Published in final edited form as:
Trends Microbiol. 2011 November ; 19(11): 557–563. doi:10.1016/j.tim.2011.07.004.

Importance of *Candida*-bacterial polymicrobial biofilms in disease

Melphine M. Harriott[1] and **Mairi C. Noverr**[2,3,*]

[1]Department of Medicine, Division of Infectious Diseases, Henry Ford Hospital, Detroit, MI 48202

[2]Department of Oral Biology, Louisiana State University School of Dentistry, New Orleans, LA 70119

[3]Department of Microbiology, Immunology, and Parasitology, Louisiana State University Health Sciences Center, New Orleans, LA 70119

Abstract

Candida albicans is the most prevalent human fungal pathogen, with an ability to inhabit diverse host niches and cause disease in both immunocompetent and immunocompromised individuals. *C. albicans* also readily forms biofilms on indwelling medical devices and mucosal tissues, which serve as an infectious reservoir that is difficult to eradicate, and can lead to lethal systemic infections. Biofilm formation occurs within a complex milieu of host factors and other members of the human microbiota. Polymicrobial interactions will likely dictate the cellular and biochemical composition of the biofilm, as well as influence clinically relevant outcomes such as drug and host resistance and virulence. In this manuscript, we review *C. albicans* infections in the context of *in vivo* polymicrobial biofilms and implications for pathogenesis.

Keywords

Candida albicans; Polymicrobial; Biofilm; Infection; Morphogenesis; Infection

Candida albicans monomicrobial and polymicrobial biofilms

Recently there has been a tremendous interest in the role of biofilms on infectious diseases. The NIH estimates that 80% of human infections result from pathogenic biofilms (http://grants.nih.gov/grants/guide/pa-files/PA-99-084.html). Biofilms are heterogeneous communities of microorganisms that attach to abiotic or biotic surfaces and are encased in an extracellular matrix (ECM), forming a complex three-dimensional architecture. Clinically, biofilm infections represent an overwhelming problem due to the highly recalcitrant nature of the embedded microbes, which are resistant to both antimicrobial drugs as well as host defenses. Biofilms can form on medical devices as well as on exposed and mucosal host tissues. As such, biofilms are often polymicrobial in nature, formed from

Corresponding Author: Mairi C. Noverr, Ph.D., Department of Oral Biology, LSUHSC School of Dentistry, New Orleans, LA 70119, Tel: 504-941-8055, FAX: 504-941-8467, mnover@lsuhsc.edu.
Authors: Melphine M. Harriott, Department of Medicine, Division of Infectious Diseases, Henry Ford Hospital, Detroit, MI 48202, Tel: 313-916-1995, mponnia1@hfhs.org

members of the endogenous microbiota as well as nosocomial pathogens. An emerging finding in polymicrobial biofilm research is the presence of both eukaryotic and prokaryotic pathogens. Therefore, these biofilms can be difficult to both diagnose as well as treat, requiring complex multi-drug treatment strategies. Polymicrobial biofilms represent an understudied and clinically relevant health problem, with the potential to serve as an infectious reservoir for a variety of microorganisms, including bacteria and fungi.

The most prevalent fungal biofilm-forming pathogen is *Candida albicans*, which can cause both superficial and systemic infections. Much of what is known about *C. albicans* biofilm development has been learned from *in vitro* monomicrobial biofilm studies. *In vitro*, biofilm formation can be divided into several growth phases: early, intermediate and mature [1, 2]. During the early phase, yeast cells adhere to an appropriate surface and undergo morphogenesis, which is essential for normal biofilm formation; mutants of *C. albicans* that are deficient in hyphal growth form less adherent biofilms that lack the normal three-dimensional architecture of wild-type biofilms [3, 4]. It should be noted that *in vitro* biofilm assays are typically performed using media that promotes morphogenesis, which could skew the importance of the role of morphogenesis in biofilm formation. Indeed, mutants in the transcriptional regulator *BCR1*, form thin, weakly adherent biofilms but are able to form hyphae [5]. The intermediate phase is characterized by continued hyphal growth and ECM production, which consists of cell wall polysaccharides and protein [6, 7]. Mature biofilms consist of a yeast base, with hyphal elements forming a complex network encased in ECM extending away from the surface. Although it has not been definitively proven in fungal biofilms, it is predicted that a dispersal phase exists [8]. This is supported by evidence that farnesol, a quorum sensing molecule that inhibits germination and biofilm formation, increases in concentration in aging cultures [9]. Farnesol does not inhibit growth of mature biofilms, but does inhibit germination of new blastospores, thus potentially promoting dispersal [10]. In addition, negative regulators of hyphal formation could play a role in biofilm dispersal by promoting yeast growth. Once such regulator, *NRG1*, was shown to promote dispersal when overexpressed during biofilm growth [11].

