



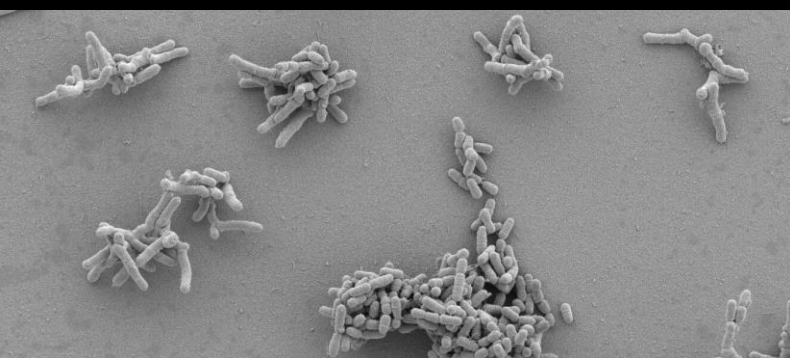
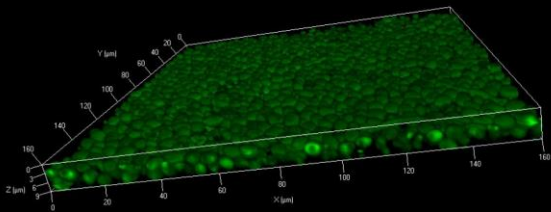
الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
يُونِيسْكَوِي اِسْلَامِي اِنْتَارَا اَبْحَثِيَا مَلِيسِيَا

Garden of Knowledge and Virtue

IJOHS

*International Journal
of Orofacial and
Health Sciences*

A scientific journal
published by Kulliyah of
Dentistry, IIUM, Malaysia



ISSN 2716-5434



9 772716 543003

VOL. 1 ISSUE 1

2020



International Journal of Orofacial and Health Sciences

Editorial Board

Chief Editor	: Professor Dr. Zainul Ahmad Rajion
Editor	: Dr. Salwana Supa'at
	: Dr. Basma Ezzat Mustafa Alahmad
	: Assoc. Prof. Dr. Khairani Idah Mokhtar@Makhtar
	: Assoc. Prof. Dr. Solachuddin J.A. Ichwan
	: Assoc. Prof. Dr. Muhannad Ali Kashmoola
	: Dr. Widya Lestari
	: Dr. Azlini Ismail
	: Dr. Mohd Hafiz Arzmi
	: Dr. Mohamad Shafiq Mohd Ibrahim

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.

Published by:

Kulliyah of Dentistry,
International Islamic University Malaysia (IIUM),
Indera Mahkota,
25200 Kuantan, Pahang, Malaysia.

Printed and distributed by:

Kulliyah of Dentistry,
International Islamic University Malaysia (IIUM),
Indera Mahkota,
25200 Kuantan, Pahang, Malaysia.

All rights reserved; No part of this publication maybe reproduced, stored in a retrieval system, or transmitted in any form, or by any means, electronic or otherwise, without prior permission of the publisher.

CONTENTS

EDITORIAL

Introduction to IJOHS 3

REVIEW ARTICLES

Genetics of malocclusion: A review 4

ORIGINAL ARTICLES

Potential antibacterial effects of flaxseed and *Nigella sativa* extracts on *Streptococcus pyogenes* 11

Dental treatment needs among patients undergoing screening at a university-based dental institution in Kuantan, Pahang, Malaysia 18

Analysis of the anti-cancer effect of ethyl-p-methoxycinnamate extracted cekur (*Kaempferia galanga*) on cancer cell lines with wild-type and null p53 28

Radiographic findings in panoramic radiographs of patients attending Kulliyah of Dentistry, IIUM 34

Isolation of *Candida* species in children and their biofilm-forming ability on nano-composite surfaces 40

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

Harina Akila Che Hussin¹, Nadiah Arshad¹, Siti Nor Humaira Nor Azemi¹, Raja Izzatun Nisa' Raja Sahrul Hishan¹, Wan Nur Fatimah Wan Mohd Kamaluddin², Nurul Alia Risma Rismayuddin², Mohd Haikal Mohd Halil³, Ahmad Faisal Ismail⁴, Fatimah Maryam Ali¹, Mohd Hafiz Arzmi^{1*}

¹Department of Fundamental Dental and Medical Sciences, Kulliyah of Dentistry, International Islamic University Malaysia, Kuantan, Pahang, Malaysia.

