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Research Article

Biofilm Formation between Species Associated with Denture Stomatitis

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ABSTRACT

Denture stomatitis (DS) can affect about 70% of denture wearers causing inflammation of oral mucosa underneath the removable denture mostly seen at the palatal area. *Candida albicans* is a well-known as the main causative agent associated with denture stomatitis. Studies shown that DS-related biofilm consists of complex structured of microbial communities. Presence of co-existing bacteria in these biofilms may also contribute to the infection. In order to understand the microbial interaction between *C. albicans* and bacteria, this study focus on the ability of bacteria species which are *Streptococcus mutans* and *Staphylococcus aureus* to form biofilm with and without the presence of *C. albicans*. For this study, two groups were observed which are the single species biofilm and the mixed species biofilm. Biofilm assay was utilized to measure biofilm mass using spectrophotometer. From the observation, single species groups formed a better biofilm when compared to fungal-bacterial biofilm. Even though biofilm assay is unable to determine the number of cell available, both species were present when fungal-bacterial biofilm sample was observed microscopically using Gram stain technique. This experiment showed that both fungal and bacteria species are able to form biofilm together and this finding can be investigated further for better understanding of DS-related biofilm.

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Introduction

Microbial biofilms are associated with medical device infection and it is known that *Candida*- associated denture stomatitis (DS) is caused by *Candida* spp. that coexist with oral bacteria [1, 2]. From literature, denture stomatitis are caused by yeasts, typically *Candida albicans*, although other species such as *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. guilliermondii* and *C. dubliniensis*, may contribute to the pathogenesis of the disease [3]. Besides that, there were bacteria from several genera such as *Streptococcus*, *Veillonella*, *Lactobacillus*, *Prevotella* and *Actinomyces* spp. isolated in DS samples [4]. *Staphylococcus aureus* and *Streptococcus mutans* are the common colonizers of DS from the samples and there were other bacterial species identified on denture surfaces such as *S. sanguinis*, *S. gordonii*, *A. odontolyticus* and *A. viscosus* [3, 5, 6]. Oral cavity provides a complex environment that consist of variety of species, and its population densities are most likely dynamic. Therefore, it is necessary to understand if the presence of bacteria with *C. albicans* will improve the

biofilm formation. The objective of this study is to investigate the biofilm formation of single and mixed species communities by comparing biofilm mass between single species biofilm and fungal-bacterial biofilm using crystal violet assay.

Materials and Methods

There are three relevant oral microorganisms associated with denture stomatitis were used in this study which are *Candida albicans* ATCC 14053, *Streptococcus mutans* ATCC 25175, and *Staphylococcus aureus* (clinical isolates). *Candida albicans* ATCC 14053 was cultured on sabouraud dextrose agar (OXOID, UK) (65g/L) and incubated aerobically in incubator (Mettler, Germany) 37°C for 24 hours. *Streptococcus mutans* ATCC 25175 and *Staphylococcus aureus* was cultured on brain heart infusion agar (OXOID, UK) (52g/L). *S. mutans* ATCC 25175 was then incubated anaerobically in anaerobic chamber (Ruskinn Bugbox) at 37°C for 24 hours whereas *S. aureus* was incubated aerobically in incubator (Mettler, Germany) at 37°C for 24 hours.

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Routine gram staining of each sample were done to ensure no contamination and single species were obtained. An overnight culture of all three were used for experiment as following *S. mutans* ATCC 25175 (7×10^8 cfu/mL), *S. aureus* (3×10^9 cfu/mL) and *C. albicans* ATCC 14053 (3×10^8 cfu/mL) [7].

Biofilm assay using crystal violet employed a colorimetric method to measure the formation of biofilm in 96-well plates [8]. This technique was used to investigate the biofilm mass of single species and mixed species biofilm. For single species biofilm, a total of 210µL consist of 205µL of tryptone soy broth (TSB) (OXOID, UK) (30gL^{-1}) and 5µL of each species of *S. mutans*, *S. aureus* and *C. albicans* were added into 96 well plate. While in a mixed species biofilm wells, 200µL of media and 5µL of *C. albicans* and 5µL of either *S. mutans* or *S. aureus* were added into 96 well plate (with total of 210µL). Each sampling was done in triplicate. Distilled water and TSB were used as control. The plate was covered and incubated aerobically at 37°C overnight in a humid

environment. Once incubation period is finished, the cell was removed by flicking, and 100µL crystal violet (0.5% v/v) was added into 96 well plate and incubated at room temperature for 15 minutes. The wells then were rinsed three times with 200µL of PBS. 100µL of 7% (v/v) acetic acid were added in order to dissolve the dye. The absorbance reading was done using microtitre plate reader (Infinite M200 PRO, Tecan, USA) at 570nm.

Results

Result in (Figure 1) showed that all species has the ability to form biofilm as previously reported. The single species of bacteria formed better biofilm compare to *Candida* only biofilm. The *C. albicans*-*S. mutans* mixed biofilm is 1/3 of biofilm mass more than *Candida* only biofilm. While in mixed *C. albicans*-*S. aureus*, there is not much difference shown compared to *Candida* only biofilm mass.

