MINIREVIEW

Polymicrobial Candida biofilms: friends and foe in the oral cavity

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One sentence summary: An examination of where and how Candida interacts with bacteria in the oral cavity

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ABSTRACT

The role of polymicrobial biofilm infections in medicine is becoming more apparent. Increasing number of microbiome studies and deep sequencing has enabled us to develop a greater understanding of how positive and negative microbial interactions influence disease outcomes. An environment where this is particularly pertinent is within the oral cavity, a rich and diverse ecosystem inhabited by both bacteria and yeasts, which collectively occupy and coexist within various niches as biofilm communities. Studies within this environment have however tended to be subject to extensive independent investigation, in the context of either polymicrobial bacterial communities or yeast biofilms, but rarely both together. It is clear however that they are not mutually exclusive. Therefore, this review aims to explore the influence of candidal populations on the composition of these complex aggregates and biofilm communities, to investigate their mechanistic interactions to understand how these impact clinical outcomes, and determine whether we can translate how this knowledge can be used to improve patient management.

Keywords: Candida; biofilm; polymicrobial

INTRODUCTION

Candida biofilms, in particular Candida albicans, are an important healthcare issue due to ineffective clinical management strategies. Over the past 20 years we have learned a great deal about their clinical importance, including the mechanisms used by members of the genus to form biofilms and resist the challenge of host and antimicrobial molecules (Nett 2014; Ramage, Robertson and Williams 2014). However, as our levels of knowledge have increased, in part through the development of more sophisticated technologies, there has been a growing awareness that Candida rarely exist within a monospecies environment, and that heterogeneous biofilm populations consisting of aggregates of other fungi and bacteria (Gram positive and Gram negative) are in fact a highly prevalent and clinically important entity (Fig. 1).

One location within the body where Candida species are readily isolated is within the oral cavity. Traditionally oral microbiologists have invested significant time and effort unravelling the importance of specific bacterial–bacterial interactions, while investigations of polymicrobial interactions have not received the same level of attention. This has led to a disparity of fundamental knowledge on the significance of candidal–bacterial interactions within the oral environment. The clinical implications of these polymicrobial biofilm interactions primarily relate to recalcitrance to antimicrobial treatment strategies. Moreover, there is growing evidence from the
literature that polymicrobial interactions may synergize the pathogenic potential of one or other microorganism (Stacy et al. 2014). This only serves to highlight the importance of a dual approach to microbial analysis, where mycological and bacteriological analysis can have an equal contribution through interdisciplinary collaboration (Holmes, Cannon and Jenkinson 1995).

This review aims to critically evaluate the available evidence as a means of appraising the clinical importance of Candida biofilms in polymicrobial environments, using key oral diseases and groups of microorganisms to illustrate these points.

**Polymicrobial candidal interactions in the oral cavity**

Oral candidosis is one of the most well-defined fungal biofilm infections of both soft and hard tissue and is characterized by complex biofilms which interact with bacteria and the host (Dongari-Bagtzoglou et al. 2009; Raaijema and Ramage 2011). The oral cavity provides a key portal of entry within the human host, and is home to a rich and diverse microbial flora. Despite being bathed in saliva, an important innate defence mechanism containing numerous antimicrobial molecules, the oral cavity is a favourable habitat for both prokaryotes and eukaryotes. Within this, it is suggested that up to $10^6$ microbes per millilitre of saliva are present (Guo and Shi 2013). The oral cavity, therefore, acts as an important incubator for a complex ‘microbial soup’, in which yeasts such as Candida interact with one another and with a plethora of cultivatable and non-cultivatable bacterial species, primarily within biofilm communities. Advances in genome sequencing are only now beginning to shed light on the importance of Candida within these complex communities (Nobbs and Jenkinson 2015). Microbiome analysis of the saliva from elderly Dutch patients showed that an increased candidal load was associated with a dysbiotic bacterial flora that favoured the coexistence with oral streptococci to the exclusion of pathogenic anaerobic species (Kraneveld et al. 2012). Candida species have been isolated from a range of oral environments involving both soft and hard tissue of biological and non-biological origin, illustrating the adaptability of candidal yeasts (Fig. 2). The sites from which C. albicans has been isolated include periodontal pockets, root canals, orthodontic appliances, enamel, dentures and mucosal surfaces (Ramage et al. 2004; de Carvalho et al. 2006; Arslan et al. 2008; Dongari-Bagtzoglou et al. 2009; Sardi et al. 2010; Freitas et al. 2014). In order for candidal biofilms to flourish in these environments, moisture, nutrients, hyphal growth and the presence of commensal bacteria are all required which influence C. albicans architecture and virulence (Bertolini et al. 2015).