²Kulliyah of Nursing, International Islamic University Malaysia, Kuantan, Pahang, Malaysia.

³Department of Restorative Dentistry, Kulliyah of Dentistry, International Islamic University Malaysia, Kuantan, Pahang, Malaysia.

⁴Department of Paediatric Dentistry and Dental Public Health, Kulliyah of Dentistry, International Islamic University Malaysia, Kuantan, Pahang, Malaysia

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 °C. *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 7.7×10^3 cells mL⁻¹. In contrast, the nano-composite that contained bulk-filled nanohybrid (cC) was the most colonised with 14.3×10^3 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

*Corresponding Author:

Telephone: +6013-260 1445

Email: hafizarzmi@iium.edu.my

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. tropicalis*, *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 °C 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 (Biomérieux, 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 °C, 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 $\text{OD}_{620\text{nm}}$ 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 ultrasonicator (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.

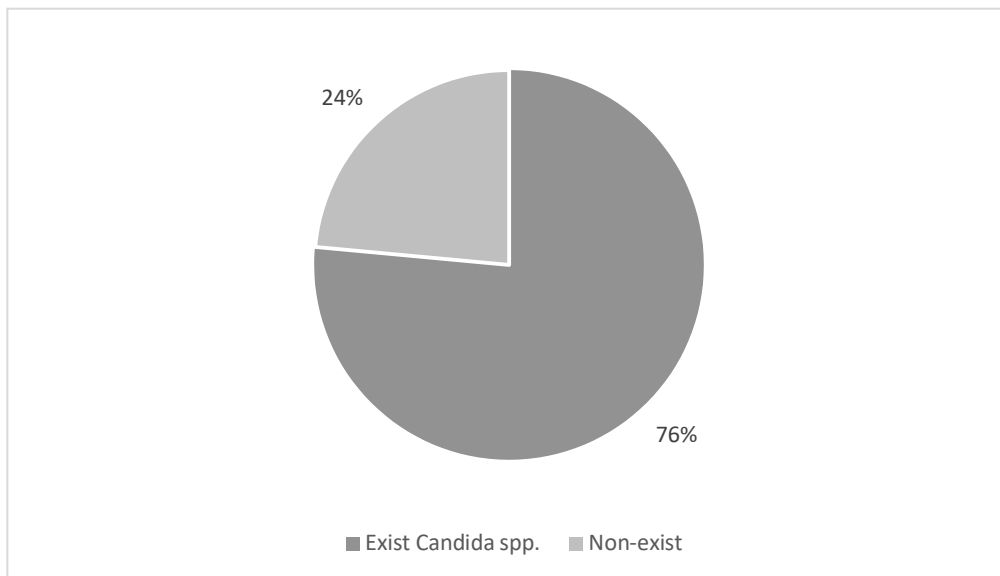


Figure 1. Percentage of caries children with *Candida* spp. (N=15).

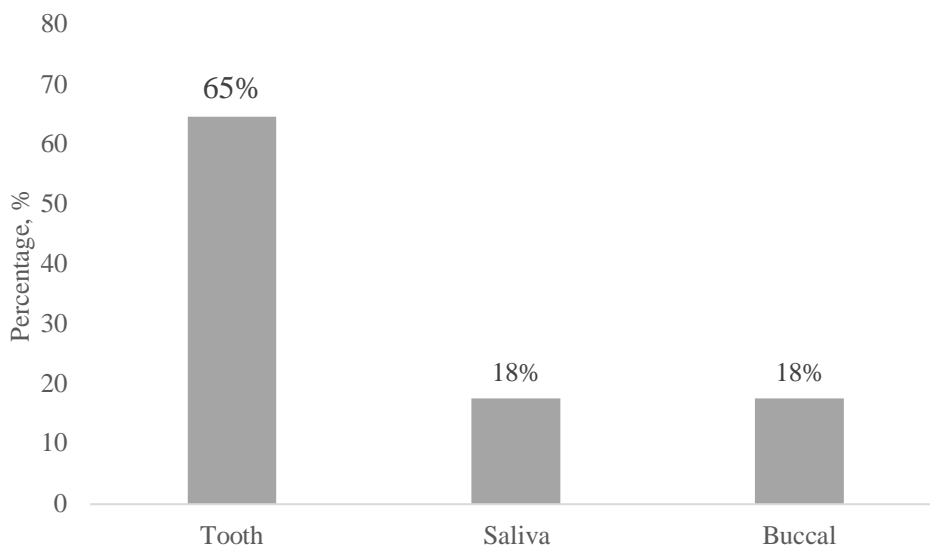


Figure 2. Percentage distribution of *Candida* spp. in children based on location; tooth, saliva and buccal mucosa of children with dental caries (N=15). No *Candida* spp. was isolated from palate.