Candida biofilms have been studied primarily on abiotic surfaces [8, 12]. Significant attention has been given to *Candida* biofilm formation of indwelling catheters, which can lead to life-threatening systemic infections [13-15]. More recently, biofilm formation on biotic surfaces has been reported, including both oral and vaginal tissues [16, 17]. The mucosa provides an excellent environment for biofilm development (reviewed in [18]), and biofilm formation phases are similar to that observed *in vitro*, even in the absence of morphogenesis inducing media [17]. Microbes easily adhere to the mucosal surface and aggregate with other microbes. Because of the heterogeneity of species within polymicrobial biofilms, it has been difficult to assess the relevance and contribution of individual species to pathogenesis and disease. However, with the use of advanced molecular and microscopic techniques to identify and characterize clinically relevant biofilms, it has become clear that interspecies interactions play a role in colonization and infection dynamics, as well as the host response, which will be discussed below. There have been numerous reports concerning the effect of bacterial species on both *C. albicans* germination and *in vitro* biofilm formation. *Candida*-bacterial interactions have been reviewed previously, and both agonistic and antagonistic relationships can occur [19-21]. Therefore, the focus of this review is the clinical significance and *in vivo* infection models of polymicrobial biofilms with *C. albicans*.

Systemic infections

An estimated 27-56% of nosocomial *C. albicans* bloodstream infections are polymicrobial [22, 23]. In a survey of 372 patients with candidemia, the top three most commonly co-

isolated bacterial species were *Staphylococcus epidermidis, Enterococcus spp., and Staphylococcus aureus* [22]. *In vitro*, using a catheter disc biofilm model, the presence of *C. albicans* enhanced the growth of *S. epidermidis*, with extensive association of the bacteria and fungal hyphae [24]. ECM production by *S. epidermidis* inhibited penetration of fluconazole into the mixed species biofilm; similarly, vancomycin resistance was increased in the presence of *C. albicans* [24-26]. Whether this increase in drug resistance occurs *in vivo* in unknown. However, if this is the case, it would help explain why concomitant bacteremia is associated with a poor prognosis despite antimicrobial therapy compared with monomicrobial candidemia [27, 28].

S. aureus does not form biofilms as readily on abiotic surfaces, requiring pre-coating and nutrient supplementation [29]. Although *S. aureus* forms poor monoculture biofilms in serum, it forms a substantial polymicrobial biofilm in the presence of *C. albicans* (Figure 1). In terms of architecture, *S. aureus* is found throughout the biofilm and forms microcolonies on the surface of the biofilm, with *C. albicans* serving as a scaffold upon which *S. aureus* adheres [30]. Ability to form hyphae is required for the interaction, as *S. aureus* preferentially adheres to hyphae as opposed to yeast forms [31, 32]. *S. aureus* resistance to vancomycin was also enhanced within the polymicrobial biofilm, required viable *C. albicans*, and was in part mediated by the *C. albicans* ECM, and required the ability of *C. albicans* to adhere to the substratum [30, 31]. While no single fungal adhesin was identified as being required for vancomycin resistance, there could be a combination involved as many *C. albicans* adhesins have overlapping function [33]. In addition, proteomic analysis demonstrated that interspecies interaction influences protein expression, which could help explain modulation of antimicrobial resistance and virulence [32].

Using an *in vivo* model of systemic intraperitoneal infection, co-inoculation of *C. albicans* and *S. aureus* had a synergistic effect on mortality in mice [34]. Infection with sublethal doses of either species alone resulted in no mortality, but with the same doses, co-infection resulted in 100% mortality; the effect was abrogated when either organism was heat-inactivated. Similar results were observed with non-lethal doses of *Serratia marcescens, Streptococcus faecalis, and Escherichia coli* during experimental peritonitis [35, 36]. Strains of *S. aureus* expressing toxic shock syndrome toxin (TSST) or delta toxin had the most dramatic effects on morbitidity and mortality [37, 38]. Culture filtrate of toxin producing strains of *S. aureus* also mediated this effect, which could be ameliorated with non-steroidal anti-inflammatory drug (NSAID) treatment [38]. Interestingly, when mice were injected with either organism at opposite injection sites (subcutaneous vs. intraperitoneal inoculation), mixed infection was observed at the fungal injection site, but not vice versa [39]. Histological analysis showed that *S. aureus* was associated throughout areas of fungal growth, interspersed as opposed to at the periphery. This indicates that *C. albicans* could facilitate *S. aureus* growth and infection within host tissues and each species could influence gene expression *in vivo*, resulting in increased virulence and resistance to host defenses as was reported *in vitro* [32].