**Caries**

Dental caries is one of the most common diseases worldwide, impacting 2.43 billion (36% of the global population) (Vos et al. 2012). Largely influenced by diet caries has a multifactorial aetiology involving behavioural, environmental and immunological factors. Microbial dental plaque biofilms adherent to tooth
Figure 2. Oral sites of polymicrobial Candida biofilm diseases. The schematic diagram illustrates site within the oral cavity typically where Candida-bacterial polymicrobial biofilms are observed (clockwise from top position): caries, periodontitis, orthodontic, endodontic, angular cheilitis, denture stomatitis.

surfaces play a key role in the development of dental caries, through carbohydrate metabolism (predominantly sucrose) leading to production of large quantities of lactic acid, and ultimately the dissolution of tooth surfaces. Typically, caries has been associated primarily with Streptococcus mutans and Lactobacillus species (Loesche 1986; Badet and Thebaud 2008), although more recently, oral microbiome studies have highlighted the polymicrobial aetiology of carious lesions (Belda-Ferre et al. 2012; Simon-Soro, Guillen-Navarro and Mira 2014). Historically, candidal yeasts have been isolated in patients with caries (Krasse 1954; Koo and Bowen 2014), though the evidence for their direct role has yet been shown directly. There is now growing evidence that C. albicans actively participates in cariogenic biofilms, through synergistic interaction with St. mutans (Metwalli et al. 2013; Koo and Bowen 2014). Evidence of enhanced exopolymeric matrix production, facilitated by the increased surface area associated with hyphal networks, supports mixed biofilm growth of dense communities cemented to tooth enamel. Based on this and other studies, the interaction between candidal yeasts and streptococci is an important area requiring further extensive investigation.

Periodontal disease

Periodontal disease (PD) is a complex disease orchestrated by host-pathogen interactions. It affects almost 50% of the US population under 30 years old, and by the time they reach 65 years of age approximately 70% are affected (Eke et al. 2012). In its mild and reversible form (gingivitis), the gingival tissues are characterized by swelling, an inflamed gum line and
bleeding, whereas in its severe and irreversible form (periodontitis) there is destruction of the supporting periodontal ligaments and progressive bone resorption. While dysregulated inflammatory responses are pivotal with respect to periodontitis, the initial catalytic stimulus common to both forms of the disease comes from complex microbial biofilms. These initially establish themselves above the gum line (supragingival plaque), alter the microenvironment and drive a lower redox and pH, thus enabling capnophiles and anaerobes to colonize and produce subgingival plaque biofilms. The microbiology of supra- and subgingival plaque is extremely well characterized, with the influence of defined groupings of commensal and pathogenic species accurately mapped to clinical outcomes (Ximenes-Fyvie et al. 2000; Shi et al. 2015). With this historical focus on defined bacterial groupings defined by Socransky’s traffic light analogy (Socransky et al. 1998), there has been minimal interest with respect to the influence of candidal species (Holmstrup 1999). This is surprising given that Candida species have also been isolated from subgingival mixed biofilm consortia in patients with severe chronic periodontitis, where quantitatively high levels of C. albicans were shown to correlate with moderate and severe chronic periodontitis (Canabarro et al. 2012). There is, however, a lack of direct evidence for causality, although in diabetes patients the relationship between subgingival candidal colonization and periodontitis is more apparent (Sardi et al. 2012; Hammad, Darwazeh and Idrees 2013). This relationship maybe a consequence of metabolic requirements, with elevated blood sugar levels supporting the growth of Candida species. Further evidence for Candida’s involvement follows the use of oral contraceptives (OC), by which several studies have found an increased prevalence of Candida spp. carriage, as well as higher incidences of oral and vaginal candidiasis amongst OC users (Spinillo et al. 1995; Kazi, Saleem and Kazi 2012; Zakout, Salih and Ahmed 2012). Furthermore, the prevalence of severe periodontitis is higher amongst OC users, suggesting that the hormones lead to the development of a dysbiotic biofilm, enabling Candida yeast to colonize (Brusca et al. 2010). Irrespective of why Candida spp. are present in this environment, we do know that the subgingival environment represents a highly diverse microbial ecosystem comprised of a variety of commensals and pathogens, ranging from benign streptococcal species to virulent Porphyromonas gingivalis (Socransky and Haffajee 2005; Haffajee and Socransky 2006). Here, competition for nutrients, gases and space dictates biofilm structure, and it is likely that the larger Candida cells play a significant physical and chemical role.

Endodontic infection

Endodontitis can result from direct tooth trauma, carious lesions on the enamel surface, or from periodontal infection progressing to the root apex. It is characterized by an infection of the pulp within the dental root canal system and is the major aetiologic agent of apical periodontitis. The American Association of Endodontists estimate that over 15 million root canal treatments are performed annually in the USA, and these are primarily of an infectious origin. Endodontic infections are typically of biofilm aetiology and are associated with oral bacterial pathogens from up to 100 different bacterial genera (Siqueira and Rocs 2009), and by large from four key phyla (Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria), although Enterococcus faecalis is considered the primary aetiologic agent. Nonetheless, due consideration should be made to the method employed (culture versus non-culture) when assembling the snapshot of the dominant microbiota, as this heavily biases our perception of which species are important. In fact, this is a pertinent point to all oral diseases. Endodontic biofilms tend to reflect their origin, i.e. those from cariogenic lesion on occlusal surfaces may be more similar to supragingival plaque whereas those in periapical infection may reflect a predominantly anaerobic environment. There is increasing evidence for the involvement of Candida species in endodontic infections (Siqueira and Sen 2004). Its role as a dentophilic pathogen is highlighted through in vitro studies of dentine, where penetration of dentine tubules with C. albicans was demonstrated (Sen, Safavi and Spangberg 1997). Subsequent studies have confirmed the presence of C. albicans from clinical root canal specimens (Baumgartner, Watts and Xia 2000), with subsequent studies showing an association between C. albicans and E. faecalis (Peciuliene et al. 2001). In spite of this evidence of polymicrobiality, there are no studies describing the candidal–bacterial interactions in the root canal environment.