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).

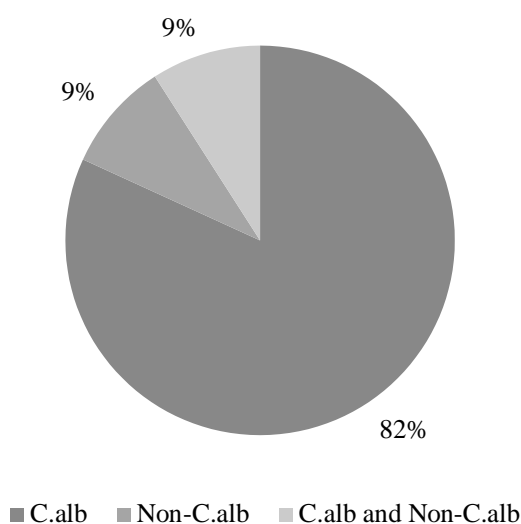


Figure 3. Percentage of caries children possess *C. albicans* only (C.alb), non-*C. albicans* only (Non-C.alb) or both *C. albicans* and non-*C. albicans* (C.alb and Non-C.alb) on the caries tooth surfaces (N=15).

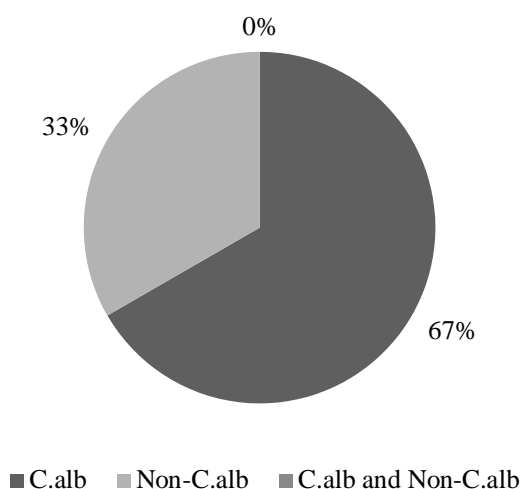


Figure 4. Percentage of caries children with *C. albicans* only (C.alb), non-*C. albicans* only (non-C.alb) or both *C. albicans* and non-*C. albicans* (C.alb and Non-C.alb) isolated from the buccal mucosa of caries patients (N=15).

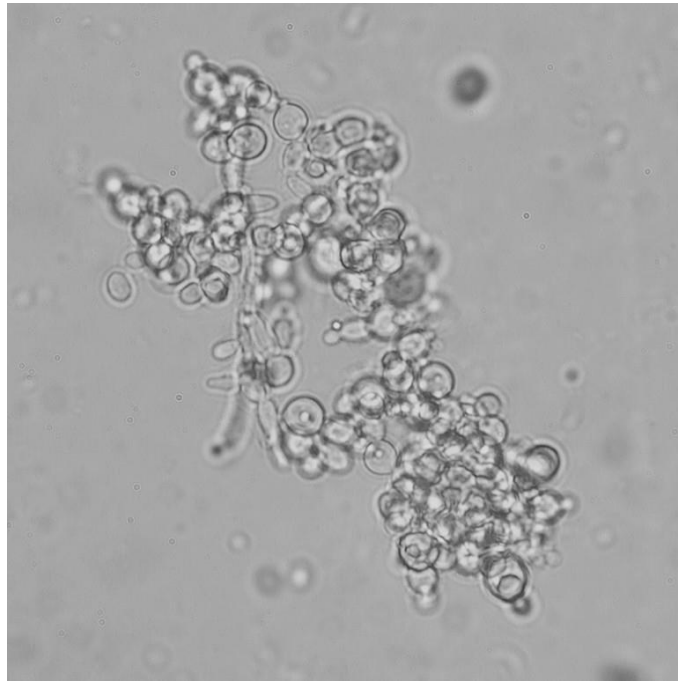


Figure 5. *C. albicans* isolated from saliva of caries children as observed under light microscope at 1000x magnification.

