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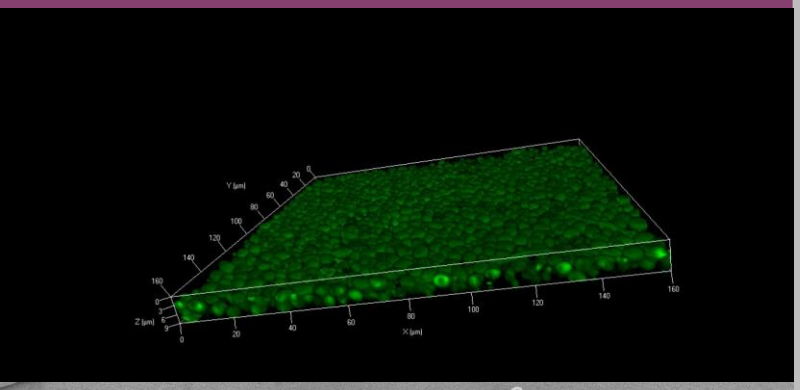

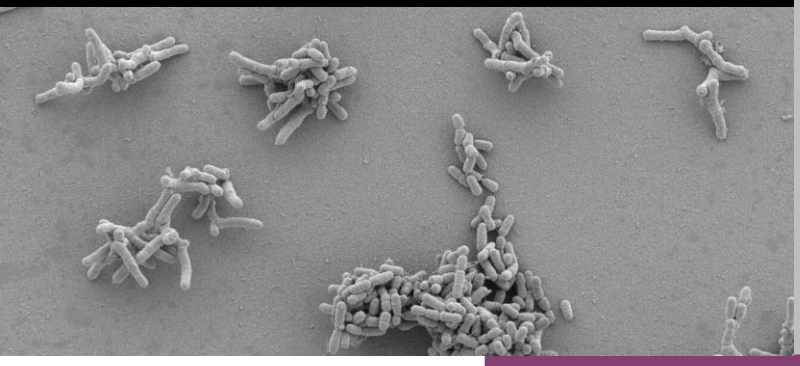

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Aims and Scope:

International Journal of Orofacial and Health Sciences (IJOHS) is a peer reviewed biannual international journal dedicated to publish high quality of scientific research in the field of orofacial sciences, health sciences and interdisciplinary fields, including basic, applied and clinical research. The journal welcomes review articles, original research, case reports and letters to the editor. Areas that are covered include but are not limited to dental sciences, oral microbiology and immunology, oral maxillofacial and craniofacial surgery and imaging, dental stem cells and regenerative medicine, dental biomaterial, oral maxillofacial genetic and craniofacial deformities.

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CONTENTS

Isolation of *Candida* species in children and their biofilm-forming ability on nano-composite surfaces

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Abstract

Candida species including *Candida albicans*, *Candida krusei* and *Candida glabrata* are opportunistic microorganisms that inhabit oral cavity. The objective of this study is to determine the effect of dental caries on *Candida* spp. biofilm-forming ability on nano-composite with the hypothesis that dental caries enhances the colonization of *Candida* spp. To assess *Candida* spp. colonisation in the oral cavity of the paediatric patient, samples were obtained from 30 subjects aged five to six years old from Kuantan, Pahang, Malaysia. The samples were collected from buccal mucosa, palate and tooth surfaces using sterile swabs. 10 mL of patient's saliva suspension was also collected. Following that, the samples were inoculated on CHROMagar and incubated for 24 h at 37 ºC. *Candida* biofilm of caries isolate *C. albicans* (HNFC2), and *C. albicans* ATCC 32354 were developed on three different types of nano-composites. The study showed that no *C. albicans* was isolated from the caries-free oral cavity while 76% of children with caries possessed *Candida* spp. 65% of the yeasts were isolated from the tooth surface. Only 35% of the total isolates were obtained from soft tissues, including palatal and buccal mucosa. *C. albicans* is the most isolated *Candida* spp. with 82% and 67% of the yeast were obtained from the tooth surface and buccal mucosa, respectively. Besides, HNFC2 significantly colonised the nano-composites more than the ATCC ($P < 0.05$). In the comparison of the three types of nano-composites, nano-hybrid-based containing pre-polymerised filler (cB) exhibited the least *C. albicans* HNFC2 cells colonisation with 7.7 x 10^3 cells mL^{-1}. In contrast, the nano-composite that contained bulk-filled nanohybrid (cC) was the most colonised with 14.3 x 10^3 cells mL^{-1}. In conclusion, dental caries enhances the colonization of *Candida* spp. in children's oral cavity, and that caries isolate form more biofilm on nano-composites compared to the lab strain *C. albicans*.