Denture stomatitis

Edentulousness is an irreversible clinical condition that can be described as an ultimate marker of oral disease burden and is often associated with socioeconomic factors (Jeganathan and Lin 1992; Cunha-Cruz, Hujoel and Nandanovsk 2007). Denture stomatitis (DS) refers to inflammation of the oral mucosa and pathological changes associated with denture surfaces adjacent to tissue (Jeganathan and Lin 1992). Approximately two thirds of individuals who wear removable complete dentures suffer from DS, though most individuals are asymptomatic (Gendreau and Loewy 2011). With 15 million dentures wearers in the UK, this is not an inconsequential disease (Coulthwaite and Verran 2007). Many factors influence its onset and severity, including salivary flow and denture cleanliness amongst others (Oksala 1990; Soysa, Samarayake and Ellepola 2004, 2006; Soysa and Ellepola 2005), although microbial factors remain one of the most important. Dentures support the growth of microbial biofilms (denture plaque) within tiny cracks and fissures. These polymicrobial biofilm communities dominate the denture surface, with up to 10^{11} microbes per milligram of denture plaque (Nikawa, Hamada and Yamamoto 1998), which take advantage of the varied topography associated with denture acrylics and resins (Ramage et al. 2004). Some of the bacterial species isolated include periodontal pathogens such as Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans and P. gingivalis (Sachdeo, Haffajee and Socransky 2008; Yasui et al. 2012), although caries-associated species such as Streptococcus and Lactobacillus species predominate (Teles et al. 2012), possibly through their ability to coaggregate with C. albicans hyphae (Bilhan et al. 2009; Ribeiro et al. 2012). Here they form biofilms analogous to that of the enamel surface through pioneer species, followed by coaggregation and maturation of complex polymicrobial biofilms (Fig. 3).

Unlike the oral diseases described above, DS is generally considered to be of yeast aetiology, with the literature disproportionately focused on Candida spp. (Coleman et al. 1997; Bagg et al. 2003; Redding et al. 2004; Li, Redding and Dongari-Bagtzoglou 2007). Candida albicans is the most frequently isolated yeast from the denture, but C. glabrata, C. dubliniensis, C. tropicalis, C. krusei and a range of other Candida species have been frequently isolated (Coco et al. 2008; Williams et al. 2011). Candida albicans accounts for the majority of the inflammatory pathology observed clinically (Salerno et al. 2011). It exists as a commensal in the oral cavity of 25–50% of the healthy population, and can become pathogenic under optimal conditions, such as when the immune response is compromised (Dagistan et al. 2009). This is
not surprising given its dimorphic capabilities, i.e. the ability to form hyphae and yeast interchangeably, a requisite of biofilm formation (Ramage et al. 2002b). The hyphal form has been more commonly isolated in DS sufferers and is assumed to be the more invasive form of the organism, with an enhanced ability to adhere to and colonize the prosthesis surface (Gendreau and Loewy 2011; Verran et al. 2014). Collectively, these polymicrobial biofilms actively release proteolytic and lipolytic enzymes that induce inflammation of the palatal surface (Marcos-Arias et al. 2011; Ramage et al. 2012), ultimately leading to DS. The scanning electron micrograph (SEM) in Fig. 4 illustrates that C. albicans interacts with bacteria on the surface of denture acrylic, with the associated confocal micrograph, showing bacteria coaggregating with C. albicans hyphae.

Angular cheilitis
Angular cheilitis is an inflammation of one, or more commonly both, corners of the mouth. It is a disease of multifactorial aetiology that includes anatomical issues, dry mouth, immunosuppression and the wearing of poor fitting dentures, amongst many others. Although not particularly common per se, this disease is of interest as it is often associated with the coisolation of Candida species with Staphylococcus aureus, microorganisms not unaccustomed to one another within the human host (Tawara, Honma and Naito 1996; Adam, Baillie and Douglas 2002; Baena-Monroy et al. 2005). Both species are leading pathogens in blood borne and systemic infections, a major cause of morbidity and mortality in hospitalized patients. These species are of significant interest because of the escalating development of antimicrobial resistance and their increasing involvement in chronic and systemic polymicrobial biofilm infections (Perlroth, Choi and Spellberg 2007), and have been shown to coaggregate together and exist within a dynamic and interactive state (Shirtliff, Peters and Jabra-Rizk 2009; Peters et al. 2012a, b). The relationship between these two has been described as mutualistic, synergistic and antagonistic, yet most of the evidence indicates synergy, as the majority of their interactions are associated with...
enhanced pathogenicity and disease severity (Peters and Noverr 2013; Schlecht et al. 2015).