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 $14.30 \pm 4.98 \times 10^3$ cells mL⁻¹ which was significantly higher than *C. albicans* ATCC 32354 which exhibited $3.80 \pm 1.14 \times 10^3$ 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 (Zijngel *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

Candida 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 (Beldüz *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	<ul style="list-style-type: none"> • SphereTEC fillers • Non-agglomerated barium glass fillers • Ytterbium fluoride Eethacrylic polysiloxane nano-particles
cB	Bulk fill nano-hybrid composite	<ul style="list-style-type: none"> • Fluoro-alumino-silicate glass • Bis-GMA • UDMA Bis-MPEPP • TEGDMA • Reaction initiator
cC	a good blend of both nanotechnology and hybrid technology	<ul style="list-style-type: none"> • 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.

Acknowledgement

This project is funded by International Islamic University Malaysia (P-RIGS18-036-0036).

References

- Akdeniz B.G, Koparal E., Sen B.H., Ates M. & Denizci A.A. (2002) Prevalence of *Candida albicans* in oral cavities and root canals of children. *Journal of Dentistry for Children*, 69(3), 289-292.
- Alnuaimi A.D., O'Brien-Simpson N.M., Reynolds E.C., McCullough M.J. (2013) Clinical isolates and laboratory reference *Candida* species and strains and have varying abilities to form biofilms. *FEMS Yeast Research*, 13(7), 689-699.
- Arzmi M.H., Abdul Razak F., Yusoff Musa M., Harun W. & Aznita W.H. (2012) Effect of phenotypic switching on the biological properties and susceptibility to chlorhexidine in *Candida krusei* ATCC 14243. *FEMS Yeast Research*, 12(3), 351-358.
- Arzmi M., Alshwaimi E., Harun W., Razak F.A., Farina F., McCullough M. & Cirillo N. (2014) Gaining more insight into the determinants of *Candida* species pathogenicity in the oral cavity. *European Journal of Inflammation*, 12(2), 227-235.
- Arzmi M.H., Alnuaimi A.D., Dashper S., Cirillo N., Reynolds E.C., McCullough M. (2016) Polymicrobial biofilm formation by *Candida albicans*, *Actinomyces naeslundii*, and *Streptococcus mutans* is *Candida albicans* strain and medium dependent. *Medical Mycology*, 54(8), 856-864
- Arzmi M.H., Cirillo N., Lenzo J.C., Catmull D.V., O'Brien-Simpson N., Eric C. Reynolds E.C., Dashper S.G., McCullough M.J. (2018) mono-species and polymicrobial biofilms differentially regulate the phenotype of genotype-specific oral cancer cells. *Carcinogenesis*, 40(1), 184-193.
- Beldüz N., Kamburoğlu A., Y. Yılmaz, İ. Tosun, M. Beldüz, C. Kara. 2017. Evaluation of *Candida albicans* biofilm formation on various dental restorative material surfaces. *Materials*, 20(3), 431-445.
- Bowen, W. H., Burne, R. A., Wu, H., & Koo, H. (2017). Oral biofilms: pathogens, matrix, and polymicrobial interactions in microenvironments. *Trends in Microbiology*, 26(3). 229-242.
- Burgers R., Schneider-Brachert W., Rosentritt M., Handel G., Hahnel S. 2009. *Candida albicans* adhesion to composite resin materials. *Clinical Oral Investigations*, 13(3), 293-299.
- Chaffin W.L. (2008) *Candida albicans* cell wall proteins. *Microbiology & Molecular Biology Reviews*, 72(3), 495-544.
- Correa, M.B., Peres M.A., Peres K.G., Horta B.L., Barros A.D., Demarco FF. 2012. amalgam or composite resin? factors influencing the choice of restorative material. *Journal of Dentistry*, 40(9), 703-710.
- Cortelli, J. R., Aquino, D. R., Cortelli, S. C., Nobre Franco, G. C., Fernandes, C. B., Roman-Torres, C. V. G., & Costa, F. O. (2008). Detection of periodontal pathogens in oral mucous membranes of edentulous individuals. *Journal of Periodontology*, 79(10), 1962-1965.
- Cramer N.B., Stansbury J.W., Bowman C.N. (2011). Recent advances and developments in composite dental restorative materials." *Journal of Dental Research*, 90(4), 402-416.
- de Carvalho, F.G., Silva, D.S., Hebling, J., Spolidorio, L.C. and Spolidorio, D.M. (2006) Presence of mutans *Streptococci* and *Candida* spp. in dental plaque/dentine of carious teeth and early childhood caries. *Archives of Oral Biology*, 51(11), 1024–1028.
- Dewhirst F.E., Chen T., Izard J., Paster B.J., Tanner A.C.R., Yu W.H. & Wade W.G. (2010).The human oral microbiome. *Journal of Bacteriology*, 192(19), 5002-5017.
- Donovan, P. D., Gonzalez, G., Higgins, D. G., Butler, G., & Ito, K. (2018). Identification of fungi in shotgun metagenomics datasets. *PloS one*, 13(2): e0192898.
- Harriott, M. M., & Noverr, M. C. (2011). Importance of *Candida*-bacterial polymicrobial biofilms in disease. *TRENDS in Microbiology*, 19(11), 557-563.
- Hashim, Noor Azlina. 2017. "responsiveness to change of the malay-ecohis following treatment of early childhood caries under general anaesthesia." *PhD thesis, University of Malaya*.
- Haynes, K. (2001). Virulence in *Candida* species. *TRENDS in Microbiology*, 9(12), 591-596.
- Hofer U (2016) Fungal pathogenesis: *candida*'s toxic relationship with its host. *Nature Reviews Microbiology*, 14(5), 268-268.
- Kim, D., Sengupta, A., Niepa, T. H., Lee, B. H., Weljie, A., Freitas-Blanco, V. S., & Koo, H. (2017). *Candida albicans* stimulates *Streptococcus mutans* microcolony development via cross-kingdom biofilm-derived metabolites. *Scientific Reports*, 7: 41332.
- Kiyora, Yusuke & Toshimasa, N. (2000). Formation of dental plaque-like masses consisting of