Keywords: Paediatric, dental caries, Candida species, biofilm formation, nano-composite

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Introduction

Oral microbiome exists in both planktonic and biofilm (plaque) forms (*Kolenbrander et al., 2010*). More than 2000 groups of pathogens present in the oral cavity with opportunistic pathogens encompass a substantial number of them. These opportunistic pathogens have been shown to involve in the establishment of several oral or systemic disease (Dewhirst *et al.,* 2010; Warinner *et al.,* 2014). Currently, the categorization of this microbiome is based on shotgun metagenomics and comparative 16S rRNA gene sequencing. Formerly, they were divided according to simple sugar fermentation, their morphology and chemical contents (Chaffin, 2008; Donovan *et al.,* 2018).

Candida is a yeast that belongs to the kingdom fungi. It is known as imperfect fungi within the family of *Cryptococcaceae* (Rybalkin *et al.,* 2014). It has sizes ranged from two to five micrometre, and it also can be presented in hyphae and yeast form (Samarayanake, 2002; Rós Ásmundsdóttir *et al.,* 2008). A few types of *Candida* spp. for instance, *C. albicans* and *Candida dubliniensis* have aptitude in septate hyphae formation. This ability is essential during pathophysiology of disease as *Candida* spp. in hyphae form is more active in tissue invasion and more capable of injuring the invaded tissue (Samaranayake, 2006; Sudbery, 2011).

Candida spp. are part of a healthy microbiome. There are 200 recognized species in the genus of *Candida* with seven species are reported to play an essential role in pathogenesis of human diseases including *C. albicans, Candida kefyr, C. glabrata, C. krusei, Candida parapsilosis, C. dubliniensis and Candida stellatoidea* (Samarayanake, 2002*;* Rós Ásmundsdóttir *et al.,* 2008; Sida *et al.,* 2016). Among all these *Candida* spp., *C. albicans* has been testified to be the most predominant in the oral cavity (Akdeniz *et al.,* 2002; Nejad *et al.,* 2013). Virulence factors of *Candida* vary between species, and they include the phenotypic switching ability, hydrophobic cell surface, biofilm formation as well as hydrolytic enzymes, candidalysin and quorum sensing molecules production (Haynes, 2001; Williams *et al.,* 2011; Arzmi *et al.,* 2012; Arzmi *et al.,* 2014; Kragelund *et al.,* 2016; Sida *et al.,* 2016).

Candida spp. mainly *C. albicans* is one of the causes of many harmful diseases, including oral candidiasis and oral squamous cell carcinoma (Arzmi *et al., 2018*). *C. albicans* is a normal commensal of the human body and causes no damage. However, when the host defences are weakened, it is capable of becoming pathogenic and causing severe problems (Ramirez-Garcia *et al.,* 2013). *C. albicans* is an innocuous dimorphic fungus. However, it can become pathogenic and harmful when the balance of microbial flora has been disturbed, or immune system of the host has been debilitated (Zunt, 2000; Byadarahally Raju *et al., 2011*).

A study conducted in Singapore showed that *C. albicans* was the most prevalent species isolated from the blood of disease-ridden patients. Aside from *C. albicans*, *C. tropicali*s, *C. glabrata C. parapsilosis*, and *C. krusei* were also reported to be isolated from the subjects. Besides that, *C. albicans* was frequently found in the paediatric patient, too (Yang *et al.,* 2003; Pereira *et al.,* 2010). Besides, a retrospective study conducted in Lyon, France from 1998 to 2001 revealed that candidal infection was mainly caused by *C. albicans* (49.5%) and followed by *C. glabrata* with 12.6% and *C. parapsilosis* with 12.1%. The same study also showed that in onco-haematology patients, candidemia was majority caused by *C. krusei* and *C. albicans* (Martin *et al.,* 2005). A study in Malaysia has discovered that the most dominant *Candida* spp. isolated from the bloodstream was *C. albicans* (44.2%). They were also able to isolate other *Candida* spp. aside from *C.*

albicans such as *C. parapsilosis* (26.0%) and *C. tropicalis* (17.7%) (Ng *et al.*,1999).

Nano-composite has become the choice of dental practitioners in modern dentistry to replace other restorative materials such as amalgam due to a better aesthetics quality and abrasion resistance (Cramer *et al.,* 2011). Furthermore, in combination with good bonding, nano-composite presents adhesive properties which can preserve the tooth structure during cavity preparation which is not possible with amalgam restoration (Correa *et al.,* 2012). In order to improve its mechanical and physical properties, conventional composite has been improved by incorporating different compositions that possess different properties (Burgers *et al.,* 2009).

