% glucose, and a pool of microorganisms. The final density of inoculum in each well of the microtiter plate was 10^5 CFU/ml. The microtiter plates were then incubated at 37°C for twenty-four hours under anaerobic conditions.

Saline solution and a 0.2% chlorhexidine gluconate solution were used as negative and positive controls, respectively. The biofilms were exposed to controls or the tested oils for 1 min. During this time the fluid was pulled up and down 20 times. Then the oils and unattached cells were removed and the biofilms were washed three times in PBS, and then scrapped and sonicated. Serial dilutions of sonicated cells were cultivated in Mitis-Salivarius Bacitracin agar (Difco, USA), Rogosa SL agar (Difco, USA) and Sabouraud dextrose agar (Difco, USA) to determine the number of *S. mutans*, *L. casei* and *C. albicans*, respectively.

Statistical analysis

All experiments were performed in triplicate and results were expressed as mean ± standard deviation. Statistical analysis of the differences between mean values obtained for the experimental groups were analyzed by one-way analysis of variance, followed by Tukey's test using SPSS 11.5 software. A p-value less than 0.05 was taken as statistically significant.

RESULTS

Multi-species biofilm of *S. mutans, L. casei* and *C. albicans* were prepared and then exposed to the tested oils for 1 min. Responses of the microbial populations in biofilm models to different oils are summarized in Table 1. Coconut oil exhibited antimicrobial activity against *S. mutans* (2.5 log reductions) and *C. albicans* (1 log reduction). Sesame oil had antibacterial activity against *S. mutans* (1 log reduction) and sunflower oil had antifungal activity against *C. albicans* (1 log reduction). For chlorhexidine, which was used as a positive control, *S. mutans* was completely removed from the biofilm and 1.5 log reductions of *C. albicans* were observed. *L. casei* was found to be resistant to all tested oils, whereas three log reductions were shown after chlorhexidine exposure

DISCUSSION

Oil-pulling therapy with sesame oil or sunflower oil has been extensively used as a traditional Indian folk remedy for many years. It is claimed to have advantages over commercial mouthwashes since it causes no staining, has no lingering after taste, causes no allergic reactions and is readily available in the household [5]. The mechanisms of oil-pulling action are not known. It has been proposed, however, that the viscosity of the oil can inhibit bacterial adhesion and plaque coaggregation[6]. The other possible mechanism might be the saponification process that occurs as a result of alkali hydrolysis of oil by bicarbonates in saliva [9]. These soaps are good cleansing agents and might be effective in removing microorganisms or plaque materials. Sufficient scientific research has not been carried out to evaluate the beneficial effect of oil pulling therapy on oral health and this needs to be addressed. This study was therefore planned to investigate the effect of oil-pulling with several edible oils on oral microorganisms related to dental caries using biofilm models.

Table1 Effect of oil-pulling on oral microorganisms in biofilm models

	S. mutans		***L. casei***		***C. albicans***	
		p-value		p-value		p-value
coconut oil	2.59±0.13	0.008*	5.44±0.16	0.950	2.03±0.12*	0.022*
corn oil	5.05±0.17	0.596	5.61±0.14	0.734	3.27±0.10	0.772
palm oil	4.91±0.12	0.631	5.52±0.06	0.702	3.34±0.14	0.853
rice bran oil	5.15±0.06	0.940	5.67±0.21	0.598	3.11±0.08	0.750
sesame oil	4.01±0.14	0.039*	5.58±0.13	0.821	3.50±0.13	0.387
sunflower oil	5.13±0.13	0.805	5.06±0.12	0.405	1.95±0.19	0.014*
soy bean oil	4.79±0.24	0.568	5.56±0.05	0.840	3.31±0.20	0.826
saline solution	5.11±0.13		5.42±0.21		3.30±0.14	
chlorhexidine§	0		2.27±0.10		1.84±0.08	

Data presented as mean log10 CFU/ml of microorganisms
* significantly different from saline solution, negative control
§ chlorhexidine was used as positive control

In the present study it was found that coconut oil exhibited antimicrobial activity against *S. mutans* and *C. albicans* whereas sesame oil had activity against *S. mutans* and sunflower oil showed only antifungal activity. Other oils such as corn oil, palm oil, rice bran oil and soy bean oil showed no antimicrobial activity against tested microorganisms.

