



Fungi at the Scene of the Crime: Innocent Bystanders or Accomplices in Oral Infections?

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Abstract

Purpose of Review Over the last decade, microbiome studies have enhanced our knowledge and understanding of the polymicrobial nature of oral infections. Recently, profiling of the fungal microbiome has expanded our conventional understanding of oral ecology, revealing the critical importance of yeasts within this complex microbiome. This review aims to explore our current appreciation of interkingdom interactions in oral disease.

Recent Findings There is a growing evidence base of interactions and pathogenic synergy and antagonism with bacterial species within oral disease. Recent studies have helped to develop our knowledge of how *Candida albicans*, alongside bacteria such as *Porphyromonas gingivalis*, *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Lactobacillus* species, influence overall pathogenicity.

Summary Clinical and experimental evidence makes a compelling case for a role for *C. albicans* in a number of oral infections, though whether its role is an active accomplice or passive bystander remains to be determined.

Keywords *Candida* · Biofilm · Oral cavity · Polymicrobial · Interkingdom

Introduction

The oral ecosystem is a critical element in oral health, with dysbiosis perceived as the primary driver of disease phenotypes [1]. Recent studies have shown that ecobiological heterogeneity of the salivary ecosystem, its functionality, and its interaction with host-related biochemical salivary parameters are also important considerations in understanding disease processes in the oral cavity [2••]. However, a notable omission

from all these studies is the importance of pathogenic fungi expressed in terms of the mycobiome, and how these eukaryotes impact the progression of various oral diseases. Indeed, fungi are often neglected in oral microbiology due to their relative low quantitative contribution within diseased sites of the oral cavity. Nevertheless, consideration of their size may lead to a rethink, as conservative estimates for a yeast cell biovolume is approximately $70 \mu\text{m}^3$, whereas a bacterium is $0.5 \mu\text{m}^3$ [3]. This almost 150-fold difference in the occupancy

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of available space upon orally relevant surfaces for fungi suggests that it is at least a bystander, but whether innocent or not remains to be determined.

The oral cavity contains numerous different micro-environments, ranging from enamel, mucosa, periodontal pockets, acrylic and metal substrates, and dentine, that are inhabited by tens to hundreds of bacterial species [4]. Polymicrobial communities are able to form biofilms upon this vast variety of substrates [5•, 6, 7]. It is now generally well understood that dental plaque biofilms contain many resident species that modulate one another, their environment, and also the host response [8–10]. Despite the widely acknowledged presence of yeasts within the oral cavity, their active role and participation in oral diseases is generally perceived to be restricted to mucosal-related diseases. Yet, in excess of 100 fungal species have been reported to colonise the oral cavity and co-exist within complex biofilm populations within aggregates alongside resident bacterial microbiomes [11]. The emergence of these datasets has been the catalyst for a number of studies investigating the importance of fungi within oral biofilm infections. This has fuelled the concept of interkingdom communities, which continues to grow above and beyond our traditional viewpoint of bacterial-bacterial interactions [12–14]. Indeed, the advancement in sequencing technologies has facilitated the characterisation of the fungal oral microbiome [11, 15•, 16]. Within this review, the current evidence regarding fungal-bacterial interactions in the oral cavity and their clinical relevance will be evaluated, which are illustrated in Fig. 1.

Identifying the Usual Suspects and Beyond

The oral cavity contains one of the most diverse microbiomes of the human body, second only to that of the gastrointestinal tract [17]. The oral microbiome plays host to hundreds of eukaryotic and prokaryotic species. It is estimated that more than 600 species of bacteria [18] and 100 fungal species [16] can inhabit this “biome.” Sequencing of “hypervariable” regions, such as the 16S rRNA (v1–9), for identifying genera of bacteria within communities has become commonplace within oral microbiology [19, 20]. Within recent years, there has been a number of studies concerned with the compositional makeup of the oral microbiome, which varies dramatically between health and various diseases, as well as distinct ecological niches [4, 21, 22•]. The heterogeneity and diversity between niches observed within the oral microbiome is in part due to the numerous surfaces that exist within the oral cavity [4]. Microbiome studies in oral disease have demonstrated the existence of distinct microbial communities compared to health. In periodontitis microbiome studies, in contrast to other clinical host sites (e.g. gastrointestinal tract), an increased bacterial diversity and richness in disease has been reported compared to health [23, 24]. Additionally, these studies have