The objective of this study is to determine the effect of dental caries on *Candida* spp. biofilm-forming ability on nano-composites with the hypothesis that dental caries enhances the colonization of *Candida* species.

Materials and Methods

Sample isolation

Before the commencement of sample collection from children at International Islamic University Malaysia (IIUM) Dental Polyclinic and Adik Arif Kindergarten in Kuantan, Pahang, Malaysia, ethical approval (IREC 2018-172) was obtained from the IIUM Research Ethics Committee (IREC) on the 8th May 2018.

Briefing regarding this study was given to the parents, and written consent was obtained before sample taking. The data was recorded on clinical examination sheet. A total of 30 healthy pre-schooled children (15 caries-free patients and 15 patients with caries) aged five to six years old consented by parents were included in the study. The exclusion criteria were children with co-morbidity and not consented by parents. The samples were collected in the presence of dental clinicians. The oral rinse technique was conducted to isolate microbial samples from the subject. In brief, patients were requested to have their mouth rinsed with 10 mL of sterile saline for one minute and spit in a sterile container. Following that, the surface of the teeth, palate and buccal mucosa were swabbed with a sterile swab, and the samples were transported immediately to the laboratory for identification of *Candida* spp.

Identification of *Candida* species

The samples that were previously collected from paediatric patients were inoculated onto *Candida* spp. selective CHROMagar (BD, USA) aseptically which was prepared beforehand for identification of *Candida* spp. Prior inoculation, each petri dish was divided into four parts and labelled with saliva suspension (S), tooth (T), palate (P) and buccal mucosa (B). To identify *Candida* spp. from saliva suspension, the transport mediums which contain microbial isolates were vortexed vigorously using a vortex mixer (Biologix, Singapore) followed by inoculation on CHROMagar using sterile swabs.

Meanwhile, to identify *Candida* spp. from other oral sites, the collection swab which was used to collect the sample from the oral surfaces of children was swabbed on CHROMagar aseptically. The plates were incubated at 37 ºC for 24 h to 48 h, aerobically. The colour of the colony grown was recorded. The colony which exhibited green, dark pink and white were identified as *C. albicans*, *C. krusei* and *C. glabrata*, respectively. The species of *Candida* spp. were finally confirmed using API 20C AUX (Biomerieux, USA).

Enumeration of *Candida* species from saline suspension

The method by Alnuaimi et al. (2013) was conducted to enumerate the number of *Candida* spp. In brief, saliva suspension collected from the patient was vortexed vigorously. Later, 100 µL of the

suspension was serially diluted in 900 µL sterile saline. Following that, 10 µL of the diluted suspension was pipetted onto haemocytometer, and a clean glass coverslip was placed to secure the sample. Finally, the number of *Candida* spp. was enumerated by observing the haemocytometer under a light microscope (Olympus, Japan). The morphology of *Candida* spp. was also recorded based on the observation under the microscope.

Identification of *Candida* species colony morphology

To identify the colony morphology of *Candida* spp., a loopful of *Candida* spp. that was previously cultured on CHROMagar was inoculated onto a fresh CHROMagar using single dilution streaking method to obtain a single colony of *Candida* spp. Later, the plate was incubated for 24 h to 72 h at 37 ºC, aerobically. Finally, the colony morphology, including margin, elevation and form, were observed and recorded.

Preparation of nano-composite beads

Three different types of nano-composites (cA, cB and cC) as described in Table 1 were prepared using a round plastic mould (6 mm diameter x 5 mm height). Each bead was then polished beforehand. Following that, all nano-composites were sterilized using ultra-violet (UV) light radiation technique.

Growth of *Candida albicans*

C. albicans American Type Culture Collection (ATCC) 32354 was sub-cultured on yeast peptone dextrose (YPD) agar and incubated at 37 °C for 24 h. Following that, a single colony of *C. albicans* was inoculated in YPD broth and standardized using a spectrophotometer to obtain an OD_{620nm} 0.1 that was equivalent to 10^6 cells mL^{-1}. Finally, the 1.5 mL of the suspension was aliquoted into 2 mL sterile Eppendorf tube and stored at -20 °C (Figure 4). A similar protocol was repeated to grow *C. albicans* isolated from the oral cavity of children with caries (HNFC2).