Oropharyngeal and respiratory infection

As described, Candida is one of the main colonizers of the oral cavity and plays an important role in many oral diseases. However, there is thought to be a potential link between oral and pulmonary colonization of Candida, which could contribute toward respiratory infection. Studies have identified respiratory pathogens colonizing the oral cavity, as well as oral pathogens colonizing the lungs (El-Soh 2011; Bansal, Khatri and Taneja 2013; Vadiraj et al. 2013; Przybylowska et al. 2015). Amongst these, Candida has been found to be one of the most predominant pathogens in the lungs, particularly in those suffering from lung cancer and chronic pulmonary disease (Biswas et al. 2010; Laroumagne et al. 2011, 2013). Aspiration of oral material into the lungs is thought to be the primary entry route of oral pathogens. Therefore, given that the oral carriage rate of Candida is approximately 50% (Darwazeh, Hammad and Al-Jamaei 2010), and that roughly 45% of healthy individuals aspirate oropharyngeal contents into their lungs whilst sleeping, this puts a high number at risk of pulmonary colonization by Candida (Gleeson, Eggli and Maxwell 1997). Yet, despite the potential to cause infection, Candida colonization of the lungs is not necessarily detrimental, particularly when Pseudomonas aeruginosa is also isolated (Ader et al. 2011). Pseudomonas aeruginosa is frequently related with ventilator-associated pneumonia and cystic fibrosis, with C. albicans often coisolated. Many studies have investigated their interactions, yet have produced conflicting results with some identifying a synergistic relationship (Roux et al. 2009); however, the vast majority provide stronger evidence for an antagonistic relationship (Morales et al. 2010; Bandara et al. 2013).a Pseudomonas aeruginosa gains the upper hand in the majority of the time by preventing biofilm formation via killing of C. albicans hyphal filaments (Hogan and Kolter 2002; Hogan, Vik and Kolter 2004). Nonetheless, it recently has been shown in a murine model that lung injury caused by Ps. aeruginosa infection is alleviated if preceded by a short-term C. albicans colonization (Ader et al. 2011). This was due to C. albicans activation of innate lymphoid cells, which produced IL-22, providing protection against P. aeruginosa induced injury (Mear et al. 2014).

Candida polymicrobial biofilm formation is the predominant problem associated with voice box prosthesis (VP) (Talpaert et al. 2015). Silicone is the most commonly used material used for VP; however, silicone is a favourable material for microbial attachment and can very quickly become colonized (Busscher, Geertsema-Doornbusch and van der Mei 1997). Biofilm formation can lead to valve malfunctioning, causing seepage of oesophageal contents into the trachea, which could potentially cause aspiration pneumonia (van Weissenbruch et al. 1997a,b). Candida albicans is the most common yeast associated with VP colonization, though C. glabrata and C. tropicalis are also frequently isolated (Bauters et al. 2002). Streptococcus spp. and Lactobacillus spp. are the predominant bacterial species isolated (Neu et al. 1994); however, the majority of mature biofilms had Candida and lactobacilli as their primary components (Buijssen et al. 2007). The success of polymicrobial biofilms forming on VP is likely due to the location, which is difficult for host immune defences to access. For the most part, it is very unusual to find a biofilm from a VP that is not comprised of both fungal and bacterial components. Before Candida can colonize the VP, there is strong evidence that bacteria must be adhered first; thus, such fungal–bacteria interactions are critical for biofilm formation (Millsap et al. 2001). The more intricate details involved in these interactions require further investigation; however, what is clear is that disease resulting from microbial colonization of a VP is very much polymicrobial in nature.

Mechanisms of polymicrobial biofilm interaction

Staphylococcal interactions

The interaction between C. albicans and S. aureus has been associated with enhanced pathogenic behaviour, disease severity and morbidity (Nair et al. 2014). They form mixed polymicrobial biofilms in which S. aureus cells are found attached to C. albicans hyphal filaments (Peters et al. 2010; Lin et al. 2013) (Fig. 5). Their colocalization within biofilms is still unclear, as some describe them interspersed throughout the biofilm three-dimensional structure (Peters et al. 2010), whereas others describe them as only found attached within the upper layers of the biofilm (Harriott and Noverr 2009). This disparity could be explained by different experimental conditions (e.g. growth medium). The initial colonizing species plays a key role in dictating their interaction, as it has been shown that C. albicans biofilm formation was delayed when S. aureus colonized first, yet when added simultaneously biofilms formed rapidly (Lin et al. 2013). The reason for this inhibition is unknown; perhaps S. aureus secretes an inhibitory molecule preventing Candida adhesion.

Studies in S. epidermidis have shown that extracellular DNA (eDNA) release through autolysis is an important entity in supporting mixed biofilm growth (Pammi et al. 2013), and is also a critical feature for C. albicans biofilm extracellular matrix (ECM) integrity (Rajendran et al. 2014; Sapaaar et al. 2014). Therefore, it is not surprising that eDNA and the ECM from both C. albicans and S. aureus biofilms are both involved in affecting the action of antibacterial agents. In fact, it has been shown that S. aureus is protected against vancomycin treatment using concentrations as high as 1600 mg/mL within the mixed biofilm environment,
through *C. albicans* ECM preventing diffusion and access to *S. aureus* (Harriott and Noverr 2009). There are, however, other adaptive resistance mechanisms that play a role in this resistance phenotype (Harriott and Noverr 2010).