elucidated previously unappreciated species. Next-generation sequencing (NGS) technologies have revealed a greater complexity within the periodontal disease microbiome, well beyond the traditional “red complex” that is comprised of *Tannerella forsythia*, *Porphyromonas gingivalis*, and *Treponema denticola* [25]. Microbiome studies have shifted this dogma, showing the prevalence of many disease-associated genera and species, including but not limited to *Spirochetes*, *Filifactor*, and *Fusobacterium*, highlighting a more diverse disease community than previously considered [19, 26].

In contrast, limited oral fungal microbiome, or “mycobiome,” studies have been published and disseminated, despite their increased recognition as an important pathogen in oral disease. As recently as 2010, a study led the way in characterising the fungal component of the oral microbiota via amplification of the internal transcribed spacer (ITS). Unlike 16S sequences with fixed length amplicons, ITS sequences can produce variable sequence lengths, which make bioinformatic processing more challenging. The authors observed that the most common genera of fungi were *Cladosporium*, *Aureobasidium*, and *Saccharomycetales* [16], many of which were subsequently confirmed by Dupuy and colleagues [27]. Interestingly, amongst the observed operational taxonomic units (OTUs) were a number of genera that were not in consensus with the initial study, including *Saccharomyces* and a number of components, including the *Saccharomycetales* order that were not found in as high frequency as reported initially [16].

In more recent studies, comparisons between oral health and disease have been made. For instance, a study comparing the mycobiome of periodontal disease and healthy individuals reported similar levels of fungal species (over 100) compared to the first report [16]. However, in their cohort, it was observed that *Candida* and *Aspergillus* were the most frequent genera, being present in 100% of samples. Interestingly, there was no significant difference in the overall diversity of fungal taxa between periodontal disease and the healthy cohort, or the overall composition. An increase in abundance the *Candida* genus in periodontal disease compared to health was observed, although this was not found to be statistically significant [11]. Notably, this was a pilot study and was perhaps hampered by its limited cohort size. Future mycobiome studies with larger cohorts and more stringent classification of disease state and other metadata would provide a more comprehensive interpretation of the mycobiome in oral health.

With 16S now being well established, and ITS amplicon sequencing becoming more readily available, there is now the ability to perform co-occurrence studies [12, 15•]. Dual bacterium and mycobiome analysis of sites within oral cavity (endodontic) infections has been demonstrated by Persoon and colleagues (2017). This group demonstrated a co-occurrence of acidogenic bacteria in the presence of fungal