Static biofilm formation

The study of static biofilm was conducted according to the modified protocol by Arzmi et al. (2016). Initially, 750 µL of YPD broth was pipetted into each well of 12-well plate. Following that 750 µL of *C. albicans* suspension standardized at 10^6 cells mL^{-1} in YPD broth was added in the same well. The suspension was mixed using a sterile pipette. Wells that contained only YPD broth were representing as the negative control. Finally, the sterile nano-composite bead was placed into each well aseptically, and the plate was incubated at 37 °C for 24 h.

Enumeration of *Candida albicans*

Following incubation, the growth medium was discarded, and each well was rinsed twice with 1 mL of sterile distilled water. Later, the nano-composite bead was transferred into a sterile 15-mL tube containing 3 mL of sterile distilled water. The tube was sonicated using ultra-sonicator (Amsonic, USA) for 60 s. Finally, 10 µl of the suspension was pipetted onto haemocytometer to measure the cell number (Alnuaimi *et al.*, 2014). All protocols were repeated in three biological replicates to confirm reproducibility.

Data analysis

All data were statistically analysed using SPSS Statistic software version 25.0. Independent T-test was used to compare lab strain, and caries isolates *C. albicans* and analysis of variance (ANOVA) associated with *post hoc* Tukey test was used to compare three different nano-composites. The data were considered statistically significant when $p < 0.05$.

Results

Colonization of *Candida* species in paediatric patients

Our data showed no *Candida* spp. was isolated from caries-free children.

However, 76% of the total samples from caries patients contained *Candida* spp. isolates (Figure 1). Of these, 65% of *Candida* spp. were isolated from the teeth while 18% were isolated from saliva suspension. Besides, 18% of the samples were also isolated from buccal mucosa (Figure 2). No *C. albicans* was isolated from the palate.

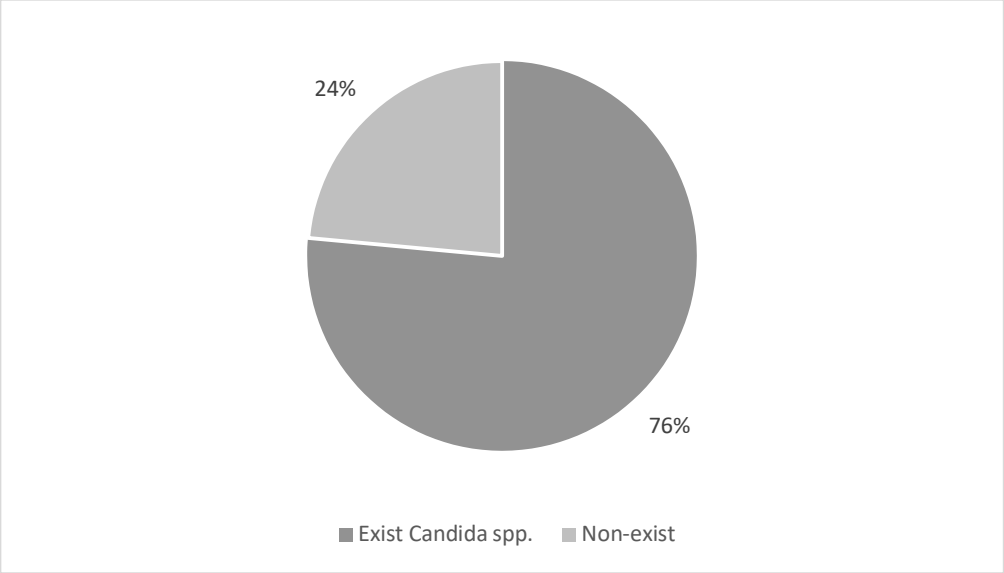

Figure 1. Percentage of caries children with *Candida* spp. (N=15).

