frontiers in Dental Medicine

REVIEW
published: 22 March 2022
doi: 10.3389/fdmed.2022.849274

Candida albicans and Early Childhood Caries

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Early childhood caries (ECC) is a highly prevalent and costly chronic oral infectious disease in preschool children. *Candida albicans* has been frequently detected in children and has demonstrated cariogenic traits. However, since ECC is a multifactorial infectious disease with many predisposing non-microbial factors, it remains to be elucidated whether the presence and accumulation of *C. albicans* in ECC is merely a consequence of the adaptation of *C. albicans* to a cariogenic oral environment, or it plays an active role in the initiation and progression of dental caries. This review aims to summarize the current knowledge on *C. albicans* and the risk of ECC, with a focus on its synergistic relationship with the cariogenic pathogen *Streptococcus mutans*. We also highlight recent advances in the development of approaches to disrupt *C. albicans-S. mutans* cross-kingdom biofilms in ECC prevention and treatment. Longitudinal clinical studies, including interventional clinical trials targeting *C. albicans*, are necessary to ascertain if *C. albicans* indeed contributes in a significant manner to the initiation and progression of ECC. In addition, further work is needed to understand the influence of other bacteria and fungi of oral microbiota on *C. albicans-S. mutans* interactions in ECC.

Keywords: *C. albicans*, early childhood caries, *S. mutans*, dental biofilm, cross-kingdom interactions

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Pediatric Dentistry, a section of the journal Frontiers in Dental Medicine

Received: *05 January 2022*
Accepted: *28 February 2022*
Published: *22 March 2022*

Citation:
Menon LU, Scoffield JA, Jackson JG and Zhang P (2022) Candida albicans and Early Childhood Caries. Front. Dent. Med. 3:849274. doi: 10.3389/fdmed.2022.849274

INTRODUCTION

Early childhood caries (ECC), formerly referred to as nursing bottle tooth decay, remains a significant chronic childhood infectious disease in developing as well as industrialized countries (1). It is defined as "the presence of one or more decayed, missing (due to caries), or filled tooth surfaces in primary dentition in children under the age of six" (1). If a child younger than 3 years of age has any sign of smooth-surface caries; a child from ages three through five has one or more cavitated, missing, or filled smooth surfaces in primary maxillary anterior teeth; or a child from ages three through five has a decayed, missing or filled score larger than the child's age, the child is considered to have severe ECC (S-ECC) (1). ECC is a major public health problem, causing tooth pain and loss, masticatory dysfunction, poor nutritional status, disrupted growth and development, as well as impaired learning ability in children. It also constitutes a substantial economic burden, particularly for individuals from low socioeconomic backgrounds due to high treatment costs, emergency room visits, and hospitalizations (1, 2).

The oral microbiome plays a critical role in the etiology of dental caries (2). *Streptococcus mutans* and *Lactobacillus* species were consistently associated with the initiation and progression of ECC. However, latest development in molecular microbiology provide novel insights into the complexity of the oral microbiome and the association of other microbial species with the risk of developing ECC. In the last two decades, increasing evidence indicates that *Candida*, the only genus of fungi

demonstrated to reach a significant biomass in the oral cavity, thrives in lower oral pH and ECC (3). At the species level, many studies have shown that the prevalence and abundance of *Candida albicans* is positively associated with the incidence and severity of ECC, suggesting that *C. albicans* may also constitute a major etiological risk of ECC (2, 4). The present review aimed to provide an update of the current knowledge on the association of *C. albicans* with ECC, its individual virulence and synergistic interactions with *S. mutans*. Recent advances in the development of approaches to disrupt *C. albicans-S. mutans* cross-kingdom biofilms in ECC prevention and treatment were also summarized.

C. albicans AND ITS ASSOCIATION WITH ECC

C. albicans is a polymorphic yeast from the *Candidaceae* family and is a normal resident of the skin, vaginal tract, oral cavity, and gastrointestinal tract in most healthy humans (5). However, conversion of this microorganism into an opportunistic pathogen occurs when there is dysbiosis in the microbial community or alterations in host immunity, and as such, is considered a pathobiont. Multiple host and environmental factors may affect the growth of *C. albicans* in the oral cavity, such as host age, diet, geographic location, socioeconomic status, gender, immunosuppression, and antibiotic use (4). An overgrowth of *C. albicans* in the oral cavity usually causes mucosal infection and leads to oral candidiasis or thrush. Virulence factors involved in the pathogenesis of *C. albicans* infection include biofilm formation, evasion of host innate immunity, yeast to hyphae transition, and production of candidalysin (6).

Although commonly present in the oral cavity, it was reported previously that *C. albicans* could not colonize to the tooth surfaces effectively on its own, instead, it mainly adheres to oral mucosa and acrylic surfaces, causing mucosal infection (7). However, others showed that *C. albicans* is capable of adhering to enamel and dentine effectively, and that dental plaque biofilms from children with ECC frequently contain *C. albicans* (8). These findings have led to the postulation that *C. albicans* is involved in caries development. Although some pilot clinical studies with limited subjects did not observe significant differences in the prevalence of oral *C. albicans* between children with or without dental caries (9, 10), multiple lines of evidence have shown that the presence of *C. albicans* in saliva and dental plaque is strongly associated with dental caries, especially ECC. In this regard, a number of cross-sectional studies have revealed that the prevalence of oral *C. albicans* in ECC children is significantly higher than that in caries-free children (4, 11–14). In addition, several studies indicate that both the prevalence and carriage of oral *C. albicans* is positively correlated with the severity of ECC (12, 15–17). Furthermore, a higher *C. albicans* detection rate was noted in plaque samples collected close to carious lesions than those collected from sound tooth surfaces (18). Moreover, a 2018 systematic review based on fifteen cross-sectional studies (4) indicated that children with oral *C. albicans* have over five times greater risk of developing ECC than those children without oral *C. albicans* carriage, reinforcing the belief that oral *C. albicans* may be considered as a potential risk factor for ECC. In line with these reports, it was found that children with oral thrush in the first year of life are three times more prone to have ECC (19). Interestingly, on the other hand, it has also been suggested that decayed teeth that harbor *C. albicans* may act as a reservoir for recurrent oral and non-oral candidiasis (4), highlighting a bidirectional relationship between soft and hard tissue oral *Candida* infection.