- Candida krusei* and oral streptococci. *Ohu University Dental Journal*, 27, 191-198.
- Klinke, T., Guggenheim, B., Klimm, W. and Thurnheer, T. 2011. Dental caries in rats associated with *Candida albicans*. *Caries Research*, 45(2), 100–106.
- Kolenbrander P.E., Palmer R.J., Periasamy S et al. (2010). Oral multispecies biofilm development and the key role of cell–cell distance. *Nature Reviews Microbiology*, 8(7), 471–480.
- Koo, H., & Bowen, W. H. (2014). *Candida albicans* and *Streptococcus mutans*: A potential synergistic alliance to cause virulent tooth decay in children. *Future Microbiology*, 9(12), 1295-1297.
- Kragelund C., Reibel J. & Pedersen A.M.L. (2016) Oral candidiasis and the medically compromised patient. *Oral Infections and General Health*, Springer, 65-77.
- Kim J., Sudbery P. 2011. *Candida albicans*, A major human fungal pathogen. *Journal of Microbiology*, 49(2), 171-177.
- Lackhe, S. A., Joly, S., Daniels, K. & Soll, D. R. (2002). Phenotype switching and filamentation in *Candida glabrata*. *Microbiology*, 148(9), 2661-2674.106
- Lalla R.V., Latortue M.C., Hong C.H., et al. (2010). A Systematic review of oral fungal infections in patients receiving cancer therapy. *Support Care Cancer* 18(8): 985–92.
- Lawaf S., Azizi A. 2009. *Candida albicans* adherence to glass ionomer restorative dental materials. *Journal of Dental Research, Dental Clinics, Dental Prospects*, 3(2), 52-55.
- Ma, C., Chen, F., Zhang, Y., Sun, X., Tong, P., Si, Y., & Zheng, S. (2015). Comparison of oral microbial profiles between children with severe early childhood caries and caries-free children using the human oral microbe identification microarray. *PLoS One*, 10(3), e0122075.
- Marsh, P. D., Moter, A., & Devine, D. A. (2011). Dental plaque biofilms: communities, conflict and control. *Journal of Periodontology* 2000, 55(1), 16-35.
- Martin, D., Persat, F., Piens, M. A., & Picot, S. (2005). *Candida* species distribution in bloodstream cultures in Lyon, France, 1998–2001. *European Journal of Clinical Microbiology & Infectious Disease*, 24(5), 329-333.
- Maza, J.L., Elguezabal, N., Prado, C., Ellacurí, J., Soler, I., and Ponto'n, J. (2002). *Candida albicans* Adherence to resin-composite restorative dental material: influence of whole human saliva. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology & Endodontology*, 94(5), 589-92.
- Nejad B.S., Rafiei A. & Moosanejad F. (2013) Prevalence of *Candida* species in the oral cavity of patients with periodontitis. *African Journal of Biotechnology*, 10(15), 2987-2990.
- Ng, K. P., Madasamy, M., Saw, T. L., Baki, A., He, J. & Soo-Hoo, T. S. (1999). *Candida* biotypes isolated from clinical specimens in Malaysia. *Mycopathologia*, 144(3),135-140.
- O'Donnell, L. E., Millhouse, E., Sherry, L., Kean, R., Malcolm, J., Nile, C. J., & Ramage, G. (2015). Polymicrobial *Candida* Biofilms: friends and foe in the oral cavity. *FEMS Yeast Research*, 15(7).
- Pereira T., Cury A.A., Cenci M.S. Rodrigues-Garcia R.C., (2007). "In vitro *Candida* colonization on acrylic resins and denture liners: influence of surface free energy, roughness, saliva, and adhering bacteria." *International Journal of Prosthodontics*, 20(3), 308-310.
- Pereira, G. H., Müller, P. R., Szeszs, M. W., Levin, A. S., & Melhem, M. S. (2010). Five-year evaluation of bloodstream yeast infections in a tertiary hospital: the predominance of non-*C. albicans* *Candida* Species. *Sabouraudia*, 48(6), 839-842.
- Raja, M., Hannan, A. and Ali, K. (2010) Association of oral candidal carriage with dental caries in children. *Caries Research*, 44(3), 272–276.
- Ramirez-Garcia A., Arteta B., Abad-Diaz-de-Cerio A., et al. (2013). *Candida albicans* increases tumor cell adhesion to endothelial cells in vitro: intraspecific differences and importance of the mannose receptor. *PLoS One*, 8(1), e53584.
- Richards, V. P., Alvarez, A. J., Luce, A. R., Bedenbaugh, M., Mitchell, M., Burne, R. A., & Nascimento, M. M. (2017). The microbiome of site-specific dental plaque of children with different caries status. *Infection & Immunity*, 85(8), e00106-e00117.
- Rybalkin, M. V., Filimonova, N. I., Strilets, O. P. & Strelnikov, L. S. (2014). The study of protective properties of associated antigens of *Candida albicans* and *Candida tropicalis*. *Journal of Chemical & Pharmaceutical Research*, 6(4), 954-957.
- Sachdeo, A., Haffajee, A. D., & Socransky, S. S. (2008). Biofilms in the edentulous oral cavity. *Journal of Prosthodontics*, 17(5), 348-356.
- Salerno, C., Pascale, M., Contaldo, M., Esposito, V., Busciolano, M., Milillo, L., Guida A, Petrucci M, Serpico R. (2011). *Candida*-Associated denture stomatitis. *Medicina Oral Patologia Oral y Cirugia Bucal*, 16(2), e139-e143.
- Samaranayake L.P., K. Cheung L. & Samaranayake Y.H. (2002) Candidiasis and other fungal diseases of the mouth. *Dermatologic Therapy*, 15(3), 251-269.
- Sida H., Shah P., Pethani J., Patel L. & Shah H. (2016) Study of biofilm formation as a virulence marker in *candida* species isolated from various clinical specimens.