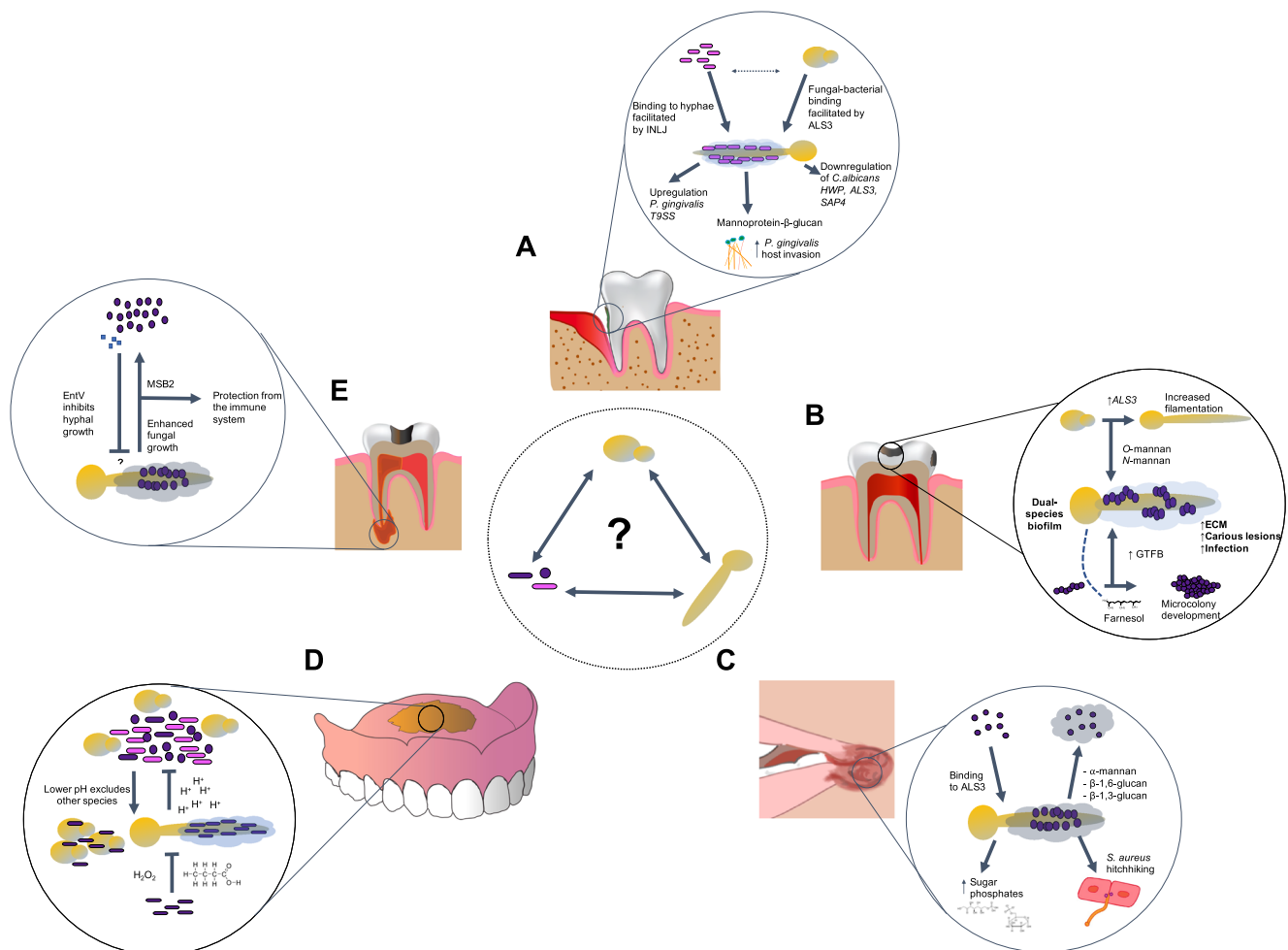


Fig. 1 Bi-directional interactions between fungal and bacterial species in oral infections. The diagram illustrates the sites of polymicrobial infection. **a** Periodontal pockets. **b** Caries. **c** Angular cheilitis. **d**

Denture stomatitis. **e** Endodontic infection. At each of these sites, differing levels of fungal biofilm formation, hyphal switching and interactions occur as well as interactions with bacterial oral pathogens

species, and an overall positive correlation of *C. albicans* with bacterial species. Negative correlations of bacterial species were observed with increased *C. dubliniensis*. These authors highlight the current limitations of these approaches, including increased difficulty in DNA extraction, PCR amplification due to length variation of ITS, and inconsistent fungal nomenclature. Despite these issues, as these types of studies grow and analytical pipelines become more developed, then the possibilities for understanding complex interkingdom interactions will become more fully realised.