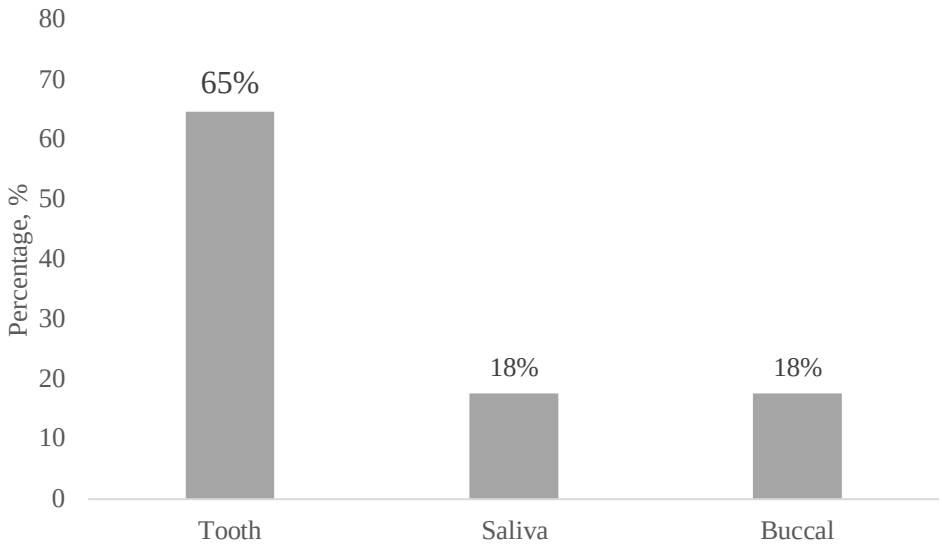

Figure 2. Percentage distribution of *Candida* spp. in children based on location; tooth, saliva and buccal mucosa of children with dental caries (N=15). No *Candida* spp. was isolated from palate.

Distribution of *C. albicans* and non-*C. albicans* in the oral cavity of children with dental caries

Candida spp. were observed to colonise the tooth surface, saliva suspension and buccal mucosa of paediatric patients with dental caries. There were 82% of the samples isolated from tooth surface of caries patients exhibited as mono-species *C. albicans*, while 9% was non-*C. albicans* (Figure 3). There were only 9% of patients with dental caries possessed both *C. albicans* and non-*C. albicans* in the tooth surface.

In addition, 67% of the paediatric patients with dental caries had mono-species *C. albicans* isolated on the buccal mucosa, whereas 33% of the isolates had non-*Candida albicans* (Figure 4). However, in saliva isolates, all samples that were isolated from patients with caries exhibited as mono-species *C. albicans* with the cell morphology was predominantly by the yeast form (Figure 5).

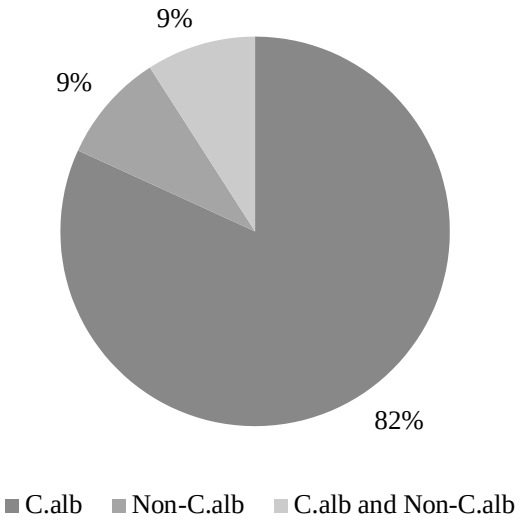

Figure 3. Percentage of caries children possess *C. albicans* only (C.alb), non-*C. albicans* only (Non-C.alb) or both *C. albicans* and non-*C. albicans* (C.alb and Non-C.alb) on the caries tooth surfaces (N=15).

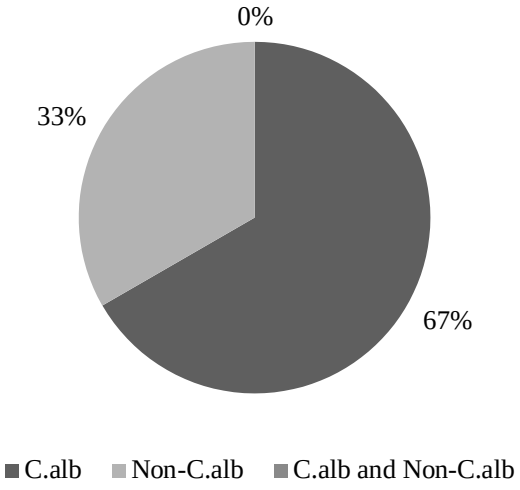

Figure 4. Percentage of caries children with *C. albicans* only (C.alb), non-*C. albicans* only (non-C.alb) or both *C. albicans* and non-*C. albicans* (C.alb and Non-C.alb) isolated from the buccal mucosa of caries patients (N=15).