Vaginal infections

Vulvovaginal candidiasis (VVC) affects a significant number of women predominantly in their reproductive years [40-42]. An estimated 75% of all women will experience an episode of acute VVC in their lifetime with another 5-10% developing recurrent VVC (RVVC) [40, 41]. Vulvovaginitis involves infections of the vaginal lumen as well as the vulva. Symptoms include burning, itching, soreness, an abnormal discharge, and dyspareunia. Signs include vaginal and vulvar erythema and edema. Acute VVC has several known predisposing factors including antibiotic and oral contraceptive usage, hormone replacement therapy, pregnancy, and uncontrolled diabetes mellitus [40-42]. Antiobiotic use reduces the levels of bacteria

that prevent fungal overgrowth. In particular, members of the genus *Lactobacillus*, which is the most prevalent bacterial group in the vagina, can prevent hyphal growth and exert direct antifungal activity [43, 44]. The antagonistic interactions of vaginal lactobacilli with *Candida* and potential use in therapeutic applications have been reviewed elsewhere [45, 46].

Vaginal mucosal bacterial biofilms have been previously described for *Gardnerella vaginalis*, which causes vaginosis [47-49]. However, in VCC and RVVC it was unknown whether *C. albicans* exist as biofilms on the vaginal mucosa and if so, whether they play a role in the immunopathogenesis. To this end, *in vivo* and *ex vivo* murine vaginitis models were recently used to examine mucosal biofilm formation by scanning electron and confocal microscopy [17]. Wild-type *C. albicans* strains formed biofilms on the vaginal mucosa *in vivo* and *ex vivo* as indicated by high fungal burden and microscopic analysis demonstrating typical biofilm architecture and ECM that co-localized with the presence of fungi. In contrast, mutants in a regulator of hyphal formation (*efg1*/*efg1)* and biofilm formation (*bcr1*/*bcr1)* exhibited weak to no biofilm formation and ECM production in both models despite comparable colonization levels. This raises an interesting question; does the presence of a biofilm determine whether *C. albicans* behaves as a pathogen and allows the switch from commensalism? In addition, does a biofilm influence the host response? Further, does growth in a biofilm allow other bacterial species to co-colonize and contribute to pathogenesis or resistance? Previous studies have shown that approximately 20-34% of RVVC samples contain vaginal bacterial pathogens such as *Streptococcus agalactiae* and *G. vaginalis* [50, 51]. Interestingly, one of the more effective therapies for recurrent and resistant vaginitis is boric acid. While the mechanism of action of this chemical in unknown, it effectively inhibits morphogenesis and biofilm formation *in vitro* [52].

While *C. albicans* biofilm formation on the mucosal surface represents biotic biofilm formation, there is the possibility of fungal or polymicrobial biofilm formation on vaginal or intrauterine devices (IUD) serving as a reservoir for various types of infections. A survey of biofilms formed on IUDs confirmed the presence of multiple bacterial pathogens as well as *C. albicans* [53]. The longer the IUD was in place, the greater the total microbial burden and heterogeneity of species isolated from the device. There is also a significantly higher prevalence of infections such as bacterial vaginosis in IUD users in comparison with users of other contraceptive methods [54, 55]. In addition, there is a higher risk of pelvic inflammatory disease (PID) immediately following IUD insertion, which is caused by several bacterial pathogens including *Chlamydia trachomatis* and *Neisseria gonorrhoeae* [56, 57]. Another type of contraception that has recently been introduced is the combined contraceptive vaginal ring (CCVR), which releases ethinyl estradiol and etonogenstrel hormones locally. *C. albicans* readily adheres to the ring *in vitro*, and estrogen is known to promote hyphal formation [58, 59]. Clinically vaginitis was reported more often in women using the ring as opposed to oral hormonal contraceptives [58]. Therefore monomicrobial or polymicrobial biofilm formation on these devices might contribute to incidence and recurrence of genital infections.