Coconut oil has a unique role in the diet as an important physically functional food. Besides the health and nutritional benefits, coconut oil has been shown to have anti-carcinogenic effects against colon tumors [10]. What makes coconut oil different from most other dietary oils are the basic building blocks, or fatty acids, making up the oil. The predominant composition of coconut oil is a

medium chain fatty acid, whereas the majority of common edible fats in our diet are composed almost entirely of long chain fatty acids. This influences the physical and chemical properties of the oil. Coconut oil contains 92% saturated acids, approximately 50% of which is lauric acid [11].

Recognition of the antimicrobial activity of coconut oil has been reported since 1982 by Hierholzer and Kabara [12]. This early research was directed at the virucidal effects because of possible problems related to food preservation. Recently, results from many studies revealed that monolaurin, the monoglycerides of lauric acid from coconut oil had antimicrobial activity against various gram positive and gram negative organisms, including *Escherichia vulneris, Enterobcater* spp.[13], *Helicobacter pylori* [14], *Staphylococcus aureus* [15], *Candida* spp., including *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. stellatoidea* and *C. krusei* [16] , as well as enveloped viruses [17-18]. Electron microscopic images showed that 15 minutes exposure to monoglycerides caused gram positive cocci cell shrinkage and cell membrane disintegration. Data from clinical studies confirm the efficacy of coconut oil in the treatment of atopic dermatitis and removing *S. aureus* from colonized skin lesions [15]. In addition, soap made from coconut oil had antimicrobial properties against *Escherichia coli* [19]. Though the exact antibacterial mechanism of the action of coconut oil is still unclear, it was hypothesized that monolaurin and other medium chain monoglycerides had the capacity to alter bacterial cell walls, penetrate and disrupt cell membranes, inhibit enzymes involved in energy production and nutrient transfer, leading to the death of the bacteria [15]. In the case of viruses, the virucidal effects of monolaurin appeared to be that of solubilizing the lipids and phospholipids in the envelope leading to disintegration of the virus particles [20].

Sesame oil was demonstrated to have antibacterial activity against *S. mutans*. It contains high amounts of unsaturated fatty acids. Linoleic acid and oleic acid are the predominant compositions. Oil-pulling therapy with sesame oil significantly reduced *S. mutans* counts in plaque and saliva of adolescents within one week [6]. In accordance with these previous investigations, our result showed that sesame oil reduced *S. mutans* to approximately 1 log compared with saline solution.

Oil-pulling therapy with sunflower oil was shown to reduce plaque scores after 45 days [4]. However, the pre- and post- values of bacterial count reduction were not statistically different. To our knowledge, little information regarding antimicrobial properties of sunflower oil exists. Infants treated with sunflower oil are less likely to experience bacterial skin infections than are control infants [21-22]. This may be the result of skin barrier enhancing effects, or some antimicrobial actions of the oil. Ozonized sunflower oil (oleozon) showed antimicrobial acitivity against *S. aureus, E. coli, Pseudomonas aeruginosa, Enterococcus faecalis, Mycobacterium* spp., *Streptococcus pyogenes* and *C. albicans* [23-24]. However, the activity might be due to the powerful oxidant properties of the ozone itself.

Considering the antimicrobial effect of other oils, including corn oil, palm oil, rice bran oil and soy bean oil, it was proposed that small amounts of saturated fatty acid, i.e. lauric acid, in these oils may play a role in their antimicrobial properties.

In conclusion, results from the present study could be scientific evidence to demonstrate that oil-pulling therapy with some edible oils could be used as a preventive home therapy to maintain oral hygiene, especially in developing countries. However, further studies are needed to investigate the mechanisms of the action of the oil on these microorganisms or dental plaque, as well as long-term effects in clinical trials.

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