Maternal transmission has been considered one of the factors that contribute to early detection of *Candida* spp. in children. Studies have shown that oral colonization of *C. albicans* in infants was positively correlated with mother's oral *C. albicans* carriage, and that more than 60% of children with S-ECC carry the same *C. albicans* strains as their mothers (15, 20). In addition, the mothers of S-ECC children have a significantly higher oral *C. albicans* detection rate than the mothers of caries-free children, suggesting that maternal carriage of oral *C. albicans* might be a risk prediction for children's *C. albicans* carriage and ECC development.

Based on the amplified DNA band lengths determined with a specific PCR primer designed to span a transposable intron region in the 25S rRNA gene, previous studies were able to classify clinical *C. albicans* strains into four to five genotypes, including genotypes A, B, C, D, and E (21, 22). It was found that genotypes A, B, and C are typically detected in the dental plaque of children, genotype D is found in the periodontal pocket of patients with periodontitis, while genotype E is rarely present in the oral cavity (13). Others found that genotype A is mostly predominant in plaque biofilms of children with S-ECC, followed by genotypes C and B (11). In addition, genotype A was predominantly associated with proximal lesions, whereas genotypes B and C were mainly isolated from occlusal cavities.

Interestingly, by comparing pooled plaque mycobiome of 40 children with ECC with the same number of caries-free children, a recent study showed that it was *C. dubliniensis*, but not *C. albicans*, that dominated the mycobiome of children with caries (23). Using a site-specific analysis of the mycobiome associated with ECC, another study showed a trend for decreased mycobiome diversity as caries severity increased and found that both *C. dubliniensis* and *C. albicans* were positively correlated with ECC, with *C. albicans* being only associated with severe disease, while *C. dubliniensis* increasing steadily as caries severity increased (24). *C. dubliniensis* is closed related to *C. albicans* but is less pathogenic in mucosal disease models (3). Further studies are needed to determine if *C. dubliniensis* plays a contributory role in caries development (3).

ACIDOGENICITY AND ACIDURICITY OF *C. albicans*

Ecological theory of dental caries states that caries lesion-associated biofilm have high amounts of acidogenic and aciduric microorganisms. *S. mutans*, *Lactobacilli* and *Candida* spp. are believed to be the major acid producing organisms associated with ECC. Although analysis of the number and biomass of microorganisms associated with dental caries revealed that *S.*

mutans dominates in number followed by *Lactobacilli* and *C. albicans*, the biomass of *C. albicans* is much larger than that of *Lactobacilli* and *S. mutans* (25–27). Clinical studies have shown that the presence of *C. albicans* in the oral cavity is associated with a highly acidogenic and aciduric bacterial community in S-ECC (11, 20). Despite being lower in abundance, *C. albicans* dissolved hydroxyapatite crystals at a 20-fold faster rate than *S. mutans* (26). Others have noted that when the biofilm pH fell below 5.5, acidification by *S. mutans* dropped substantially and stopped at pH around 4.2, whereas *Lactobacilli* and *C. albicans* continued to acidify even at pH 4 (11, 25). Additionally, the main organic acid produced by *C. albicans* is pyruvic acid, and pyruvic acid is more potent than lactic acid in decreasing the pH of an already intensely acidified environment (25). In terms of aciduricity, *C. albicans* is highly aciduric and can survive at a pH 4 or even 3 in the presence of glucose and sucrose (8, 11). The acid tolerance ability of *C. albicans* has been postulated to favor its high frequency in dentinal caries, the highly acidic part of a decayed tooth (25). However, others have reported that *C. albicans* does not invade carious human dentine (28). Therefore, the role of *C. albicans* in dentinal caries pathologies need to be further investigated.

Interestingly, different results have been reported related to the acidogenicity and aciduricity of *C. albicans* isolated from caries-free and caries-active children. It was reported that *Candida* is more acidogenic and aciduric in nature when isolated from children with ECC than those isolated from children without caries (11). In contrast, others showed that the yeast isolated from children without caries caused significant higher percentage of demineralization *in vitro* in the presence of sucrose, compared with those isolated from children with caries (29). It was speculated that *C. albicans* from children without caries may metabolize sucrose faster to adapt to a sucrose-rich environment, whereas *C. albicans* from individuals with caries should already accustom to an environment rich in sucrose. In addition, it was also reported that biofilms originating from children with and without caries have similar cariogenicity when subjected to the same cariogenic challenge (30).

Unlike *S. mutans*, *Candida* does not metabolize sucrose efficiently due to the lack of invertase activity, and grows at much higher rate when cultures in glucose or fructose than in sucrose (31). Glucose is an essential factor for *Candida* to produce high amount of acid. In a glucose limited environment, *C. albicans* is comparatively less acidogenic than *Lactobacilli* (25). In addition, it was suggested that carious activity of *C. albicans* may depend not only on the presence of fermentable carbohydrates, but also on the proportion of sucrose and glucose in the diet (32).

In addition to the acidogenicity and aciduricity, *C. albicans* produces multiple proteolytic enzymes such as proteases, hemolysins, phospholipases, collagenases (8, 33). These extracellular enzymes are particularly active in an acidic milieu, and may play a significant role in dentinal caries progression via destroying dentinal collagen, or assisting the yeast to penetrate deep into dentine through dentinal tubules (8). It has been shown that the activity of aspartyl proteinases (Saps) in dental plaque biofilm in the S-ECC group were significantly higher than those in caries-free group (33, 34). More studies are necessary to understand whether *C. albicans* from caries-free and caries-active children have identical or different virulence.

SYNERGISTIC INTERACTIONS BETWEEN *C. albicans* AND *S. mutans* IN ECC

A biofilm is a an assemblage of surface-associated microbial cells that is enclosed in a self-produced extracellular polymeric matrix (35). Microorganisms living within a biofilm have increased resistance to environmental stress, such as antibiotics and the host immune response. *Candida* mostly exists in a polymicrobial environment, and such a heterogeneous biofilm population is a crucial and clinically important element for the growth, proliferation and survival of *C. albicans*. It also enhances bacterial colonization and biofilm formation by interacting with environmental and host factors, which enhances biofilm virulence and drug protection/resistance (36). Recent studies suggest that *C. albicans* may act as an essential "keystone" component in oral biofilms (37).