Periodontal Disease: An Uncontrolled Gangland

Periodontal disease is a disease or group of diseases characterised by a complex host inflammatory response that is stimulated by microbial interactions from complex polymicrobial biofilm plaque. This subsequently leads to damage to the surrounding gingival tissues and supporting structures of the tooth. It varies in severity from reversible gingivitis to severe irreversible periodontitis, where the

periodontal ligaments and alveolar bone are destroyed [28]. Despite our ability to easily prevent this disease with oral hygiene measures, it remains one of the most prevalent diseases in the world, with nearly half of adults in the USA developing periodontitis [29]. Periodontal disease has a well-understood bacterial aetiology, with *P. gingivalis* considered the keystone pathogen within periodontitis [30] (Fig. 1a). Several other bacteria have also been implicated, including but not limited to *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum* [31, 32]. Given the diversity and numbers of other bacteria in and around the periodontal neighbourhood, then it is difficult to say with absolute certainty that particular periodontal pathogens are the sole cause of periodontitis. Indeed, the concept of oral ecotypes and pathotypes suggests that the sum of different varieties of periodontal pathogens and their functional capacity within the periodontal environment, despite functionally redundant metabolic processes, are more likely to drive synergised virulence leading to clinical disease [2••].

Several fungal species have been isolated from the periodontal pockets of patients with periodontitis, with *Candida albicans* generally being the most prevalent [33, 34]. Notably, the presence of *C. albicans* has also correlated with the severity of periodontitis [33]. Whether it is simply innocently colonising this environment and playing no active pathogenic role is unknown, yet mounting evidence suggests that it has the capacity to interact with periodontal pathogens and influence their behaviours. Specific bacteria frequently co-isolated with *C. albicans* in periodontal pockets include the anaerobes *F. nucleatum* and *P. gingivalis*. Remarkably, it has been shown that fungi are able to rapidly deplete oxygen within mixed species environments, which may explain why obligate anaerobes and yeasts are observed together [35]. For instance, *P. gingivalis* modulates and enhances the germ tube formation of *C. albicans* [36], whereas *F. nucleatum* has been demonstrated to inhibit *C. albicans* hyphal morphogenesis [37]. Other *P. gingivalis*-related studies however noted an antagonistic effect on the yeast-hyphal transition in *C. albicans*, with *P. gingivalis* notably downregulating hyphal related genes *ALS3*, *HWP1*, and *SAP4* [38, 39]. Most recently, it has been demonstrated that the attachment of *P. gingivalis* to *C. albicans* is facilitated by the virulence factor InlJ from the internalin protein family, which interacts with the *C. albicans* adhesin *ALS3* [40]. Additionally, co-adhesion specific interactions were observed, where adhesive interactions between these pathogens appear to induce the type 9 secretion system of *P. gingivalis*, a system characterised as having an increased community pathogenicity [41].

Further studies have shown that enhanced invasion of a gingival epithelial cell line and gingival fibroblasts by *P. gingivalis* is enhanced by pre-incubation with heat-killed cells and the mannoprotein- β -glucan complex from *C. albicans* [42]. The mechanism by which *C. albicans* facilitates this invasion is unclear, though the authors hypothesise that the recruitment of *C. albicans* cell wall components increases the recruitment of clathrin in epithelial cells. This is a mechanism by which *P. gingivalis* has been shown to invade host cells [14]. Further physical interactions have been investigated with *F. nucleatum*. This contact-dependent interaction is mediated by the FLO9 *C. albicans* cell wall protein and the RadD *F. nucleatum* membrane protein, which prohibits the morphological switching from yeast to hyphae [37]. Only recently has this interaction of FLO9 and RadD have been shown to be necessary for co-aggregation of *F. nucleatum* and *C. albicans* under both planktonic and biofilm conditions [43]. It has additionally been shown that this co-aggregation with *F. nucleatum* has a modulatory effect on the innate immune response. MCP-1 and TNF- α production are reduced during co-aggregation, which Bor et al. (2016) conclude has the potential to provide a mutualistic protection from macrophage killing and recruitment of monocytes resulting in an increased persistence [37].