It has also been shown that *S. aureus* adhere to hyphal filaments by relying on the adhesion to the *C. albicans* agglutinin-like sequence 3 protein (Als3p) (Peters et al. 2010, 2012), though it is likely that other proteins are involved. Staphylococcus epidermidis has also been shown to preferentially adhere to hyphae, with forces between single bacterial and fungal germ tubes showing large adhesion forces (~5 nN) (Beaussart et al. 2013). Studies have shown that *S. aureus* binding to *C. albicans* hyphae was significantly stronger than all other bacteria tested, including *P. aeruginosa* (Peters et al. 2010). Interestingly, it was reported that none of the members of the ALS family of adhesins (ALS1–7 and ALS9), including ALS3, are involved in interspecies adhesion (Harriott and Noverr 2010). Thus, further insight is required before we can fully understand the mechanisms responsible for adherence, yet it is likely that this is a complex process in which a multitude of proteins are involved. Nevertheless, it is thought that adhesion to hyphae may assist *S. aureus* in penetrating into the host (Schlecht et al. 2015), a manner analogous to injection from a needle-stick injury. This has been demonstrated in mice studies, in which mixed infections with *C. albicans* als3A strains together with *S. aureus* were unable to invade the tongue, whereas the wild-type infections demonstrated coinfection (Peters et al. 2012). The ramifications of this enhanced invasive capacity have been shown historically to impact mortality, where synergism between the coinfected species administered intraperitoneally in a mouse model lead to 100% mortality, whereas monospecies infections caused no mortality whatever (Carlson 1983). Whether or not the relationship between the two organisms is physical or chemical remains to be determined, although there is evidence that growth-related synergy is an important factor in their cohabitation of microniches (Carlson and Johnson 1985). Indeed, the physical relationship between the organisms is important, but not fundamental. Recent studies indicated that morphogenesis, i.e. the presence of hyphae, is not critical for their pathogenic potential, as demonstrated in some intricate murine studies using *C. albicans* genetically locked into the yeast state (Nash et al. 2014). This suggests that physical cellular interactions are not solely responsible.

Metabolic signalling between *C. albicans* and *S. aureus* may play an important role in orchestrating this relationship. Chemically mediated signalling in the form of quorum sensing (QS) could potentiate both positive and negative interactions between these two microorganisms, which may inadvertently impact clinical outcomes. *Candida albicans* secretion of farnesol, a QS molecule, decreases *S. aureus* biofilm formation, as well as increasing its susceptibility to antibiotics (Akiyama et al. 2002; Jabra-Rizk et al. 2006; Unnanuntana et al. 2009). Moreover, it was shown to competitively inhibit *S. aureus* lipase activity (Kuroda et al. 2007). However, Lin et al. (2013) found that *S. aureus* conditioned media had a striking impact on *C. albicans* biofilm growth rate, indicating that *S. aureus* secretes a reciprocal QS molecule that stimulates *C. albicans* growth (Lin et al. 2013). Nonetheless, whether *C. albicans* secretes sufficient farnesol in vivo to have an effect on *S. aureus* remains unknown. Yet despite these conflicting results, the majority of studies support the idea of a synergistic relationship between the two.

Indeed, affinity panning of a *S. aureus* phage display library against *C. albicans* biofilms demonstrated that *S. aureus* released extracellular fibrinogen-binding protein (Efb) during the interaction. This was shown to coat *C. albicans* yeast cells and reduce phagocytosis by granulocytes (Fehrmann et al. 2013). In order to gain a better understanding of the molecular interaction between *C. albicans* and *S. aureus*, Peters et al. (2010) undertook a proteomics approach to identify proteins upregulated during their interaction. The majority of the 27 proteins that were upregulated were involved in processes, including stress and growth responses, and metabolism. *Staphylococcus aureus* upregulated stress-related genes in response to both yeast and hyphae, yet interestingly most of these genes were upregulated in response to yeast rather than hyphal biofilms. As for *C. albicans*, yeast cells increased a number of stress-related proteins such as Tsa1p and aconitate hydratase, yet *C. albicans* in hyphal formation showed minimal changes in gene expression in response to *S. aureus*. These results suggest that both organisms induce a stress response on their initial encounter with one another, particularly whilst *Candida* exists in yeast form. However, as they mature and develop into a hyphal biofilm, they may downregulate these genes as a survival strategy, facilitating survival within the host.

Clearly, these two pathogens have the ability to influence one another’s behaviour, so care must be taken in their clinical management. Broad-spectrum antimicrobial activity is crucial, accounting for both prokaryote and eukaryote. The use of ethanol has been shown to be effective at preventing both mono-and polymicrobial biofilms (Peters et al. 2013). However, the successful use of miconazole in angular cheilitis is interesting given no precise mechanism of action for this azole to *S. aureus* (Sud and Feingold 1982). It could therefore be hypothesized that given the polymicrobility of the disease miconazole acts by exhibiting *C. albicans* activity, thereby destabilizing *S. aureus* colonization, which is physically supported by the hyphal biofilm meshwork.

What is clear though is that these organisms are no strangers to one another.

**Streptococcal interactions**

Streptococci are amongst the primary colonizers of the oral cavity and compromise a large proportion of the overall flora (Syed and Loesche 1978; Moore et al. 1982). Oral streptococcal species are often termed as the mitis group streptococci (MGS), which include *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus gordonii*, *Streptococcus sanguinis* and *Streptococcus parasanguinis* species (Kawamura et al. 1995). MGS streptococci are traditionally known to be early colonizers of dental surfaces, comprising approximately 60–80% of the flora (Diaz et al. 2012), although use of high-throughput gene sequencing technology has revealed them to also be predominant colonizers of oral mucosal surfaces (Diaz et al. 2012).