S. mutans is one of the most predominant microbial pathogens associated with ECC. *C. albicans* is frequently co-isolated with *S. mutans* from the dental plaque biofilms. Studies have demonstrated that the abundance of salivary *S. mutans* in infants positively correlates with infants' *C. albicans* levels (38). In addition, the emergence of *S. mutans* is much higher in infants who have early colonization of oral *Candida* compared to those who are free of oral *Candida* (38). Similarly, the adherence of *S. mutans* to oral biofilm and tooth surfaces also increases with the prevalence of *Candida* species (38).

The association of *S. mutans* and *C. albicans* may result in highly cariogenic biofilms that are readily associated with ECC. Yeast, without co-existence with *S. mutans* showed weak ability to colonize smooth surfaces of teeth, and the average number of DMFT/DMFS in individuals with co-existence of *S. mutans* and *C. albicans* were higher than those infected with *S. mutans* alone (15). In addition, the total count of *C. albicans* and *S. mutans* in the supragingival dental plaque of children with ECC increases with an increase in the percentage of active carious lesions and the severity of dental caries (39). Furthermore, co-existence of *C. albicans* and *S. mutans* in saliva and dental plaque is also strongly associated with caries recurrence in children (40).

In vitro studies have illustrated that the *C. albicans-S. mutans* co-species biofilms present greater 3D complexity, and are more resistant to stress conditions (41). The co-species biofilms not only exhibit protection against antibacterial drugs, but also protect yeast cells from antifungal agents (42). Saliva contains a wide variety of antimicrobial peptides (AMPs) that resists *S. mutans*. Thus, in the presence of purified saliva, *S. mutans* fail to form mature biofilms (43). However, the co-species biofilms showed rapid maturation, maintained acidogenicity in saliva, and caused severe enamel demineralization *in vitro*, while *S. mutans* single species biofilms displayed poor development, failed to create an acidic environment, and caused minimal damage to the enamel surface (43, 44). In addition, the co-species biofilms also have a positive effect on the survival of *C. albicans*, increasing its biomass, thereby increasing the overall

biofilm formation. However, it is necessary to highlight that the synergistic interactions between *C. albicans* and *S. mutans* may be variable and tend to be dictated by environmental conditions and population density (45). Furthermore, other species of oral microbiota may also influence the synergistic interactions between *C. albicans* and *S. mutans* (46, 47).

Animal studies further showed that co-infection with *S. mutans* and *C. albicans* can cause more severe and extensive caries in rats exposed to a cariogenic diet than the rats infection either species alone (44). Others have shown that exposure to *C. albicans* alone significantly increases the advanced fissure lesions in rats than non-infected controls in the presence of 40% of sucrose or 40% glucose; however, co-infection with *C. albicans* and *S. mutans* did not increase the incidence of occlusal caries (32). Although these animal studies suggest that *C. albicans* could have a pathological role in caries development, the potential effect of indigenous microbiome members, either enriched or depleted by a cariogenic diet in dental plaque of the animal models, has not been considered (3). Recent studies have shown that a high-sucrose diet is associated with a significant reduction in indigenous enterococci in a murine candidiasis model (48).

In addition to *S. mutans*, studies have shown that *C. albicans* may also synergize with *Actinomyces* to increase the biomass and cariogenic virulence of the *C. albicans-Actinomyces* dual species biofilm (49). However, decreased levels of salivary/plaque *Actinomyces* were identified in children with S-ECC with increased abundance of *C. albicans* (20). How *C. albicans* influences the composition and diversity of oral biofilm and the role of multiple species biofilms on *C. albicans-S. mutans* interactions and caries development require further investigation.

MECHANISMS INVOLVED IN *C. albicans-S. mutans* SYNERGY

Various mechanisms are proposed to be associated with the synergistic effect between *S. mutans* and *C. albicans* in the pathogenesis of dental caries. One critical mechanism is the co-adhesion of *S. mutans* and *C. albicans* to tooth surfaces via glucan synthesis. One of the key virulence factor of *S. mutans* in dental caries is its ability to convert sucrose into a wide range of soluble and, especially, insoluble extracellular polysaccharides (EPS), namely glucan (7). The formation of glucan is catalyzed by the exoenzymes glucosyltransferases (Gtfs). EPS creates an acidic environment due to its diffusion-limiting barrier, and forms the foundation for dental biofilms. EPS and Gtfs have a pivotal role in the *S. mutans-C. albicans* partnership within dental plaque biofilms (36, 50). Microscopic in situ analysis of intact clinical biofilm samples from subjects with caries provides further evidence that the interactions between the bacteria and yeast is mediated by extracellular EPS (51). *C. albicans* has multiple Gtf binding sites on the cell wall. Mannans on the *C. albicans* surface have been identified as key binding sites for GtfB, and the presence of *S. mutans* significantly upregulates genes associated with *C. albicans* mannan production (52). Therefore, *C. albicans* utilizes Gtf to adhere to tooth surfaces indirectly via its interaction with *S. mutans*. In addition, *C. albicans* also upregulates *gtfB* gene expression by *S. mutans*, leading to an increase in EPS production (53). This matrix forms a scaffold for microbial adhesion and acts as a gradient by modulating chemical and nutrition diffusion. Additionally, the matrix blocks access of saliva to the interior of the biofilm and prevents acid within biofilm from diffusing outward, thereby resulting in acidification and demineralization of the teeth (7). Moreover, as yeast is highly acidogenic, acid production is even more enhanced in this cospecies biofilm.

Another mechanism involved in the synergistic interactions between *C. albicans* and *S. mutans* is metabolic communication/cross-feeding, wherein one organism uses metabolites secreted by another for nutrition. As described above, *C. albicans* significantly enhances *S. mutans* carbohydrate utilization and glucan biosynthesis in the mixed biofilm by upregulating Gtf expression (31). This modification is critical for *S. mutans* survival and proliferation. On the other hand, *S. mutans* can metabolize sucrose to release free glucose and fructose, allowing *C. albicans* to utilize the monosaccharide efficiently in the mixed biofilm. This cross-feeding from *S. mutans* therefore compensates the inefficiency of *C. albicans* to utilize sucrose, leading to an enhanced fungal growth and acid production under cariogenic conditions (31). The reduced environmental pH in turn favors *S. mutans* survival. In addition, lactate is one of the major metabolites detected in the *C. albicans* and *S. mutans* co-cultures, and it favors the growth of *C. albicans* by acting as a carbon donor thereby reducing oxygen tension (50). The reduced level of oxygen tension, in turn, favors the growth of *S. mutans*. Thus, *C. albicans* and *S. mutans* benefit mutually from a symbiotic bacterial-fungal sugar metabolism, leading to an enhanced biofilm virulence under cariogenic conditions.