Skin infection and wound healing

Biofilms contribute to chronicity and delayed healing of most, if not all, chronic wounds [60]. Recently, the contribution of fungal pathogens to mixed species biofilms has been assessed due to the extremely recalcitrant nature of many wounds to antibacterial agents. Using molecular diagnostics, a survey of 915 clinical specimens taken from chronic wounds revealed 23% were positive for fungal species [61]. The most abundant fungi were yeasts in the genus *Candida*; however, *Curvularia*, *Malessezia*, *Aureobasidium*, *Cladosporium*, *Ulocladium*, *Engodontium* and *Trichtophyton* were also found to be prevalent components

of these polymicrobial infections. Quantification of bacteria versus fungi in these chronic wounds demonstrated that fungi contributed to >50% of the microbial burden in the majority of the wounds. In several cases, identification of fungal pathogens in the chronic wound biofilm led to the addition of an antifungal drug to the treatment regimen and gradual healing of the wound site. This clinical survey demonstrates that the incidence of fungal pathogens in wound biofilm infection is more significant than previously reported. However, to date, there are no reports of *in vivo* models to investigate fungal or bacterial-fungal biofilms in wounds. Nevertheless, new cost-effective diagnostics have been developed [62], which can aid in identification of underreported fungal pathogens in chronic wounds, thereby promoting targeted therapies and improving healing trajectories.

Oral infections

C. albicans is responsible for several types of oral infections, including oropharyngeal candidiasis (OPC) or thrush, and denture stomatitis (DS). OPC encompasses infections of the hard and soft palate, tongue, buccal mucosa, and floor of the mouth, and can present as reddened patches (erythematous) or white curd-like lesions (pseudomembranous). Although OPC occurs with several immunocompromising conditions, it appears to be much more common in HIV-infected persons than under any other condition [63-66]. The pseudomembranous lesions on oral mucosa consist of blastospores, pseudohyphae, and hyphae attached to underlying epithelium [67]. It has been hypothesized that these plaques are biofilms, however, this concept had not been studied *in vivo*. Using an immunosuppressed mouse model, researchers were able to induce development of characteristic white lesions on the surface of the tongue after inoculation with *C. albicans* [16]. Three-dimensional confocal imaging revealed a complex architecture similar to *in vitro* biofilms with considerable β-glucan being exposed during invasion of the epithelium. This masking and unmasking of β-glucan *in vivo* is hypothesized to play a role in immunomodulation, because it serves as a pathogen recognition receptor ligand [68]. In particular, β-glucan signals via dectin-1 and TLR2 to promote Th17 cytokine production, which has been proposed to play a protective role against mucosal fungal infections [69-72]. Another interesting observation was the contribution of host components to the biofilm and ECM. Both keratin and polymorphonuclear leukocytes (PMN) aggregates were observed within the ECM, suggesting that the ECM might inhibit migration of effector cells. PMNs exert potent antifungal defenses, and if they become trapped in the ECM, this would limit the ability of these cells to control the fungal infection. The contribution of bacterial species to the fungal biofilm was also investigated. Using fluorescent in situ hybridization (FISH), several groups of bacteria were identified on the basis of 16S RNA using probes that recognize *Lactobacillus/Enterococcus* spp. and *Staphylococcus* spp. They noted that mice were generally positive for only one bacterial group and were most often associated with the apical surface of the biofilm [16]. How these bacterial species play a role in disease remains to be determined.

DS is an inflammatory fungal infection affecting approximately 50-75% of otherwise healthy denture wearers and the most common form of oral candidiasis [73-75]. Recently, a new rat model of oral device associated infection was developed to study oral biofilm formed *in vivo* on dental material [76]. In this model, non-custom fitted oral devices were installed in mice, which were constructed with a 1 mm gap between the palate and device, to allow inoculation. This design does not permit contact between the device and the oral tissue. *C. albicans* formed biofilms on the device *in vivo*, and bacteria were found to be associated with the fungi. However, there was no evaluation of palatal biofilm formation and no palatal inflammation [76]. Therefore, the model does not represent a clinically relevant model of denture stomatitis. To investigate the role of biofilms in DS, a novel contemporary rat model was recently developed using a custom fitted denture system composed of both

fixed and magnetic removable plates [77]. The denture system was installed against the hard palate of rats without alteration of the dental architecture. The novel design of this denture system (removable portion) allows for longitudinal studies to evaluate the progression of the disease. Biofilm formation was analyzed on the denture and palate via scanning electron and confocal microscopy. Biofilm formation on the denture occurred by week 4 post-inoculation, characterized by the presence of yeast and hyphae coated with ECM. However, on the palate tissue, only blastospore colonization was observed and no clinical evidence of disease was observed. By week 6 post-inoculation, biofilm formation was observed on both the denture and the palate tissue and palatal erythema was evident [77]. This suggests that during DS, *C. albicans* biofilm formation occurs initially on the denture plate, which in turn seeds the palatal tissue, resulting in mucosal biofilm formation and signs of disease. Microscopic analysis of the infected denture and tissues revealed co-association of bacteria (Figure 2). Therefore, further investigation at the species level is warranted to determine whether these species play a role in disease.