The relationship between *Candida* and streptococci is generally considered to be synergistic, with advanced microscopy showing streptococcal interactions with the hyphal filaments of *Candida* (Dutton et al. 2014). Streptococci provide *Candida* with nutrients from the salivary pellicle, such as lactate and glucose, which *Candida* utilizes as a source of carbon (Holmes et al. 2006). Furthermore, streptococci are acidic and thus create an acid environment through the fermentation of carbohydrates (Takahashi and Nyvad 2011). At low pH, *Candida* grows in its yeast form. However, when colonized with streptococci, *Candida* can grow and survive at a lower pH (~4.5), and the H₂O₂ produced by streptococci can induce hyphal growth by inducing oxidative stress (Jenkinson, Lala and Shepherd 1990; Nasution et al. 2008). This interaction is bidirectional, as *C. albicans* can promote the survival of streptococci by lowering oxygen tension levels to that more acceptable for streptococcal growth, as well as providing nutrients to stimulate bacterial growth (Douglas 2002). This
synergistic relationship can prove disparaging for the host. Studies have shown that streptococci augment the persistence of Candida spp. Xu et al. (2014) demonstrated that coinfection with C. albicans and St. oralis resulted in a more pathogenic inflammatory response compared with infection with either microorganism alone, as demonstrated through an exaggerated upregulation of TLR2-dependent inflammatory genes (Dutton et al. 2014). Adherence to mucosal surfaces occurs through binding interactions with components of the salivary pellicle; however, there is a limited number of niches for C. albicans to inhabit. Thus, C. albicans has to compete with other microbes (Kolenbrander et al. 2002). To overcome this problem, C. albicans has evolved a mechanism allowing it to bind directly to MGS species, including St. oralis, St. mitis and St. gordonii (Jenkinson, Lalra and Shepherd 1990). This interaction is mutually beneficial as C. albicans can support the outgrowth of streptococci by enabling them to form more robust oral biofilms (Xu et al. 2014). Adherence between these two species occurs via interactions of the C. albicans hyphal cell wall protein Als3, and the streptococcal cell surface adhesins SspA and SspB (Holmes, McNab and Jenkinson 1996), proteins that belong to the antigen I/II polypeptide family (Bamford et al. 2009). Als3p is one of eight Als protein family members expressed in C. albicans (Als1p-7p, Als9p). Direct binding of SspB and Als3 is required for bacterial–fungal attachment. Interaction between these molecules is associated with the N-terminal domain of Als3 (Bamford et al. 2015), as deletions at the N-terminus abrogated binding to St. gordonii. Hoyer et al. (2014) have demonstrated that this interaction may be more complex than originally thought by showing that the peptide-binding domain (PBD) of C. albicans is essential for C. albicans–St. gordonii adherence. The PBD functions by binding to the free C-terminus; however, in St. gordonii the SspB C-terminus is covalently linked to pep- tidoglycan, and is thus unavailable to bind. Further investigation is required before we can fully understand the mechanism behind this interaction, though recent studies suggest that the early stage of cell wall O-mannosylation may be important in the development of these polymicrobial communities (Dutton et al. 2014).

An important component of a biofilm is the ECM, which confers protection to antimicrobials (Xu et al. 2014). The ECM of streptococcal biofilms is composed of α-glucans (Gregoire et al. 2011), whereas Candida biofilm ECM is primarily composed of β-glucans (Al-Fattani and Douglas 2006; Taff et al. 2012). Streptococcus mutants utilizes its ECM components to enhance adhesion to fungal cells by depositing α-glucans on the surface of hyphae (Gregoire et al. 2011). Moreover, interaction between St. mutans and C. albicans is promoted by glucosyltransferase-derived ECM and expression of the St. mutans virulence gene gtfB (Falsetta et al. 2014). It was also shown in this study that Candida-derived β1,3-glucans contribute to ECM matrix structure, whilst fungal β-glucan and mannan provide sites for GtfB binding and activity. Furthermore, β-glucans are found on the surface of hyphae as well as in the matrix (Donhar-Bagzoglou et al. 2009), thus suggesting that streptococci utilize these proteins to adhere to candidal hyphae. Collectively, this suggests that the biofilm ECM contributes to this mutualistic behaviour, favouring their coexistence in the oral environment to the detriment of the host.

As with Candida–S. aureus interactions, QS is an important factor in the relationship between Candida and streptococci. Farnesol, a tetraprenoid alcohol and a key intermediate in the sterol biosynthetic pathway in eukaryotic cells, represents the primary QS molecule associated with C. albicans, its main role being repression of hyphal growth and biofilm formation (Ramage et al. 2002a). However, one study has suggested that St. gordonii is able to suppress farnesol induced inhibition of biofilm formation, via autoinducer 2 (AI-2), as luxS mutants were less effective at permitting hyphal formation; however, the mode of action has yet to be elucidated (Bamford et al. 2009). Farnesol has also been shown to inhibit St. mutans biofilm accumulation and polysaccharide production (Koo et al. 2003). Based on this and further work, it has been suggested that it may be used to control its competitiveness in mixed species biofilms and could be used as a means of a chemotherapeutic strategy (Jeon et al. 2011). AI-2 is the primary QS molecule secreted by bacteria that allows interspecies communication (Vendeville et al. 2005). The luxS gene is associated with AI-2 production and luxS streptococcal mutants can form monospecies biofilms. However, when cocolonized with C. albicans, biofilm formation becomes abrogated, suggesting that this molecule is involved in cellular communication (McNab et al. 2003; Bamford et al. 2009).