Quorum sensing is also important in *S. mutans-C. albicans* interactions within the co-species biofilm (53, 54). *C. albicans*-derived farnesol, a quorum-sensing molecule, has a main role in the yeast morphological switching by inhibiting hyphae production (55). Farnesol and its derivatives/analogs usually exhibit anti-biofilm, anti-bacterial and fungicidal activity (55). However, farnesol at concentrations of 25–50 μM was found to enhance Gtf activity and *S. mutans* microcolony development (53). It was only when the farnesol production reached a higher concentration (>100 μM), inhibition of the growth of *S. mutans* occurred. The presence of *S. mutans* tends to control the level of farnesol production by *C. albicans,* which may contribute to hyphal formation typically seen in the co-species biofilm. In addition, farnesol can incorporate into *S. mutans* cell membrane due to its fatty acid-like structure (53, 56). These observations suggest that farnesol is an important modulator in resolving the potential antagonism between *S. mutans* and *C. albicans*, and that a well-controlled mechanism exists between *S. mutans* and *C. albicans* to maintain farnesol at levels that promote the symbiotic relationship in the cross-kingdom biofilm.

In addition to the above-mentioned mechanisms, *in vitro* studies found that *S. mutans*-derived AgI/II is also important

for incorporating *C. albicans* into the two-species biofilms and increasing acid production (57). Therefore, with these highly complex symbiotic communications between *S. mutans* and *C. albicans*, it is plausible that this cross-kingdom biofilm has an enhanced virulence under cariogenic conditions, resulting in increased risk of ECC. More studies are needed to better understand the molecular mechanisms that govern the cross-kingdom interactions between *C. albicans* and *S. mutans* in ECC, which may help define novel approaches to prevent and treat ECC.

APPROACHES TO DISRUPT *C. albicans-S. mutans* BIOFILM

Due to the association of the *C. albicans-S. mutans* biofilm with ECC, various approaches have been attempted to disrupt this cross-kingdom interactions. Numerous *in vitro* studies have tested the efficacy of using antimicrobial agents, especially plant extracts or plant-derived compounds to inhibit the cross-kingdom biofilm formation and virulence. Curcumin, a food-grade natural product extracted from the root of turmeric, was found to be able to downregulate Gtf and quorum sensing-related gene expression of *S. mutans*, reduce EPS production, and decrease biofilm biomass and viability in *C. albicans-S. mutans* dual-species biofilms (58). The expression of genes related to *C. albicans* adhesion and aggregation in biofilm, the agglutinin-like sequence (Als) family members *als1* and *als2,* was also suppressed after curcumin treatment. Polyphenol extracts from green tea or cranberries have also been shown to effectively inhibit the acidogenicity and metabolic activity of *C. albicans-S. mutans* biofilms, decrease EPS and microbial biovolumes, and disrupt biofilm structure (59, 60). Thymol, the major constituent of thyme essential oil, is another nature product that showed ability to diminish the *C. albicans-S. mutans* dual-species biofilm formation and virulence (61). In addition, thymol was found to be effective in diminishing *C. albicans-S. mutans* dual-species virulence in the invertebrate model *Galleria mellonella* (61). Other agents, such as chitosan nanoparticles (62), gymnemic acids (63), Rhamanus prinoides (gesho) stem extracts (64) and candy derived from *Melaleuca cajuputi* essential oil (65), have also been shown to be effective in the inhibition of *C. albicans-S. mutans* dual-species biofilm formation. However, the efficacy of these antimicrobial agents in inhibiting cariogenic biofilms and preventing ECC *in vivo* needs to be further verified.

Lactobacillus spp. are part of human microbiome and are natural competitors of *Candida* in the vaginal environment (66). This concept has prompted studies to probe the effect of *Lactobacilli* in preventing or treating ECC by targeting the *C. albicans-S. mutans* biofilm. Studies have shown that *L. salivarius* can inhibit the dual-species biofilm formed *in vitro* with clinical isolates from dental plaque of children with ECC (47). The addition of *L. salivarius* decreased biofilm biomass, *S. mutans* and *C. albicans* abundance, and fungal morphological transformation. It was also found that *L. plantarum* 108 culture supernatants can downregulate the expression of *S. mutans* Gtf genes, *C. albicans* hyphal specific genes, inhibited biofilm formation, and reduced the pre-formed biofilm *in vitro* (67). In addition, *L. plantarum* CCFM8724 was found to be capable of preventing and treating *C. albicans-S. mutans*-induced caries in a rat model, and it exhibited a better inhibitory effect than 0.02% chlorhexidine (68). A number of clinical trials have also shown the beneficial effect of using probiotic bacteria in caries reduction (69). These probiotic strains typically do not colonize the oral cavity permanently, either following early-in-life interventions or in individuals with a mature microbiota. In contrast, other clinical studies have shown a positive correlation between *C. albicans* and *Lactobacillus* spp. in ECC. Yeast and *Lactobacilli* have been co-isolated from advanced caries lesions, and the presence of *C. albicans* in dental plaque is associated with increased abundance of several *Lactobacillus* spp., as well as the risk of ECC (20). In addition, *L. casei* was shown to stimulate *C. albicans* hyphal growth *in vitro*, which in turn supported the coaggregation of *Lactobacilli* and biofilm development (70). Therefore, it is possible that the interactions between *Lactobacilli* and *Candida* may depend on the environment they cohabit. In addition, a strain-specific relationship may exist between *Lactobacilli* and *Candida*.

Another approach proposed is to use oral commensal *Streptococci* to disrupt the *S. mutans* and *C. albicans* synergy in biofilms. *S. parasanguinis*, a mitis group *Streptococcus* and one of the most abundant commensals in the oral cavity, inhibits the growth of *S. mutans in vitro* (71). This commensal *Streptococcus* can also disrupt *S. mutans-C. albicans* synergy in the three species biofilms *in vitro* by altering the global metabolic signature, impairing *S. mutans* GTF activity, and blocking *C. albicans* from binding glucan (46). However, it was shown that *S. parasanguinis* is overrepresented in the saliva from children experiencing recurring decay within 6 months after being treated for ECC (40). Others have also observed more abundant *S. parasanguinis* present in dental plaque from caries-active children compared to caries-free children (72). Further studies are needed to elucidate the role of *S. parasanguinis* in the pathogenesis of ECC.