- International Journal of Medical Science & Public Health* 2016, 5(5), 842-846.
- Silletti, E., Vingerhoeds, M. H., Van Aken, G. A., & Norde, W. (2008). Rheological behavior of food emulsions mixed with saliva: effect of oil content, salivary protein content, and saliva type. *Food Biophysics*, 3(3), 318-328.
- Segal E., Lehrman O., Dayan D. (1988). Adherence in vitro of various *Candida* Species to acrylic surfaces. *Oral Surgery Oral Medicine Oral Pathology*, 66(6), 670-673.
- Thaweboon S., Thaweboon B., Nakornchai S., Jitmaitree S. (2008). Salivary secretory IgA, pH, flow rates, *Mutans Streptococci* and *Candida* in children with rampant caries. *Southeast Asian Journal of Tropical Medicine & Public Health*, 39(5), 893-899.
- Sudbery, P. E. (2011). Growth of *Candida albicans* Hyphae. *Nature Reviews Microbiology*, 9(10), 737.
- Sztajer, H., Szafranski, S. P., Tomasch, J., Reck, M., Nimtz, M., Rohde, M., & Wagner-Döbler, I. (2014). Cross-feeding and interkingdom communication in dual-species Biofilms of *Streptococcus mutans* and *Candida albicans*. *ISME Journal*, 8(11), 2256.
- Waltimo T., Tanner J., Vallittu P., Haapasalo M. 1999. Adherence of *Candida albicans* to the surface of polymethylmethacrylate-E glass fiber composite used in dentures. *International Journal of Prosthodontics*, 12(1), 83-86.
- Warinner C., Rodrigues J.F.M., Vyas R., Trachsel C., Shved N., Grossmann J., Radini A., Hancock Y., Tito R.Y. & Fiddyment S. (2014) Pathogens and host immunity in the ancient human oral cavity. *Nature Genetics*, 46(4), 336-344.
- Williams, D. W., Kuriyama, T., Silva, S., Malic, S. & Lewis, M. A. O. (2011). *Candida* biofilms and oral Candidosis: treatment and prevention. *Periodontology 2000*, 55(1), 250-265.
- Williams, D., & Lewis, M. (2011). Pathogenesis and treatment of oral candidosis. *Journal of Oral Microbiology*, 3(1), 5771.
- Xiao, J., Grier, A., Faustoferri, R. C., Alzoubi, S., Gill, A. L., Feng, C., & Gill, S. R. (2018). Association between Oral *Candida* and bacteriome in children with severe ECC. *Journal of Dental Research*, 97(13), 1468-1476
- Yang, C. W., Barkham, T. M. S., Chan, F. Y., & Wang, Y. (2003). Prevalence of *Candida* Species, including *Candida dubliniensis*, in Singapore. *Journal of Clinical Microbiology*, 41(1), 472-474.
- Yang, X.Q., Zhang, Q., Lu, L.Y., Yang, R., Liu, Y. and Zou, J. (2012) Genotypic distribution of *Candida albicans* in dental biofilm of chinese children associated with severe early childhood caries. *Archives of Oral Biology*, 57(8), 1048–1053.
- Zijinge, V., van Leeuwen, M. B. M., Degener, J. E., Abbas, F., Thurnheer, T., Gmür, R., & Harmsen, H. J. (2010). Oral biofilm architecture on natural teeth. *PLoS One*, 5(2), e9321.
- Zunt, S. L. (2000). Oral candidiasis: diagnosis and treatment. *Journal of Practical Hygiene*, 9(5), 31-33.