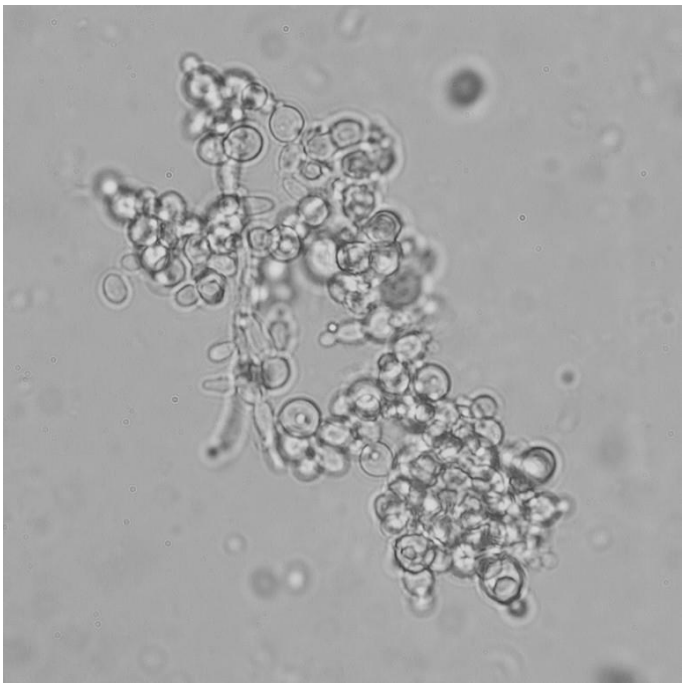

Figure 5. *C. albicans* isolated from saliva of caries children as observed under light microscope at 1000x magnification.

Colony morphology of *Candida* species

All *C. albicans* isolated from paediatric patients with dental caries exhibited as a circular shape, entire margin and convex elevation. Meanwhile, *C. krusei* that was isolated exhibited irregular shape, undulate margin and raised elevation. Finally, *C. glabrata* that were isolated from caries patients exhibited circular shape, entire margin and convex elevation.

Comparison of biofilm formation of *C. albicans* between nano-composites

C. albicans ATCC 32354 showed adhesion on cA, cB and cC nano-composites with $4.3 \pm 0.58 \times 10^3$ cells mL^{-1}, $4.3 \pm 0.82 \times 10^3$ cells mL^{-1} and $3.8 \pm 1.14 \times 10^3$ cells mL^{-1}, respectively. However, there was no significant difference observed between the three nano-composites ($p > 0.05$).

C. albicans HNFC2 exhibited $14.3 \pm 4.98 \times 10^3$ cells mL^{-1} adhered on cC nano-composite followed by cA nano-composite, which had $9.9 \pm 4.13 \times 10^3$ cells mL^{-1}. Besides, there were $7.7 \pm 0.5 \times 10^3$ cells mL^{-1} adhered on cB nano-composite with a significant difference was observed between cC and cB nano-composites ($P < 0.05$).

Comparison of biofilm formation of *C. albicans* between lab and clinical strains

C. albicans HNFC2 had significantly more cell adhered on the cA nano-composite compared to *C. albicans* ATCC 32354 ($p < 0.05$). This similar trend was also observed in both cB and cC nano-composites. There were $9.9 \pm 4.13 \times 10^3$ cells mL^{-1} of *C. albicans* HNFC2 were adhered on cA nano-composite, which was significantly higher than *C. albicans* ATCC 32354, which exhibited $4.3 \pm 0.58 \times 10^3$ cells mL^{-1}. There were $7.7 \pm 0.5 \times 10^3$ cells mL^{-1} of *C. albicans* HNFC2 cells adhered on cB nano-composite, which significantly more than the *C. albicans* ATCC 32354 cells that adhered on the same nano-composite which exhibited $4.3 \pm 0.82 \times 10^3$ cells mL^{-1} ($p < 0.05$). The

adhesion of *C. albicans* HNFC2 cells on cC exhibited 14.30 $\pm$ 4.98 x 10^3 cells mL^{-1} which was significantly higher than *C. albicans* ATCC 32354 which exhibited 3.80 $\pm$ 1.14 x10^3 cells mL^{-1} ($p < 0.05$).

Discussion

Candida spp. was observed to be isolated only from caries patients. This data supported the hypothesis of the present study, which stated that dental caries enhances the colonization of *Candida* spp. in children with dental caries. Most of the caries research were reported that a high number of *Streptococcus mutans, Lactobacillus* species, and *Scardovia* species in children diagnosed with severe early childhood caries (S-ECC) as its presence intensify the presence of these bacteria. Previous *in vitro* and *in vivo* studies have shown that the existence of *C. albicans* can lead to a complex bacterial-fungal interaction and result in the growth of a cariogenic biofilm environment (O'Donnell *et al.,* 2015; Bowen *et al.,* 2017). For example, induction of glucosyltransferase B by *C. albicans* in addition to bacterial accumulation via chemical-metabolic interactions able to promote the growth of *S. mutans* (Sztajer *et al.,* 2014*;* Kim *et al.,* 2017). Cross-feeding interaction between *S. mutans* and *C. albicans* heightens their growth further while creating a Gtf activation loop and promotes the development of highly acidified microenvironment which is a suitable environment for acidogenic-aciduric bacteria (Bowen *et al.,* 2017).