Most recently, a binding mechanism-based non-microbicidal approach using mannan-degrading enzymes was evaluated for the possibility of disrupting *S. mutans-C. albicans* cross-kingdom biofilm interactions (73). The enzymes were found to efficiently degrade mannans on *C. albicans* cell wall surfaces, therefore impairing *S. mutans* Gtf-to-mannan binding mechanism in the biofilm, leading to weakened biofilm mass, population, mechanical stability, acidity, and significantly decreased human tooth enamel demineralization *in vitro*. No microbicidal effect and cytotoxic effect on gingival keratinocytes were noted, and the enzymes were stable in human saliva, suggesting a potential application of this targeting intervention on *C. albicans-S. mutans* interkingdom ligand-receptor binding interactions in preventing and/or treating ECC. Further understanding of the proteins/structures involved in the interkingdom biofilm interactions may reveal more potential targets against the pathogenic biofilm in ECC.

Additionally, the *C. albicans-S. mutans* biofilm builds up tenaciously on various dental restorative materials with higher numbers of *C. albicans* being found on rough restorative surfaces.

In addition, different components in different restorative materials may have different effects on biofilm formation. The presence of heavy metals in restorative materials exhibits antimicrobial properties, for example, amalgam tends to liberate ions that interfere with the microorganism's ability to adhere to the tooth surfaces and form biofilm *in vitro* and *in vivo* (74, 75). Similarly, *in vitro* studies showed that glass ionomer cements reduced biofilm formation by these organisms owing to their fluoride releasing property (75). Furthermore, biointeractive dental materials incorporated with antimicrobial and ion releasing/recharging formulations have been explored for the efficacy in interfering *C. albicans-S. mutans* biofilm formation. Incorporation of dimethylaminohexadecyl methacrylate (DMAHDM) and amorphous calcium phosphate nanoparticles (NACPs) into dental sealants showed promising effect on inhibiting *C. albicans-S. mutans* dual-species biofilm formation and preventing enamel mineral loss *in vitro* (76). Thus, using these selective materials may have beneficial effect in inhibiting *C. albicans-S. mutans* biofilm formation. More *in vivo* studies including animal studies, microscopic in situ evaluation of the biomass and metabolic state of intact clinical biofilm samples from subjects with ECC, and randomized clinical trials, are necessary to further explore and verify the effectiveness of different approaches in inhibiting *C. albicans-S. mutans* biofilm formation and in controlling ECC.

CONCLUSIONS AND PERSPECTIVES

There is increasingly evident from cross-sectional studies that the presence and accumulation of *C. albicans* in dental plaque biofilms is associated with the incidence and the severity of ECC, indicating that detection of an enriched level of *C. albicans* in saliva and dental plaques may serve as an indicator of the risk of ECC. Although animal studies have suggested that *C. albicans* may have a pathogenic role in caries development, it's still a matter of debate whether the presence and accumulation of *C. albicans* in ECC is merely a consequence of the adaptation of *C. albicans* to a cariogenic oral environment, or it plays an active role in the initiation and progression of dental caries. Longitudinal clinical studies, including interventional clinical trials targeting *C. albicans*, are necessary to ascertain if *C. albicans* indeed contributes in a significant manner to the initiation and progression of ECC. In addition, further work is needed to understand the influence of other bacteria and fungi of oral microbiota on *C. albicans-S. mutans* interactions in ECC.

AUTHOR CONTRIBUTIONS

The idea of this manuscript was conceived by PZ. LM wrote the manuscript. JS, JJ, and PZ critically reviewed the manuscript. All authors have read and approved the final manuscript.

REFERENCES

1. *Policy On Early Childhood Caries (ECC): Consequences, and Preventive Strategies.* The Reference Manual of Pediatric Dentistry Chicago, Ill: American Academy of Pediatric Dentistry (2021). p. 81–4. Available online at: https://www.aapd.org/research/oral-health-policies--recommendations/early-childhood-caries-classifications-consequences-and-preventive-strategies/.
2. Hajishengallis E, Parsaei Y, Klein MI, Koo H. Advances in the microbial etiology and pathogenesis of early childhood caries. *Mol Oral Microbiol.* (2017) 32:24–34. doi: 10.1111/omi.12152
3. Diaz PI, Dongari-Bagtzoglou A. Critcally appraising the significance of the oral mycobiome. *J Dent Res.* (2021) 100:133–40. doi: 10.1177/0022034520956975
4. Xiao J, Huang X, Alkhers N, Alzamil H, Alzoubi S, Wu TT, et al. *Candida albicans* and early childhood caries: a systematic review and meta-analysis. *Caries Res.* (2018) 52:102–12. doi: 10.1159/000481833
5. Nobile CJ, HJohanson AD. *Candida albicans* biofilms and human disease. *Annu Rev Microbiol.* (2016) 69:71–92. doi: 10.1146/annurev-micro-091014-104330
6. Pellon A, Nasab SDS, Moyes DL. New insights in *Candida albicans* innate immunity at the mucosal: toxins, epithelium, metabolism, and beyond. *Front Cell Infect Microbiol.* (2020) 10:81. doi: 10.3389/fcimb.2020.00081
7. Koo H, Bowen WH. *Candida albicans* and *Streptococcus mutans*: a potential synergistic alliance to cause virulent tooth decay in children. *Future Microbiol.* (2014) 9:1295–7. doi: 10.2217/fmb.14.92
8. Pereira D, Seneviratne CJ, Koga-Ito CY, Samaranayake LP. Is the oral fungal pathogen *Candida albicans* a cariogen? *Oral Dis.* (2018) 24:518–26. doi: 10.1111/odi.12691
9. Neves A, Lobo L, Pinto K, Pries E, Requejo M, Maia L, et al. Comparison between clinical aspects and salivary microbial profile of children with and without early childhood caries: a preliminary study. *J Clin Pediatr Dent.* (2015) 39:209–14. doi: 10.17796/1053-4628-39.3.209
10. Thomas A, Mhambrey S, Chokshi K, Chokshi A, Jana S, Thakur S, et al. Association of oral *Candidas albicans* with severe early childhood caries-a pilot study. *J Clin Diagn Res.* (2016) 10:ZC109–12. doi: 10.7860/JCDR/2016/19387.8357
11. Fakhruddin KS, Perera Samaranayake L, Egusa H, Ngo HC, Pesee S. Profuse diversity and acidogenicity of the *Candida*-biome of deep carious lesions of severe early childhood caries (S-ECC). *J Oral Microbiol.* (2021) 13:1964277. doi: 10.1080/20002297.2021.1964277
12. Moraga CPL, Martinez GAR, Puente CAL, Bozo ICM, Orellana BRU. Prevalence of *Candida albicans* and carriage of candida non-albicans in the saliva of preschool children, according to their caries status. *Acta Odontol Scand.* (2017) 75:30–5. doi: 10.1080/00016357.2016.1244560
13. Qiu R, Li W, Lin Y, Yu D, Zhao W. Genotypic diveristy and cariogenicity of *Candida albicans* from children with early childhood caries and caries-free children. *BMC Oral Health.* (2015) 15:144. doi: 10.1186/s12903-015-0134-3
14. de Carvalho FG, Silva DS, Hebling J, Spolidorio LC, Spolidorio DMP. Presence of mutans streptococci and Candida spp. in dental plaque/dentine of carious teeth and early childhood caries. *Arch Oral Biol.* (2006) 51:1024–8. doi: 10.1016/j.archoralbio.2006.06.001
15. Xiao J, Moon Y, Li L, Rustchenko E, Wakabayashi H, Zhao X, et al. *Candida albicans* carriage in children with severe early childhood caries (S-ECC) and maternal relatedness. *PLoS ONE.* (2016) 11:e0164242. doi: 10.1371/journal.pone.0164242
16. Wu N, Lin J, Wu L, Zhao J. Distribution of *Candida albicans* in the oral cavity of children aged 3-5 years of uygur and han nationality and their genotype in careis-active groups. *Genet Mol Res.* (2015) 14:748–57. doi: 10.4238/2015.January.30.18
17. Baraniya D, Chen T, Nahar A, Alakwaa F, Hill J, Tellez M, et al. Supragingivival mycobiome and inter-kingdom interactions in dental caries. *J Oral Microbiol.* (2020) 12:1729305. doi: 10.1080/20002297.2020.1729305
18. Yang XQ, Zhang Q, Lu LY, Yang R, Liu Y, Zou J. Genotypic distribution of *Candida albicans* in dental biofilm of Chinese associated with severe early childhood caries. *Arch Oral Biol.* (2012) 57:1048–53. doi: 10.1016/j.archoralbio.2012.05.012