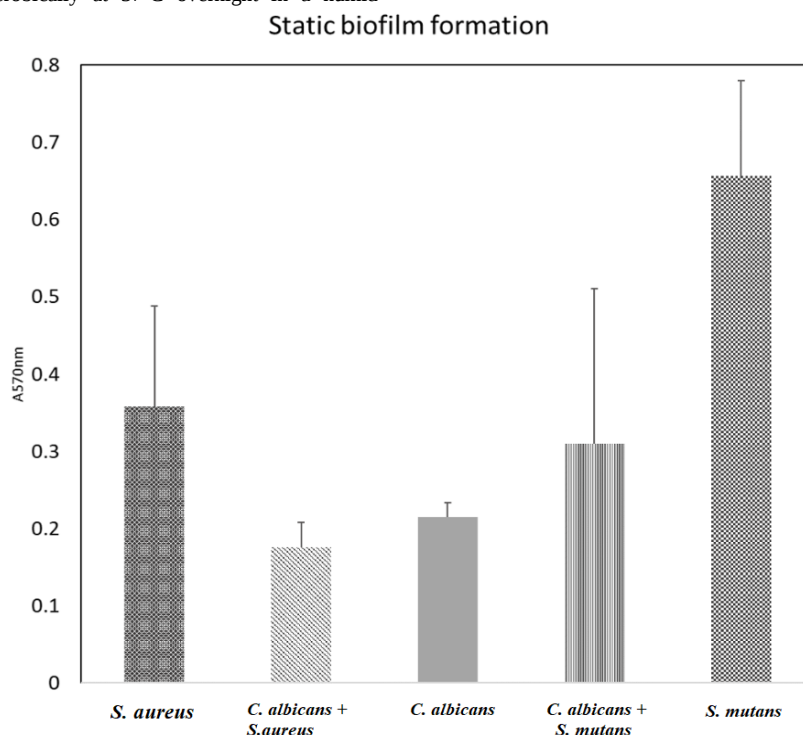


Figure 1: Biofilm mass of *Candida albicans*, *Staphylococcus aureus*, *Streptococcus mutans* single and mixed species biofilm using crystal violet biofilm assay. Bars represent mean and SDs of three independent experiments are shown.

Discussion

All the species chosen in this experiment are oral microbiome and commonly found in denture stomatitis as stated by Nair *et al.*, 2016 and this experiment has showed that both fungal and bacteria species are able to form biofilm together in the same environment [6, 7, 9]. Biofilms present on intravascular medical devices and prosthesis and become the source of infection. *C. albicans* is readily forms biofilms on a wide variety of polymers used for indwelling medical devices, such as dental materials, stents, shunts, prostheses (voice, heart valve, knee), implants, endotracheal tubes, pacemakers, and catheters [10]. There is some evidence to suggest that a large proportion of device-related *Candida albicans* infections involve biofilms [11]. *S. aureus* is the common colonizer of the skin and mucous membrane and can enter the

bloodstream and causes bacteraemia, osteomyelitis, toxic shock and many more infections. The capacity of *S. aureus* to form biofilm as well as the virulence have become problematic. From the result, biofilm mass of mixed *C. albicans* and *S. aureus* has almost similar with *C. albicans* only biofilm mass.

Studies has found that 27% of *C. albicans* bloodstream infections are polymicrobial and *S. aureus* was the common organism isolated together with *C. albicans* and the combination of these two organisms will result in synergism. *S. aureus* was found to be associated preferentially with hyphae within the biofilm and also formed microcolonies on the surface of the biofilm. It appeared that *C. albicans* served as the scaffolding, with *S. aureus* scatter among the hyphal network [12]. Interestingly, when *S. mutans* was incubated with *C. albicans*, there was an increase of biofilm

mass. This showed that *S. mutans* also have the ability to form biofilm when present in the same environment with *C. albicans* as previously reported by Kim *et al.*, 2017 [9]. This maybe due to the symbiotic relationship between *S. mutans* and *C. albicans*. Physical coadhesion of *S. mutans* and *C. albicans* is drastically enhanced in the presence of sucrose; these conditions also promote biofilm formation [13, 14]. *C. albicans* does not efficiently metabolize sucrose and is favoured by degraded products of sucrose by *S. mutans* (glucose and fructose). In contrast, the presence of *C. albicans* in the biofilm alters the physical environment, favouring the increase of exopolysaccharides and consequently, accumulation and formation of microcolonies by *S. mutans* [14].

This is a comparative pilot study and only used a simple model to mimic the biofilm formation in the oral cavity by static biofilm formation. This study can be proceeded with RT-PCR to quantify the number of microbial species presence in the biofilm and the usage of more advance biofilm model that can mimic the intra oral environment should be investigated. Thus, it can be explored further for better understanding of denture stomatitis related biofilm as there is inadequate of extensive study regarding the association of other bacteria in the biofilm associated with denture induced stomatitis.

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