Finally, *A. actinomycetemcomitans* has been associated with severe periodontitis [44]. In vitro, *A. actinomycetemcomitans* adheres to hyphae, although interactions with *C. albicans* show decreased fungal biofilm formation which is mediated by the luxS-synthesised autoinducer-2 (AI-2)-dependent mechanism. This was also mirrored using the 4,5-dihydroxy-2,3-pentanedione (DPD) synthetic molecule [45]. In *Streptococcus gordonii*, a ubiquitous oral commensal, it was shown that *C. albicans* hyphal formation was induced in a luxS-dependent manner, and that the addition of DPD had no effect on hyphal formation [13]. It is evident, as the authors concede, that more studies are necessary to elucidate the interactions between *C. albicans* and *A. actinomycetemcomitans*.

There is still many unknowns and conflicting evidence within the literature with regard to the importance of interkingdom relationships and their involvement in the progression of periodontitis. The literature does highlight some potentially important synergised pathogenicities of both the fungal and bacterial species, though further clinical studies are required to demonstrate functional dependency, perhaps starting with showing active presence of hyphae within periodontal pockets of patients with active disease.

Caries: Slimy Residents

Impacting 2.43 billion people globally, dental caries is the second most prevalent disease of humans, exceeded only by the common cold [46, 47]. Dental plaque biofilms are responsible for this cause of tooth decay, predominantly through the biofilms ability to ferment dietary sugars such as sucrose into the production of intolerable quantities of lactic acid that ultimately results in the demineralisation of the tooth surface. If left untreated, the dissolution of the enamel and dentine within the tooth results in the formation of a cavity within the tooth, providing an ideal niche for the cariogenic biofilm to thrive and proliferate to progress disease [48]. Moreover, a consequence of sugar metabolism is the production of soluble and insoluble glucan “slime” that protects and encases the acidogenic microbiome.

Historically, *Streptococcus mutans* has been regarded as the primary etiological agent of this disease; however, recent microbiome studies of dental plaque have highlighted that caries may indeed be polymicrobial in nature [49, 50] (Fig. 1b). An organism of interest in this context is *C. albicans*, a yeast frequently isolated from patients with caries. Studies have identified a higher incidence rate of *C. albicans* in children with caries compared to caries-free children [51, 52]. A recent systematic review by Xiao and co-workers (2018) indeed confirmed this co-isolation, stating that children with oral *C. albicans* colonisation, can be > 5 times more likely to develop early childhood caries (ECC) [53•]. The role for *C. albicans* colonisation as a risk factor for ECC should not be under-estimated and warrants further investigation.

Collectively, do these findings suggest incorporating antifungal therapy in the treatment of ECC? [54].

Mechanistically, there have been several interesting observations, with both physical and chemically mediated signals facilitating these interactions. Perhaps the most well-documented candidal adhesin with regard to interkingdom interactions is *ALS3*. Genetic manipulation of this adhesin has been shown to reduce bacterial binding to hyphal filaments of *C. albicans*, reducing the invasive capacity of this relationship [55]. The role of this phenotype has been shown in several bacterial pathogens including *Staphylococcus aureus*, *Streptococcus gordonii*, and *Streptococcus agalactiae* [56–58]. Interestingly, when the Δ als3 strain was grown with *S. mutans*, no reduction was observed in mixed species biofilm formation, suggestive of a different mechanism of interaction [59]. However, it has been shown that the presence of *S. mutans* in a mixed species biofilm can induce transcriptional expression of this key adhesin [60]. Instead of a protein-based interaction, Hwang and colleagues (2017) demonstrated a mannan-mediated binding, whereby the extracellular α -glucans of *S. mutans* interacted with a high affinity to the mannans of the *C. albicans* cell wall. Furthermore, they identified that mixed species biofilms formed with *C. albicans* strains defective in the outer mannan chain were unable to establish in vivo [59]. The exoenzyme glucosyltransferases (Gtfs) are predominantly responsible for the production of α -glucans from dietary sugar, and it is known that these extracellular polysaccharides are the key mediators of these dual-species cariogenic biofilms. In the absence of sucrose, *C. albicans* demonstrates a weak co-aggregation with *S. mutans*; however, secreted Gtfs from *S. mutans* establishes a co-aggregation between these two organisms, binding with high affinity [61, 62]. The increased ECM biovolume within the dual-species biofilm actively contributes to the pathogenicity of disease, with increased levels of infection and carious lesions observed in vivo [60].