19. Jean J, Goldberg S, Khare R, Bailey LC, Forrest CB, Hajishengallis E, et al. Retrospective analysis of candida-related conditions in infancy and early childhood caries. *Pediatr Dent.* (2018) 40:131–5.
20. Xiao J, Grier A, Faustoferri RC, Alzoubi S, Gill AL, Feng C, et al. Association between oral *Candida* and bacteriome in children with severe ECC. *J Dent Res.* (2018) 97:1468–76. doi: 10.1177/0022034518790941
21. McCullough MJ, Clemons KV, Stevens DA. Molecular and phenotypic characterization of genotypic *Candida albicans* subgroups and comaprison with *Candida dubliniensis* and *Candida stellatoida*. *J Clin Microbiol.* (1999) 37:417–21. doi: 10.1128/JCM.37.2.417-421.1999
22. Tamura M, Watanabe K, Mikami Y, Yazawa K, Nishimura K. Molecular characterization of new clincal isolates of *Candida albicans* and *C.* dubliniensis in Japan: analysis reveals a new gentype of C albicans with gorup I intron. *J Clin Microbiol.* (2001) 39:4309–15. doi: 10.1128/JCM.39.12.4309-4315.2001
23. de Jesus VC, Shikder R, Oryniak D, Mann K, Alamri A, Mittermuller B, et al. Sex-based diverse plaque microbiota in children with severe caries. *J Dent Res.* (2020) 99:703–12. doi: 10.1177/0022034520908595
24. O'Connell LM, Santos R, Springer G, Burne RA, Nascimento MM, Richards VP. Site-specific profiling of the dental mycobiome reveals strong taxonomic shifts during progression of early-childhood caries. *Appl Environ Microbiol.* (2020) 86:e02825–19. doi: 10.1128/AEM.02825-19
25. Klinke T, Kneist S, de Soet JJ, Kuhlisch E, Mauersberger S, Forster A, et al. Acid production by oral strains of *Candida albicans* and *Lactobacilli*. *Caries Res.* (2009) 43:83–91. doi: 10.1159/000204911
26. Nikawa H, Yamashiro H, Makihira S, Nishimura M, Egusa H, Furukawa M, et al. In vitro cariogenic potential of *Candida albicans*. *Mycoses.* (2003) 46:471–8. doi: 10.1046/j.0933-7407.2003.00888.x
27. Marchant S, Brailsford SR, Twomey AC, Roberts GJ, Beighton D. The predominant microflora of nursing caries lesions. *Caries Res.* (2001) 35:397–406. doi: 10.1159/000047482
28. Maijala M, Rautemaa R, Jarvensivu A, Richardson M, Salo T, Tjaderhane L. *Candida albicans* does not invade carious human dentine. *Oral Dis.* (2007) 13:279–84. doi: 10.1111/j.1601-0825.2006.01279.x
29. Caroline de. Abreu Brandi T, Portela MB, Lima PM, Castro G, Maia LC, Fonseca-Goncalves A. Demineralizing potential of dental biofilm added with Candida albicans and Candida parapsilosis isolated from preschool children with and without caries. *Microb Pathog.* (2016) 100:51–5. doi: 10.1016/j.micpath.2016.09.003
30. Azevedo MS, van de Sande FH, Romano AR, Cenci MS. Microcosm biofilms originating from children with different caries experience have similar cariogenicity under successive sucrose challenges. *Caries Res.* (2011) 45:510–7. doi: 10.1159/000331210
31. Ellepola K, Truong T, Liu Y, Lin Q, Lim TK, Lee YM, et al. Multi-omics analyses reveal synergistic carbohydrate metabolism in *Streptococcus mutans-Candida albicans* mixed-species biofilms. *Infect Immun.* (2019) 87:e00339–19. doi: 10.1128/IAI.00339-19
32. Klinke T, Guggenheim B, Klimm W, Thurnheer T. Dental caries in rats associated with *Candida albicans*. *Caries Res.* (2011) 45:100–6. doi: 10.1159/000324809
33. Fakhruddin KS, Samaranayake LP, Egusa H, Ngo HC, Panduwawala C, Venkatachaalam T. *Candida* biome of severe early childhood caries (S-ECC) and its cariogenic virulence traits. *J Oral Microbiol.* (2020) 12:1724484. doi: 10.1080/20002297.2020.1724484
34. Li W, Yu D, Gao S, Lin J, Chen Z, Zhao W. Role of *Candida albicans*-secreted aspartyl proteinases (Saps) in severe early childhood caries. *Int J Mol Sci.* (2014) 15:10766–79. doi: 10.3390/ijms150610766
35. Donlan RM. Biofilms: Microbial life on surfaces. *Emerg Infect Dis.* (2002) 8:881–90. doi: 10.3201/eid0809.020063
36. Koo H, Andes DR, Krysan DJ. *Candida-Streptococcal* interactions in biofilm-associated oral diseases. *PLOS Patho.* (2018) 14:e1007342. doi: 10.1371/journal.ppat.1007342
37. Young T, Alshanta O, Kean R, Bradshaw D, Pratten J, Williams C, et al. *Candida albicans* as an essential "keystone" component within polymicrobial oral biofilm models. *Microorganisms.* (2020) 9:59. doi: 10.3390/microorganisms9010059
38. Alkhars N, Zeng Y, Alomeir N, Al Jallad N, Wu TT, Aboelmagd S, et al. Oral *Candida* predicts *Streptococcus mutans* emergence in underserved US infants. *J Dent Res*. (2021) 101:54–62. doi: 10.1177/00220345211012385