Instruction to Authors

Submission

Complete manuscripts, written in English along with tables and illustrations should be submitted as softcopy to zainulrajion@iium.edu.my

Conditions

All manuscripts submitted for publication must be accompanied by a cover letter declaring originality of the work and certifies that the manuscript has not been published or submitted elsewhere, free from conflict of interest and conduct of research in accordance to ethical guidelines established for human subjects and animal welfare. In addition, a statement of agreement of all authors to transfer all the copyrights to the journal should also accompany the manuscript.

A copy of the certification form, which certifies that the manuscript has not been published or submitted before and all authors have contributed and are in agreement with the content of the manuscript, must be submitted to the journal's editorial office by uploading it as a PDF file.

Manuscripts submitted for publication are understood to be offered only to **IJOHS** and which have not been sent to other journals for consideration. There is no publication fee to submit or publish content in **IJOHS**.

Manuscript should be typed in **Arial, font size 11 with 1.5 spacing**.

The manuscript should be divided into the following sections with the sequence; Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Illustrations and Figure Legends must be incorporated near to the text they are cited

Title Page:

Title page should be arranged in the following order: (1) a concise and informative title not exceeding 80 characters; (2) authors' full names (without degrees and titles) and affiliation including city and country. Superscript numbers may be used to affiliate authors to different departments or institutions; and (3) a complete mailing address, telephone, fax and e-mail address of the corresponding author.