Another molecule worthy of consideration within the context of this interaction is the quorum sensing compound farnesol. This molecule is a key regulator of *C. albicans* biofilm formation [63], and has also been shown to augment antimicrobial therapy and decrease bacterial biofilm formation [64, 65]. These effects are also observed with *S. mutans*, with high concentrations of the compound inhibiting biofilm formation [66, 67]. Yet, interestingly, when at lower concentrations (25–50 μ M) that are representative within these dual-species biofilms, then it can stimulate *S. mutans* microcolony development and enhance biofilm formation [67]. It is therefore thought that this is a key maintenance molecule with regard to regulating microbial fitness and biofilm formation in oral plaque [68]. If indeed candidal yeasts are important physical and metabolic members of cariogenic microbiomes, then this may explain why *S. mutans* centric therapeutic approaches have failed. Taking a holistic microbiological view

of this slimy caries environment may lead to combined bacterial and fungal therapeutic approaches.

Angular Cheilitis: Partners in Crime or Peer Pressure?

Angular cheilitis is a disease characterised by inflammation and crusting of one or more commonly both corners of the mouth [69]. It is a condition of a multifaceted aetiology, including poorly fitted dental appliances, minor trauma, and allergens that account for between 0.7 and 3.8% of oral mucosal lesions in the adult population [70]. Despite being recognised as a natural inhabitant of the oral microbiome, the role for *Staphylococcus aureus* in oral disease is underappreciated, though it is commonly isolated in angular cheilitis and associated mucositis (Fig. 1c). Whether it is simply an innocent bystander or actively contributes to pathogenesis is understudied, yet a growing body of evidence yields to the latter [71–74]. A retrospective 10-year analysis by McCormack and co-workers (2015) examined clinical data based on the isolation of *S. aureus* from the oral cavity. Interestingly, the two most common isolation sites were candidal infection and angular cheilitis, both suggestive of a polymicrobial infection with *C. albicans* [72]. Furthermore, these same two organisms have been co-isolated from the oral cavity, periodontal pockets, and from denture stomatitis [75, 76]. Given this frequent association, several investigations have begun to elucidate the consequences of these interactions with regard to pathogenicity and therapeutic implications. Using a proteomic approach, Peters and colleagues (2010) identified a number of upregulated proteins involved in stress response and metabolism that confers recalcitrance to host defence strategies [77]. Furthermore, a metabolomic-based analysis of the biofilm secretome of these dual-species biofilms identified increased levels of sugar phosphates in comparison to both single-species biofilms, collectively highlighting the complex nature of this relationship [78]. Perhaps the best studied interaction is the attachment of the two microbes. One such phenomena is “microbial hitchhiking,” where intricate studies by Schlecht and co-workers (2015) identified the ability of *S. aureus* upon binding with fungal hyphae to facilitate its own invasion of host tissues [55]. This mechanism, which was dependent on the fungal adhesin *ALS3*, resulted in recovery of the bacteria from kidney tissue, whereas no recovery was observed in single-species infection. This mechanism is likely responsible for the increased pathogenicity of co-infection observed in vivo [79, 80]. Synergised recalcitrance to antimicrobials has also been observed in several studies [80, 81, 82••]. Pioneer studies from Harriot and Noverr (2009) identified a biofilm-mediated resistance against vancomycin within the dual-species biofilm [81]. More recently, the precise mechanism of this resistance has been elucidated, with a suggestive “barrier mechanism,” whereby *S. aureus* coats itself in secreted *C. albicans* matrix

molecules to impede drug penetration within the biofilm [82•]. Reciprocal resistance has also been observed against miconazole, a dual-active antifungal routinely used in angular cheilitis infections [80].