Another important signalling mechanism in streptococci, including St. gordonii, is through the comCDE operon, which encodes a sensor-regulator system (ComDE) activated by the com gene product competence stimulating peptide (CSP). Streptococcus gordonii–C. albicans biofilms formed with ΔcomCDE or ΔcomC mutants showed increased biomass compared to wild-type biofilms. Interestingly, more eDNA was observed in the mixed ΔcomCDE mutant biofilms. Although purified CSP did not affect C. albicans hyphal formation. Contrary to earlier findings (Jarosz et al. 2009), it did inhibit monospecies biofilm formation, suggesting that the St. gordonii comCDE QS system modulates the production of eDNA (Jack et al. 2015), and important component of candidal ECM (Rajendran et al. 2014).

Candidal interactions

Hyphae provide C. albicans with an advantage over many of its competitors in terms of size and surface area, enabling them to take advantage of more sites for adhesion and occupation of a variety of niches. This is why it is a more successful pathogen than other members of the genus. Nonetheless, there is hypothesis that Candida spp., in particular C. glabrata benefit from C. albicans. There have been suggestions that DS pathology may be promoted by the synergistic interaction between these species within denture biofilms. Coco et al. (2008) first reported that C. glabrata and C. albicans were often coisolated from patients, particularly those with severe inflammation. The authors hypothesized that pathogenic synergy existed between the two Candida species. Candida glabrata, devoid of hyphae, forms relatively structurally poor and unstable biofilms, yet is associated with disease. Therefore, it was hypothesized to use C. albicans as a structural scaffold to gain entry into the host. Further studies have confirmed this, where C. albicans appeared to assist the invasive capacity of C. glabrata within an in vitro reconstituted epithelial biofilm model (Silva et al. 2011). The mechanistic of this interaction is at present unknown; however, we can speculate that tissue destruction through proteolytic and lipolytic enzymes augments the invasive capacity of the hyphae and allows coaggregate C. glabrata to enter and contribute to pathogenesis. Further work by this group has shown similar data with work in a reconstituted human vaginal epithelial model, where C. glabrata individually caused minimal tissue damage, though there was a significant increase in C. glabrata colonization and invasiveness in combination with C. albicans (Alves et al. 2014). Damage was primarily dependent on the process of invasion, with key virulence genes upregulated (HWP1, PLD1 and ALS3). Further studies using in vivo models to investigate the pathogenesis of DS would be useful in this context (Nett et al. 2010).
although as described above there is mounting evidence that hitchhiking through adhesion to hyphae is not a limited phenomenon and may also be important with respect to C. glabrata using C. albicans to gain entry to the host (Schlecht et al. 2015).

Anaerobic Gram-negative interactions

Life in subgingival plaque is highly anaerobic, favouring many obligate PD pathogens such as F. nucleatum and Prevotella intermedia. However, given the undefined relationship between Candida spp. and PD, then this remains a relatively neglected area of research. Studies regarding C. albicans and P. gingivalis have produced conflicting results. It was shown that P. gingivalis suppressed Candida biofilm formation through a reduction in the number of viable yeast cells coincident with an increasing P. gingivalis concentration (Thein, Samaranyake and Samaranyake 2006). Conversely, it was also shown that P. gingivalis induces germ-tube formation in C. albicans, producing a more invasive phenotype, and thus increasing the risk of infection (Nair, Anil and Samaranyake 2001). Furthermore, both microbes appear to have an antagonistic effect on one another in relation to host cell adhesion, as P. gingivalis inhibited adhesion of C. albicans to buccal epithelial cells (Nair and Samaranyake 1996), whilst the presence of C. albicans did not enhance adhesion to gingival epithelial cells or gingival fibroblasts by P. gingivalis (Tamai, Sugamata and Kiyoura 2011). Yet, in the same study pre-exposure of gingival epithelial cells and fibroblasts to C. albicans enhanced cell invasion by P. gingivalis. Clearly, further studies are required to decipher how these microorganisms interact with one another.

As for F. nucleatum, coaggregation studies have revealed its ability to adhere to C. albicans species (Grimaudo and Nesbitt 1997), as well as C. dubliniensis (Jabra-Rizk et al. 1999). However, the interaction with C. albicans may be temperature dependent as C. albicans grown at 37 °C did not coaggregate with F. nucleatum, yet the two species did coaggregate when grown at 25 and 45 °C (Jabra-Rizk et al. 1999). The exact mechanistic behind these interactions remains unknown; however, these observations indicate that C. albicans–F. nucleatum interactions may be an important factor in oral colonization by yeasts.

Studies using lipopolysaccharide from a variety of Gram-negative strains have shown that hyphal formation is inhibited as is biofilm formation in a number of Candida spp. (Bandara et al. 2010), indicating that physical interaction may be an important factor in defining their subgingival niches. Subsequent work in Escherichia coli demonstrated that secreted elements also play an important role in affecting hyphal formation (Bandara et al. 2013b). This is also true of the relationship between the capnophilic bacterium A. actinomycetemcomitans where it has been shown that its release of AI-2 was actively involved in inhibiting C. albicans hyphal growth and biofilm formation (Bachtiar et al. 2014). Given the complexity of various metabolites and QS molecules in subgingival plaque, such as volatile sulphur compounds, fatty acids and AI-2 (Kurita-Ochiai, Fukushima and Ochiai 1995, Huang et al. 2011; Jang et al. 2013; Basic and Dahlen 2014), it is likely that these also impact hyphal formation and Candida’s ability to contribute to PD (Noverr and Huffnagle 2004). This anoxic environment has been shown to result in significant transcriptional changes in C. albicans, including the upregulation of WOR1, which is a transcriptional regulator central to phenotypic switching (Fox et al. 2014). Based on the available literature it could be surmised that subgingival plaque is most likely to antagonize yeast proliferation, except in cases where there is dysbiosis of the biofilm ecology, such as following broad-spectrum antibiotic therapy or pre-existing medical conditions, including diabetes (Rams, Babalola and Slots 1990; Sardi et al. 2010; Al Mubarak et al. 2013).