Candida spp. was observed to be isolated mostly on the tooth surface and less on the buccal mucosa and saliva suspension. A study has shown that a hard surface in constant colonization of early colonizer of oral biofilm such *as S. mutans*. *S. salivarius* dominates the microbiota in the oral cavity during the early neonate life until the appearance of the teeth (Sachdeo *et al.,* 2008). The eruption of the teeth during the first year of life leads to colonization by *S. mutans* and *S. sanguis* (Cortelli *et al.,* 2008). Oral biofilm is a structured community of microbes which adheres to oral surfaces and is compressed within extracellular polymeric substances (EPS), formed from multi-microorganisms and environment of the oral cavity (Filoche *et al.,* 2010). The ability to form biofilm on the oral surfaces is one of important virulence factor of *Candida* spp. in the oral cavity. Colonization of microorganism on hard and soft tissue surfaces is initiated by adhesion of *Candida* spp, which lead to the formation of an organized microbial community known as biofilm (Hofer, 2016). Formation of biofilm is the first step in the establishment of dental caries. *Candida* spp. have the ability to co-aggregate with other microorganisms. This ability assists the yeast in attaching on the oral surface which is pre-colonised by the early coloniser bacteria such as *S. mitis, S. oralis* and *S. sanguinis* attached on the acquired pellicle that can form easily on hard tissue surface (Kiyora *et al.,* 2000). *Candida* spp. are found to colonize the tooth surface due to formation of the salivary pellicle. Deposition of pellicle on the tooth surface is the start of the formation of the initial plaque layer (Zijnge *et al.,* 2010). This evidence supports the hypothesis of the present study that dental caries enhances the colonization of *Candida* spp. in children.

Our results also showed that *Candida* spp. were isolated from buccal mucosa. *Candida* spp. have been shown to colonize mucosal surfaces (Salerno *et al.,* 2011; Hofer, 2016). The oral cavity presents abundant surfaces for microbial colonization. Colonization of these surface was started by biofilms of divergent microbial complexity inimitable to each species. While oral biofilm can arise on dental surfaces and mucosal surfaces inside the mouth, the constitution of the microbiomes varies greatly depending on the type of surface (Marsh *et al.,* 2011). The previous study has suggested that

microbes such as *S. mutans* required hard surfaces for continuous colonization. However, they can also be detected on the soft tissues in low levels (Sachdeo *et al., 2008*). It has also been shown that *S. mutans* fundamentally disappeared from the oral cavity when all teeth were extracted and reappeared again when the denture was worn. The denture provides a hard surface for colonization (Sachdeo *et al.,* 2008). However, this does not negate the fact that microbes such as *S. mutans* present on the mucosal surface. *Candida* spp. is known to have the ability to co-aggregate with other microorganisms and adhere to the oral surface colonised by the early coloniser bacteria such as *S. mutans* (Kiyora *et al.,* 2000), which can justify the presence of *Candida* spp. on buccal mucosa.

Candida spp. were also isolated from the saliva suspension. This result was supported by the previous research, which also found *Candida* spp. such as *C. albicans* aside from other species of microbes in saliva obtained from children (Xiao *et al.,* 2018). Saliva contains proteins such as mucins and statherins, which act as adhesion receptors used by the mannoproteins exist in the *Candida* spp. Imbalance of normal microbial communities was evident in the condition of decrease or complete absence of saliva in a patient with xerostomia (Salerno *et al., 2011*). Saliva contains numerous, different proteins and peptides with different molecular mass. The proteomic study revealed over 1050 diverse kinds of proteins in saliva. One of the constituents of the saliva, which is mucins consist of highly glycosylated particles (Silletti *et al.,* 2008). More than a few protein organizations such as MUC5B networks increase the complexity of the saliva structures.

Despite the presence of *Candida* spp. on several oral surfaces sampled, none were found on the palatal surface. Williams *et al.* (2011) mentioned that *Candid*a spp. must present in sufficient amount with an adequate rate of progress to permit their sustained attachment in order for them to colonize a mucosal surface. Thus, *Candida* biofilms are not typically seen on the palatal mucosa of healthy individuals. However, in cases of commensal carriage, colonization can be detected (Williams *et al.,* 2011).

