Adhesion of *Candida albicans* to Vanillin Incorporated Self-Curing Orthodontic PMMA Resin.

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**Abstract.** It has been observed that there is an increase in *Candida* carriers during the treatment with orthodontic removable appliance. Vanillin is flavouring agent, which is known to have antioxidant and antimicrobial properties. The aim of this study was to evaluate the effect of vanillin incorporated PMMA on adhesion of *Candida albicans*. A total of 36 orthodontic self-curing PMMA resin samples were fabricated. The samples were divided into 3 groups depending on percentage of vanillin incorporated (0.1%, 0.5% and PMMA without vanillin as control). PMMA samples were coated with saliva. The adhesion assay was performed with *C. albicans* (ATCC 10231). The adherent yeast cells were stained with crystal violet and counted under microscope by random selection of 3 fields at 10X magnification. The statistical analyses performed by Kruskal Wallis and Mann Whitney non-parametric test. It was found that the PMMA resin samples with vanillin incorporation significantly reduced the adhesion of *C. albicans* as compared to the control group. This study indicates that vanillin incorporated resin can impede the adhesion of *C. albicans* to about 45 - 56 %. With further testing and development, vanillin can be employed as an antifungal agent to prevent adhesion of *C. albicans* to orthodontic self-curing PMMA resin.

1. **Introduction**

Vanillin is an important component of natural vanilla, extracted from the seeds of tropical orchid, *Vanilla planifolia*. Vanillin is mainly used as a flavouring agent for food, beverages and also used for intermediates for pharmaceuticals [1]. Additional to flavouring agent, vanillin being phenolic compound with low molecular weight, has antioxidant and antimicrobial properties [2]. Several studies reported the effectiveness of vanillin against certain food spoilage bacteria, yeasts and molds [3-5].

*Candida albicans* is a commensal pathogen that lives on various surfaces of body like genital and intestinal tracts, oral mucosa as well as skin [6-8]. The increased growth of *C. albicans* can cause...
soreness, unpleasant taste, bad breath with bleeding and inflammation of oral mucosa. Also, it can penetrate the blood stream causing candidemia which will infect the internal organs causing significant morbidity and mortality [9, 10]. For efficient colonization and development of pathogenes, the adhesion of *C. albicans* to host cells or biomaterials is necessary [10, 11].

PMMA is a transparent acrylic based resin, it is used for the fabrication of oral appliances and retainers in Orthodontics. It has been reported that with orthodontic appliances and other oral devices leads to change in oral microbiota resulting in rise of Candida density at oral mucosa [12, 13]. The microbial plaque biofilm can be controlled by mechanical and chemical aids which demand stringent patient compliance which unfortunately is unobtainable in patients with disabilities. The objective of this study is to investigate the inhibitory effect of vanillin on *C. albicans* adhesion to vanillin incorporated Orthodontic self-curing PMMA resin surface.

2. Method and materials

2.1. Collection of saliva
Unstimulated saliva was collected from a normal healthy individual (non-smoker, no medication). The subject was asked to refrain from using oral hygiene products like antimicrobial mouthwashes and the saliva was collected 2 h after consumption of meals.

2.2. Candida suspension preparation.
*C. albicans* (ATCC 10231) was obtained from Department of Oral Microbiology, Faculty of Dentistry Mahidol University, Thailand. They were maintained in Sabouraud dextrose agar at 4°C. Few colonies from the agar were inoculated in phosphate buffer saline (PBS) to yield a concentration of approximately 10^7 CFU/ml using McFarland standard No.1.

2.3. Preparation of PMMA Samples
The Orthodontic self-curing PMMA resin powder (Siam cement group, SCG) was mixed with 0.1% and 0.5% vanillin powder. Thirty-six orthodontic self-curing PMMA resin samples were prepared with dimension of 15 mm x 15 mm x 1.5 mm. The samples were divided into 3 groups depending on percentage of vanillin incorporated (0.1%, 0.5% and PMMA without vanillin as control). The tissue surfaces of these samples were unpolished to replicate an exact impression of tissue surface of retainers. The resin samples were placed in distilled water for 3 days to leach out excess monomer and sterilized with UV light for 30 min. The PMMA samples were place in sterile saliva collected from a healthy individual for 30 min.