Abstract:

Abstract should be unstructured and should not exceed 250 words. It should be a self-standing summary of the work that may be republished by information retrieval services. A maximum of 5 keywords should be listed at the end of the abstract.

Introduction:

Introduction should briefly and clearly describe the background and objective(s) of the study. Avoid exhaustive review of the literature and include only relevant recent studies.

Materials and Methods:

This section should be described in sufficient details so that it is reproducible. It should include technical information such as the study design, and specific procedures. Sub-headings may be used to enhance the clarity or to categorize the methods. Established methods or procedures should be named and cited. New methods or modifications of old methods should be described with complete details. Authors are advised to use generic names and terms rather than commercial names. Statistical methods applied, software used must also be stated clearly and concisely.

Results:

This section should be presented in a logical sequence in text, tables and illustrations. Subheadings may be used

to enhance clarity or to call attention to the most significant findings. Data appearing in tables or figures may be summarized but not duplicated in the text. Tables and figures should be numbered in the order in which they are described and cited in the text. Example, For tables, examples are Table 1, Table 2 and for figures, examples are Figure 1, Figure 2.

Discussion:

This section should summarize, explain and interpret the results with a scientifically critical view of previously published works in the field. Avoid repetition of the data already presented in the result section.

Acknowledgements:

This section is to be given to those who have provided assistance to the project and sponsors who have given financial support and funds.

References:

This section should list all sources cited in the paper. The citations should be arranged in alphabetical order by the surname of the first author without numbering in the text. Cite article as e.g. (Ahmad, 2016) for a single author, (Chu & Kim, 2019) for two authors and (Karim *et al.*, 2013) for more than three authors.

Standard journal article

References to journals should provide the name(s) of the author(s), year, title of the paper and journal, volume and issue number, page numbers. Please provide full name of journal. If there are more than six authors, the first six authors should be listed and followed by *et al.* in the list of References.

Sudbery, P. E. (2011). Growth of *Candida albicans* Hyphae. *Nature Reviews Microbiology*, 9(10), 737.

Book

References to books/monographs should give the name(s) of the author(s), year, title of book, edition number, place of publication, publisher and page numbers.

Conn, E.E., Stumpf, P.K., Brueing, G., Doi, R.H. (1987). *Outlines of Biochemistry*, 3rd edn. New York: John Wiley & Sons, pp. 45–52.

Chapter in book

References to chapter in books/monographs should give the name(s) of the author(s), year, chapter title, editors, title of book, edition number, place of publication, publisher and page numbers.

Fejerskov O, Nyvad B, Kidd EAM (2003). Clinical and histological manifestations of dental caries. In: Fejerskov O, Kidd EAM (eds.), *Dental Caries – The Disease and Its Clinical Management*. London: Blackwell Munksgaard, pp. 71–97.

Internet resources

Authors are required to include as much electronic retrieval information as needed for others to locate the sources they have cited.

López-Jornet P (2006). Labial mucocele: a study of eighteen cases. *The Internet Journal of Dental Science*, 3(2). Retrieved 7 February 2007, from <http://www.ispub.com/ostia/index.php?xmlFilePath=journals/ijds/vol3n2/labial.xml>

Tables

Each table should have a suitable title with footnotes wherever necessary. Do not use vertical lines in the table.

Illustrations

All figures and illustrations could be original photographs, artwork or high-quality digital images (submitted as CMYK - 8 bits per channel in TIFF format). Images must be at least 600 by 450 pixels (proportional height) in size when in landscape orientation with a resolution of at least 300 pixels per inch. Graphs should be approximately 500 pixels wide so that all labeling can be read with data points clearly visible. Figures should be numbered

consecutively in the order in which they appear in the manuscript, using Arabic numerals. A list of figure legends must be included on a separate page following the illustrations. The legend should explain each figure in detail. All figures will be printed as black and white. Colour figures will only appear in the PDF file.

Page proofs

On acceptance of the manuscripts for publication, page proofs should be reviewed meticulously by the contributors. Changes made in proof should be limited to the correction of typographical errors. Proofs must be returned for publication with corrections and responses to queries on the date specified by the Editor.



الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
يُونَيْتِي سَلَامًا نَبَا رَجْسًا مِلْسَنَا

Garden of Knowledge and Virtue