Given the multifaceted nature of this relationship and their frequent co-isolation from a variety of host niches, then their co-operative role within pathogenicity and disease severity should not go unrecognised. This relationship certainly appears to benefit *S. aureus*, and whether it coerces *C. albicans* by peer pressure to contribute to its success is debatable. We propose that *S. aureus* uses *C. albicans* as a physical scaffold, forming biofilms upon the 3-D hyphal biofilm network, an interaction we term mycofilms [80]. To date, it remains unclear how *Candida* benefits from the relationship, or whether it is just an unwitting accomplice.

Denture Stomatitis: Mixing in a Rough Neighbourhood

As the elderly population expands to a predicted 2 billion by 2050, the number of denture wearers will coincidentally rise. Currently, around 20% of the UK population wear removable dentures of some form, with 70% of UK adults older than 75 years old wearing dentures [83], with many of these individuals suffering from denture stomatitis (DS), an inflammation of the palate [84]. Poor oral hygiene is frequently observed within this patient group and several factors can impact the onset of DS such as salivary flow, denture cleanliness, age of denture, smoking, and diet [85]. Soft tissue inflammation below or above the denture, as a result of persistent exposure to microorganisms, is characteristic of DS [86]. Microbes frequently adhere to the denture surface and a biofilm quickly develops which can contain numerous species of bacteria and fungi. This is aided by the varied topographical landscape that promotes microbial retention within cracks and crevices of acrylic substrates [83]. Denture plaque microbiome studies by our group have identified a variety of oral pathogens, including cariogenic bacteria, such as the *Lactobacillus* species that were positively correlated with high levels of *Candida* [22•] (Fig. 1d).

Discovery of *C. albicans* and *Lactobacillus* species in denture plaques was unexpected, as these bacteria have previously displayed antagonism with *C. albicans* at other mucosal sites [87]. The interactions and mechanisms employed by *C. albicans* and *Lactobacilli* remain somewhat enigmatic. *Lactobacilli* species have demonstrated the ability to inhibit *C. albicans* growth via the release of hydrogen peroxide and fatty acids [88]. Previous to this, in an ex vivo experiment, *L. rhamnosus* and *L. reuteri* altered host responses by eliciting an increased inflammatory cytokine response in a *C. albicans* co-infection model [89]. Hypothetically, a pro-inflammatory response could exacerbate the inflammation of DS whilst ultimately assisting in the clearance of *C. albicans*. *Lactobacilli*

supernatants have been shown to considerably reduce the ability of *C. albicans* to form biofilms [90]. However, the supernatants were unable to significantly reduce the viability of mature biofilms compared to bacterial cell suspensions. It is likely that production of excreted metabolites such as hydrogen peroxide and short-chain fatty acids may interfere with initial adhesion, but direct bacterial-fungal interactions occur to disperse mature *C. albicans* biofilms. Patients with more severe DS are colonised with greater numbers of *C. albicans* and *Lactobacilli*, indicating the possibility that these organisms can detect changes in their environment and alter their behaviours appropriately to effectively colonise the oral cavity [91].

Although *Lactobacillus* species are the most commonly isolated bacteria from DS biofilms, other *Candida* species, namely *C. glabrata*, have also been detected [92]. Co-infection with *C. glabrata* results in upregulation of key virulence genes (*ALS3* and *HWPI*) in *C. albicans* [93]. This increased virulence, in return, complements the ability of *C. glabrata* to invade epithelial tissue [94]. Authors hypothesised that during penetration of tissues by *C. albicans*, *C. glabrata* is transported into host cells via forming aggregates on the hyphae, as has been shown with various bacteria.

As studies get closer to understanding the mechanisms of interactions within dual-species biofilms, this will pave the way to elucidate how interactions within multispecies denture plaque contribute to disease processes. Indeed, it has been shown in vitro that multispecies interkingdom interactions have been shown to synergise one another, with hyphae induced by *Streptococcus oralis*, and the overall biovolume of denture biofilms is further enhanced with accompanying *Actinomyces oris* [95]. Understanding the behaviours of multi-species biofilms in vivo will prove more useful in the management of DS, where rough surface topography plays an additional physical role in supporting detrimental interactions. Though, this can be mitigated by physically altering denture surfaces to prevent candida adhesion [96].