Facultative Gram-positive interactions

Candida species and E. faecalis have become increasingly noted for their coisolation within endodontic infections, both of which play an important role in nosocomial infection. Interestingly, data from a longitudinal study carried out over two years at a German teaching hospital found that Candida-positive patients (blood, CSF, skin, feaces or sputum) were twice as likely to be colonized by E. faecalis (Hermann et al. 1999). Enterococcus faecalis has been found to incorporate itself into Candida biofilms, and is the third most predominant bacterial species found in mucosal fungal biofilms (Dongari-Bagtzoglou et al. 2009; Fox et al. 2014). It was shown to adhere to Candida in both hyphal and yeast forms, yet caused a reduction in the overall biofilm biomass (Fox et al. 2014). However, Cruz et al. (2013) demonstrated that E. faecalis inhibited hyphal morphogenesis, which was partially dependent on the Fsr QS system, a major regulator of E. faecalis virulence. Collectively, these effects both impacted virulence during co-infection when compared to species infection, suggesting that they both negatively influence one another’s virulence and help maintain a commensal relationship (Garsin and Lorenz 2013). Further work has revealed that C. albicans releases a surface protein Msb2, which binds to host antimicrobial peptides as well as antibiotics, thus conferring protection to both organisms (Swidergall, Ernst and Ernst 2013). Furthermore, evaluating the influence of C. albicans on the dynamics of the bacterial microbiome following antibiotic treatment found that bacterial re-colonization was enhanced in the presence of C. albicans (Mason et al. 2012). Moreover, C. albicans reduced Lactobacillus spp. whilst enhancing E. faecalis numbers, which led to the persistence of E. faecalis long term. This effect was not apparent in subjects when C. albicans was absent. Whether this effect was due to a synergetic relationship with E. faecalis or an antagonistic interaction with lactobacilli remains to be elucidated.

There is a conceived dogma that lactobacilli antagonize candidal colonization (Young, Krasner and Yudofsky 1956). This forms the basis of why they play a key role in probiotics. It is well documented that probiotics reduces candidal levels at several sites, including oral cavity, bloodstream and urinary tract (Mendonca et al. 2012; Kumar et al. 2013). Early observations indicate that C. albicans decreased in the presence of lactobacilli through provision of nutrients for lactobacilli that leads to lactic acid production, thus hindering candidal growth through pH-dependent inhibition. This dynamic relationship suggests that there is a close association between the two, but to date this has mainly been observed in vaginal infection. Our own microbiome studies of the denture plaque have shown that C. albicans and lactobacilli are positively associated in disease (unpublished work). The role of lactobacilli in maintaining homeostasis at the vaginal mucosa initially came to light due to the occurrence of vaginal candidiasis during treatment with systemic antibiotics. The mechanisms by which Lactobacillus species inhibits growth and virulence of Candida spp. are not yet fully understood, but perhaps the production of hydrogen peroxide as it has been shown to cause anti-candidal activity, albeit in some strains of lactobacilli (Strus et al. 2005). This suggests that other interactive mechanisms are involved in disease, including the modulation of the host response whereby lactobacilli cells have been shown to upregulate inflammatory cytokines when cocultured with C. albicans (Martinez et al. 2009),
potentially assisting in the clearance of candidal infection. Despite the overwhelming evidence of an antagonistic interaction, certain species of oral Lactobacillus, namely Lactobacillus casei, have demonstrated a stimulatory effect on C. albicans hyphal growth (Orsi et al. 2014), and in fact it has been demonstrated that candidal hyphae have the capacity to coaggregate and support lactobacilli levels in patients with higher levels of oral disease (Bilhan et al. 2009). Nevertheless, further studies are required to investigate these details in more detail to determine the true extent of the dynamic relationship; particularly as the perceived antagonism may only exist for C. albicans. For example, recent studies have shown that only one of six probiotic Lactobacillus species had an inhibitory effect on C. glabrata growth (Jiang et al. 2015). This suggests that the interaction between Candida and lactobacilli may be dependent on the particular environment they cohabit.

CONCLUSIONS

Collectively, these data demonstrate that the interaction between candidal species and other microorganisms may be dependent on the nature of the interaction (chemical, physical or both) and the particular environment they cohabit. It is clear from many of these studies that the interaction between C. albicans hyphae and different bacterial species is important in defining their interaction, whether mutualistic or antagonistic in nature. The secretion of signalling molecules from the myriad of microorganisms in the oral cavity such as AI-2, farnesol and other small molecules is clearly important, with recent studies supporting the notion that the metabolome plays an integral part in defining the interaction between the host, Candida and microbiota such as lactobacilli (Romani et al. 2015). Understanding how each of these specific interactions influences one another and Candida’s pathogenicity will enable us to target this medically important yeast rationally. Though, we must be cognizant of the negative influences of changing its role within complex oral biofilm communities and the consequences of dysbiosis (McLean, 2014), as this may support the unnecessary proliferation and overgrowth of candidal yeasts that leads to oral disease.

Conflict of interest. None declared.

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