2.4. Adhesion Assay
The self–curing orthodontic PMMA samples were placed in 24-well plate. To each well containing PMMA sample, 2 mL of candida suspension was added. The 24-wellplate with PMMA sample and candida suspension was placed in shaking incubator (120r /min) at 37°C for 90 min. The PMMA samples were then washed twice with PBS to remove the non-adherent cells. The remaining adherent cells on PMMA resin surface was fixed with 95% ethanol and stained with crystal violet. They were counted under microscope by random selection of 3 fields at 10X magnification. The mean number of adherent yeast cells were expressed as cells/mm^2^.

2.5. Statistical analyses
All tests were performed triplicate on 4 separate occasions. The analysis of data was done using SPSS statistics. Kruskal Wallis and Mann Whitney U test. The significance level of 0.05 was used.
3. Results

Table 1 presents the number of adherent cells on orthodontic self–curing PMMA surface. Both the vanillin incorporated resin samples had significant reduction of candida adhesion as compared to the control. It was found that there was a reduction of about 45 to 56 % as illustrated in figure 2. Although the number of adherent yeast cells was more reduced in 0.5% than 0.1% vanillin group, there was no statistical significant difference found between these two groups.

| Vanillin | 0%       | 0.1%     | 0.5%     |
|----------|----------|----------|----------|
|          | 18.9183±1.22431 | 10.3233±1.32726 | 8.4117±1.30667 |

values are expressed as mean±SD

Figure 1 Percentage of reduction of C. albicans adhesion on vanillin incorporated self-curing orthodontic PMMA resin

4. Discussion:
The adhesion of C. albicans to host cells or polymers such as acrylic resin is a necessary step which is required for efficient colonization and progression of pathogenesis and infection [6, 14]. The adherence of the candida strain is important to prevail over the host clearance mechanisms and for colonization, it depends on the effectiveness of the adhesion mechanism and the yeast growth rate [6]. Factors affecting the adhesion of C. albicans to PMMA depends on the substrate surface (type, chemical nature and surface properties like free surface energy, surface roughness), the presence of salivary proteins and serum, the presence of other adherent microorganisms, strain variability, consumption of carbohydrate-rich diet, and culture conditions [15].

A study conducted on orthodontic patients undergoing removable appliance therapy indicated that the Candida carriers was increased from 39% before treatment to 79% during treatment in 9 months. However, following the completion of treatment, it was reduced significantly to 14% [16]. Both mechanical and chemical methods can be used to control plaque accumulation on orthodontic appliances. Despite their effectiveness, these procedures demand good patient compliance, which may not be readily achievable in those patients with disabilities. Hence, the addition of antimicrobial agents in orthodontic acrylic resin for plaque biofilm reduction is highly recommendable [9, 12].

So, in this study, the effect of vanillin on adhesion of a standard strain of C. albicans to self-curing orthodontic PMMA was investigated. The results showed that candida adhesion to 0.1 % and 0.5% vanillin incorporated orthodontic PMMA resin was significantly reduced as compared to control.

Vanillin, a major component of natural vanilla is not only an important flavour but also has antioxidant and antimicrobial properties. A study conducted using food bacteria like Escherichia coli, Lactobacillus plantarum and Listeria innocua have shown that the antimicrobial activity of vanillin is mainly due to its ability to affect the integrity of the cytoplasmic membrane with the resultant loss of ion gradients, pH homeostasis and inhibition of respiratory activity [3]. Vanillin can inhibit several strains of Cryptococcus neoformans and C. albicans. The study conducted showed that vanillin is effective against C. albicans in vitro and it can be used for treatment of infection caused by non-dermatophyte fungus[17]. It was suggested that the antifungal property of the vanillin could be due to aldehyde moiety its structure [3].

5. Conclusion
The vanillin incorporated orthodontic PMMA resin exhibited significant inhibitory effect against candida adhesion to the surface about 45-56%. Hence vanillin can be incorporated in self curing orthodontic PMMA resin to reduce candida adhesion for fabrication of intraoral orthodontic appliance.

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