Endodontic Infections: Sinister Encounters in the Dark

Apical periodontitis is a pathological condition that arises from microbial biofilm infection of the intricate anatomy of the root canal system [97, 98]. It is a common condition in primary endodontic infections, and even more so in those that have been previously root canal treated. Significantly, it is a substantial cause of dental morbidity worldwide. Under normal circumstances, the root canal is a sterile environment and any microorganism that invades this space is regarded as a potential cause of disease. Infection of the pulp can range from easy-to-treat infections such as pulpitis to apical periodontitis [99]. Root canal infections are typically of biofilm aetiology, and the causative microorganisms of endodontitis are also found as commensal organisms in the oral cavity. Several

factors can lead to the onset of endodontic infections such as diet and direct trauma. These can create a gateway to the root canal that oral microorganisms can exploit. Previous studies have shown that endodontic infections are more complex than originally thought. Using NGS techniques, bacteria, fungi, and viruses have been detected within infected root canals [100]. Fungi have been isolated from up to 55% of root canal infections with *C. albicans* being isolated with the highest frequency [101]. During a 2-year study in a German hospital, patients colonised with *C. albicans* were found to be two times more likely to be colonised by *E. faecalis* [102]. The ability of *C. albicans* to function in both monomicrobial and polymicrobial communities suggests that it could play a role in the pathogenesis of endodontic infections (Fig. 1e).

E. faecalis, a Gram-positive bacterium, is one of the most commonly isolated bacteria from infected root canals [103]. It has been presented in a recent study that fungi and bacteria can exist within infected root canals in a polymicrobial community [104]. Based on existing knowledge of polymicrobial biofilm communities, these findings could complicate treatment regimens and provide a possible explanation for recurrent root canal infections. Mechanistically, *E. faecalis* integrates into *Candida* biofilms and reduces the overall biomass [105], and also been shown to negatively affect *C. albicans* hyphal formation [106]. The protein responsible was identified as the bacteriocin EntV; this secreted protein demonstrated antihyphal and antivirulence properties without antagonising fungal growth [107]. Co-infection of these organisms has been shown to attenuate *C. albicans* virulence in vivo, particularly with an initial administration of *E. faecalis* [108, 109]. Interestingly, this effect was also mimicked in a murine oral candidiasis model, whereby initial administration of heat-killed bacteria was shown to have a protective antifungal effect [110]. Together, these studies highlight a potential immunological priming of the host response to withstand fungal infection. In contrast, the *C. albicans* membrane protein Msb2 is able to bind and inactivate host defence proteins and antibiotics, such as daptomycin [111, 112]. Production of Msb2 could subsequently provide the same protection to *E. faecalis*, leading to long-term colonisation of the root canal. It has also been shown that dual-species biofilms of these two organisms upregulate expression of the *C. albicans* transcriptional regulator *WOR1*, to which controls the phenotypic switch from the white to opaque cell type, that has been shown to be less susceptible to the innate immune system [113, 114]. *C. albicans* has also demonstrated the ability to promote the growth of *E. faecalis* in the gastrointestinal tract [115, 116]. These same interactions could likely occur in the root canal, encouraging bacterial growth and preventing resolution of endodontic infection.

Conclusions

We have highlighted how some key oral diseases are influenced by fungi, which undoubtedly play an important accessory role to a variety of bacterial pathogens. Within the myriad of different microbial interactions that occurs in the oral microbiome, then synergistic and antagonistic dynamic interactions constantly occur, with the most dominant manifesting themselves in disease outcomes. Fungi, and in particular *C. albicans*, can physically, metabolically, and through the release of soluble molecules play an important participatory role in the oral diseases outlined above. It would be naive to think of it simply as an innocent bystander—“guilt by association”; though until it is taken seriously as a keystone oral pathogen/commensal, then we cannot label it as an accomplice either.

Compliance with Ethical Standards

Conflict of Interest Christopher Delaney, Ryan Kean, Bryn Short, Maria Tumelty, William McLean, Christopher J. Nile, and Gordon Ramage declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of major importance

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