39. Sridhar S, Suprabha BS, Shenoy R, Suman E, Rao A. Association of *Streptococcus mutans*, *Candida albicans* and oral health practices with activity status of caries lesions among 5-year-old children with early childhood caries. *Oral Health Prev Dent*. (2020) 18:911–9. doi: 10.3290/j.ohpd.a45411
40. Garcia BA, Acosta NC, Tomar SL, Roesch LFW, Lemos JA, Mugayar LRF, et al. Association of *Candida albicans* and Cbp(+) *Streptococcus mutans* with early childhood caries recurrence. *Sci Rep.* (2021) 11:10802. doi: 10.1038/s41598-021-90198-3
41. Lobo CIV, Rinaldi TB, Christiano CMS, Leite LDS, Barbugli PA, Klein MI. Dual-species biofilms of *Streptococcus mutans* and *Candida albicans* exhibit more biomass and are mutually beneficial compared with sigle-species biofilms. *J Oral Microbiol.* (2019) 11:1581520. doi: 10.1080/20002297.2019.1581520
42. Kim D, Liu Y, Benhamou RI, Sanchez H, Simon-Soro A, Li Y, et al. Bacterial-derived exopolysaccharides enhance antifungal drug tolerance in a cross-kingdom oral biofilm. *ISME J.* (2018) 12:1427–42. doi: 10.1038/s41396-018-0113-1
43. Kim HE, Liu Y, Dhall A, Bawazir M, Koo H, Hwang G. Synergism of *Streptococcus mutans* and *Candida albicans* reinforces biofilm maturation and acidogenicity in saliva. *Front Cell Infect Microbiol.* (2021) 10:623980. doi: 10.3389/fcimb.2020.623980
44. Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai C, et al. Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms in vivo. *Infect Immun.* (2014) 82:1968–81. doi: 10.1128/IAI.00087-14
45. Xu H, Jenkinson HF, Dongari-Bagtzoglou A. Innocent until proven guilty: mechanisms and roles of *Streptococcus-Candida* interactions in oral health and disease. *Mol Oral Microbiol.* (2014) 29:99–116. doi: 10.1111/omi.12049
46. Huffines JT, Scoffield JA. Disruption of *Streptococcus mutans* and *Candida albicans* synergy by a commensal streptococcus. *Sci Rep.* (2020) 10:19661. doi: 10.1038/s41598-020-76744-5
47. Krzysciak W, Koscielniak D, Papiez M, Vyhouskaya P, Zagorska-Swiezy K, Kolodziej I, et al. Effect of a *Lactobacillus salivarius* probiotic on a double-species *Streptococcus mutans* and *Candida albicans* caries biofilm. *Nutrients*. (2017) 9:1242. doi: 10.3390/nu9111242
48. Souza JGS, Bertolini M, THompson A, Mansfield JM, Grassmann AA, Mass K, et al. Role of glucosyltransferase R in biofilm interactions between *Streptococcus oralis* and *Candida albicans*. *The ISME J.* (2020) 14:1207–22. doi: 10.1038/s41396-020-0608-4
49. Deng L, Li W, He Y, Wu J, Ren B, Zhou L. Cross-kindom interaction of *Candida albicans* and *Actinomyces viscosus* elevated cariogenic virulence. *Arch Oral Biol.* (2019) 100:106–12. doi: 10.1016/j.archoralbio.2019.02.008
50. Metwalli KH, Khan SA, Krom BP, Jabra-Rizk MA. *Streptococcus mutans*, *Candida albicans*, and the human mouth: a sticky situation. *PLoS Pathog.* (2013) 9:e1003616. doi: 10.1371/journal.ppat.1003616
51. Kim D, Koo H. Spatial design of polymicrobial oral biofilm in its native disease state. *J Dent Res.* (2020) 99:597–603. doi: 10.1177/0022034520909313
52. Hwang G, Liu Y, Kim D, Li Y, Krysan DJ, Koo H. *Candida albicans* mannans mediate *Streptococcus mutans* exoenzyme GtfB binding to modulate cross-kingdom biofilm development in vivo. *PLOS Patho.* (2017) 13:e1006407. doi: 10.1371/journal.ppat.1006407
53. Kim D, Sengupta A, Niepa TH, Lee BH, Weljie A, Freitas-Blanco VS, et al. *Candida albicans* stimulates *Streptococcus mutans* microcolony development via cross-kingdom biofilm-derived metabolites. *Sci Rep.* (2017) 7:41332. doi: 10.1038/srep41332
54. Sztajer H, Szafranski SP, Tomasch J, Reck M, Nimtz M, Rohde M, et al. Cross-feeding and interkingdom communication in dual-species biofilms of *Streptococcus mutans* and *Candida albicans*. *The ISME J.* (2014) 8:2256–71. doi: 10.1038/ismej.2014.73
55. Rodrigues CF, Cernakova L. Farnesol and tyrosol: secondary metabolites with a crucial quorum-sensing role in *candida* biofilm development. *Genes.* (2020) 11:444. doi: 10.3390/genes11040444
56. Jeon JG, Pandit S, Xiao J, Gregoire S, Falsetta ML, Klein MI, et al. Influences of trans-trans farnesol, a membrane-targeting sesquiterpenoid, on *Streptococcus mutans* physiology and survival within mixed-species oral biofilms. *Int J Oral Sci.* (2011) 3:98–106. doi: 10.4248/IJOS11038