Our data also showed that the majority of the isolated *Candida* spp. comprised of *C. Albicans.* Previous clinical studies revealed that compared to other *Candida* spp. such as *C. tropicalis, C. krusei,* and *C. glabrata, C. albicans* was frequently detected in high numbers in plaque-biofilms from toddlers with early childhood caries (ECC) (de Carvalho *et al.,* 2006; Raja *et al.,* 2010; Yang *et al.,* 2012; Koo *et al.,* 2014). Other *Candida* spp. such as *C. glabrata, C. krusei* and *C. tropicalis* were also detected but not as frequent or as abundant as *C. albicans* (de Carvalho *et al.,* 2006). Harriott and his colleagues also pointed out the capability of *C. Albicans* as the most dominant fungal pathogen that can cause superficial and systemic infections (Harriott *et al.,* 2011).

Our results have also shown that *C. albicans* attached directly to the surfaces of the restorative materials. Furthermore, *C. albicans* biofilm was also observed regardless of the type of nano-composite used for both lab and clinical strains. Besides, dental restoration materials have also been shown to induce biofilm formation. The biofilm accumulation of *C. albicans* on nano-composites may cause material surface deterioration, which will further help in the progression of the biofilm formation of different strains of *C. albicans*. *C. albicans* biofilm was observed to adhere firmly onto the nano-composite beads suggested that none of the nano-composites exhibited antifungal properties. These findings were similar to the previous study which indicated the

ability of *C. Albicans* to adhere on various abiotic surfaces including prosthesis, denture base, relining materials and some dental restorative materials (Segal *et al.,* 1988, Waltimo *et al.,* 1999; Maza *et al.,* 2002; Pereira *et al., 2007;* Lawaf & Azizi, 2009; Belduz *et al.,* 2017). *C. albicans* has been reported to form biofilm on various oral surfaces including prosthesis that can lead to oral pathogenesis including oral candidiasis (Akdeniz *et al.,* 2002; Blankenship *et al.,* 2006; Nejad *et al.,* 2013; Sida *et al.,* 2016). The present study showed a significant difference of cell adhesion on the same nano-composite between lab strain *C. albicans* ATCC 32354 and caries isolate *C. albicans* HNFC2, thus supported the hypothesis of the present study that caries isolate forms more biofilms compared to the lab strain. The previous study has shown that caries-free children had no *C. albicans*. Meanwhile, 82% of children with caries teeth presented *C. albicans* inside their oral cavity (unpublished data). Furthermore, caries prevalence has been reported to be correlated with the presence of *C. Albicans,* especially in children, adolescents and young adults (Klinke *et al.,* 2011). Furthermore, the acidic environment can also contribute to the growth of *C. albicans* suggesting that children with caries may possess more *C. albicans* compared to children with a healthy oral condition (Thaweboon *et al.,* 2008).

C. albicans has been shown to adhere on all nano-composites with cB exhibited the least adhesion by caries isolate *C. albicans* HNFC2. In contrast, cC exhibited the most adhered by the yeast strain. These results supported the hypothesis of the present study which *C. albicans* form different cell number in biofilm grown on different nano-composite and that *C. albicans* biofilm formation is nano-composite surface dependent. Each type of dental restorative material and its specific chemistry and their configuration including matrix and fillers arrangement suggested contributing to the different number of *C. albicans* cells adhered on different nano-composites (Beldûz *et al.,* 2016). The adhesion ability of *C. albicans* on dental nano-composite resin materials seems to vary depending on the type of matrix of the nano-composite. Even though all nano-composites that were used in this study were nano-hybrid resin composite type, however, the specific composition has been shown to differ thus affecting the level of *C. albicans* adhesion on the surface of the beads (Table 1).

Table 1. Compositions of dental materials

Manufacturer	Type	Compositions
cA	a nano-hybrid composite with pre-polymerized fillers	• SphereTEC fillers • Non-agglomerated barium glass fillers • Ytterbium fluoride Eethacrylic polysiloxane nano-particles
cB	Bulk fill nano-hybrid composite	• Fluoro-alumino-silicate glass • Bis-GMA • UDMA Bis-MPEPP • TEGDMA • Reaction initiator
cC	a good blend of both nanotechnology and hybrid technology	• Strontium glass filler type and high filler loading • Fluorescent agent

Conclusion

Dental caries enhances the colonization of *Candida* spp. in the oral cavity of children and that that caries isolate forms more biofilm on nano-composite compared to the lab strain *C. albicans* thus supported the hypothesis of the study.

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