57. Yang C, Scoffield J, Wu R, Deivanayagam C, Zou J, Wu H. Antigen I/II mediates interactions between *Streptococcus mutans* and *Candida albicans*. *Mol Oral Microbiol.* (2018) 33:283–91. doi: 10.1111/omi.12223
58. Li X, Yin L, Ramage G, Li B, Tao Y, Zhi Q, et al. Assessing the impact of curcumin on dual-species biofilms formed by *Streptococcus mutans* and *Candida albicans*. *Microbiologyopen.* (2019) 8:e937. doi: 10.1002/mbo3.937
59. Farkash Y, Feldman M, Ginsburg I, Steinberg D, Shalish M. Polyphenols Inhibit *Candida albicans* and *Streptococcus mutans* biofilm formation. *Dent J.* (2019) 7:42. doi: 10.3390/dj7020042
60. Philip N, Leishman SJ, Bandara H, Walsh LJ. Polyphenol-Rich Cranberry Extracts Modulate Virulence of *Streptococcus mutans-Candida albicans* biofilms implicated in the pathogenesis of early childhood caries. *Pediatr Dent.* (2019) 41:56–62. doi: 10.1016/j.archoralbio.2019.03.026
61. Priya A, Selvaraj A, Divya D, Karthik Raja R, Pandian SK. In vitro and in vivo anti-infective potential of thymol against early childhood caries causing dual species *Candida albicans* and *Streptococcus mutans*. *Front Pharmacol.* (2021) 12:760768. doi: 10.3389/fphar.2021.760768
62. Ikono R, Vibriani A, Wibowo I, Saputro KE, Muliawan W, Bachtiar BM, et al. Nanochitosan antimicrobial activity against *Streptococcus mutans* and *Candida albicans* dual-species biofilms. *BMC Res Notes.* (2019) 12:383. doi: 10.1186/s13104-019-4422-x
63. Veerapandian R, Vediyappan G. Gymnemic acids inhibit adhesive nanofibrillar mediated *Streptococcus gordonii-Candida albicans* mono-species and dual-species biofilms. *Front Microbiol.* (2019) 10:2328. doi: 10.3389/fmicb.2019.02328
64. Campbell M, Fathi R, Cheng SY, Ho A, Gilbert ES. Rhamnus prinoides (gesho) stem extract prevents co-culture biofilm formation by *Streptococcus mutans* and *Candida albicans*. *Lett Appl Microbiol.* (2020) 71:294–302. doi: 10.1111/lam.13307
65. Septiana S, Bachtiar BM, Yuliana ND, Wijaya CH. Cajuputs candy impairs *Candida albicans* and *Streptococcus mutans* mixed biofilm formation in vitro. *F1000Res*. (2019) 8:1923. doi: 10.12688/f1000research.20700.1
66. Zangl I, Pap I, Aspock C, Schuller C. The role of *Lactobacillus species* in the control of *Candida* via biotrophic interactions. *Microb Cell.* (2020) 7:1–14. doi: 10.15698/mic2020.01.702
67. Srivastava N, Ellepola K, Venkiteswaran N, Chai LYA, Ohshima T, Seneviratne CJ. *Lactobacillus Plantarum* 108 Inhibits *Streptococcus mutans* and *Candida albicans* mixed-species biofilm formation. *Antibiotics*. (2020) 9:478. doi: 10.3390/antibiotics9080478
68. Zhang Q, Qin S, Xu X, Zhao J, Zhang H, Liu Z, et al. Inhibitory Effect of *Lactobacillus plantarum* CCFM8724 towards *Streptococcus mutans*- and *Candida albicans*-induced caries in rats. *Oxid Med Cell Longev*. (2020) 2020:4345804. doi: 10.1155/2020/4345804
69. Hasslof P, Stecksen-Blicks C. Chapter 10: probiotic bacteria and dental caries. *Monogr Oral Sci.* (2020) 28:99–107. doi: 10.1159/000455377
70. Orsi CF, Sabia C, Ardizzoni A, Colombari B, Neglia RG, Peppoloni S, et al. Inhibitory effects of different *Lactobacilli* on *Candida albicans* hyphal formation and biofilm development. *J Biol Regul Homeost Agents*. (2014) 28:743–52.
71. Huang X, Browngardt CM, Jiang M, Ahn SJ, Burne RA, Nascimento MM. Diversity in antagonistic interactions between commensal oral *Streptococci* and *Streptococcus mutans*. *Caries Res.* (2018) 52:88–101. doi: 10.1159/000479091
72. Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, Griffen AL. Beyond *Streptococcus mutans*: dental caries onset linked to multiple species by 16S rRNA community analysis. *PLoS ONE*. (2012) 7:e47722. doi: 10.1371/journal.pone.0047722
73. Kim HE, Dhall A, Liu Y, Bawazir M, Koo H, Hwang G. Intervening in symbiotic cross-kingdom biofilm interactions: a binding mechanism-based nonmicrobicidal approach. *mBio*. (2021) 12. doi: 10.1128/mBio.00651-21
74. Netuschil L, Brecx M, Vohrer KG, Riethe P. Vital fluorescence to assess in vitro and in vivo the antibacterial effects of amalgams. *Acta Stomatol Belg.* (1996) 93:129–34.
75. Belduz N, Kamburoglu A, Yilmaz Y, Tosun I, Belduz M, Kara C. Evaluation of *Candida albicans* biofilm formation on various dental restorative material surfaces. *Niger J Clin Pract.* (2017) 20:355–60. doi: 10.4103/1119-3077.198388
76. Ibrahim MS, Balhaddad AA, Garcia IM, Hefni E, Collares FM, Martinho FC, et al. Tooth sealing formulation with bacteria-killing surface and on-demand ion release/recharge inhibits early childhood caries key pathogens. *J Biomed Mater Res B Appl Biomater.* (2020) 108:3217–27. doi: 10.1002/jbm.b.34659

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