There have been several reports investigating the interaction between *C. albicans* and oral streptococci. *Streptococcus gordonii* is a non-pathogenic oral commesal bacterium, but plays an integral role of formation of dental plaque on tooth surfaces, creating an adherent surface amenable to colonization by other oral pathogens. During early polymicrobial biofilm formation *in vitro* on saliva-coated surfaces, *S. gordonii* enhances hyphal development, binding to *C. albicans* hyphae via cell wall proteins SspA and SspB [78]. *C. albicans* hyphal specific adhesin Als3 is also required for optimal interaction with *S. gordonii* during biofilm formation [79]. A mutant lacking *ALS3* was unable to form a biofilm on a salivary pellicle or on a layer of adherent *S. gordonii* nor were the bacteria able to adhere to mutant hyphae. Heterologous expression of *C. albicans* adhesins in *Saccharomyces cerevisiae* demonstrated a similar requirement for Als3 and Ssp protein in mediating the interaction with *S. gordonii*. Screening of several fungal adhesins heterologously expressed in S. cereivisiae revealed that specific proteins mediate binding to *S. gordonii* including: Als3 and Eap1, and to a lesser degree Hwp1 [80]. Therefore, multiple adhesins with overlapping functions work to promote polymicrobial interactions. Repression of *C. albicans* hyphae and biofilm production by the fungal quorum sensing molecule farnesol was also relieved by *S. gordonii*, which was mediated in part by the bacterial quorum sensing molecule, autoinducer 2 (AI-2). These results suggest that interactions between *C. albicans* and *S. gordonii* involve physical (adherence) and chemical (diffusible) signals that influence the development of biofilms. However, it remains to be investigated *in vivo* whether *S. gordonii* can promote *C. albicans* colonization of the oral cavity.

Lung Infections

While *C. albicans* is not often associated with pulmonary colonization or infection, there are several reports documenting isolation from clinical samples in the context of bacterial infections, including ventilator associated infection, pneumonia, and cystic fibrosis related infections with *Pseudomonas aeruginosa* [81, 82]. Interestingly, *in vitro* studies have pointed to an antagonistic interaction between bacteria and fungi. *P. aeruginosa* kills yeast, hyphae, and biofilms of *C. albicans* [83, 84]. This is accomplished in part through production of bacterial phenazine derivatives, which affect cell wall integrity and allows *P. aeruginosa* to form biofilms on killed hyphae [83, 85, 86]. While the clinical relevance of this relationship is unclear, both organisms have been isolated from lung samples of patients with cystic fibrosis [81, 87]. Although there appears to be an antagonistic relationship *in vitro*, killing of highly adherent hyphae on a mucosal surface could produce a fungal scaffold upon which *P. aeruginosa* can form a biofilm, thereby parasitizing *C. albicans*. In addition, *in vivo* rat studies have demonstrated that pre-colonization of lung tissue by *C. albicans* increases rates of *P. aeruginosa* pneumonia [88]. These results are corroborated by

a clinical study of 804 ventilator patients, which showed that pulmonary *Candida* colonization (which occurred in 26% of patients) is an independent risk factor for *Pseudomonas* pneumonia [82]. Therefore, this could represent a novel mechanism whereby prior *C. albicans* mucosal biofilm formation promotes secondary biofilm formation by a bacterial pathogen, leading to disease.

Gastrointestinal Infections

Candida species are normal inhabitants of the gastrointestinal (GI) tract, with colonization rates ranging from 30-70% among healthy adults [89, 90]. Persistence in the GI tract can lead to gastrointestinal candidiasis (defined as infection of the stomach and small and large intestines). More importantly, GI colonization and infection predispose patients to systemic candidiasis due to outgrowth from the GI tract, which is also known as candidiasis of endogenous origin. GI candidiasis is not easily recognized clinically due to the lack of specific symptoms and the lack of the ability to differentiate between pathogenic and commensal *C. albicans*. However, it is clear from many human studies that two major predisposing factors are immune deficiency and antibiotic therapy [91, 92]. Therefore, both the host immune response and a normal bacterial microbiota are involved in controlling commensalism vs. disease. While it could be assumed that *Candida* exists as a biofilm in the GI tract, this has not been demonstrated. Gastrointestinal colonization with *C. albicans* has been associated with alterations in mucosal immunity, promoting atopic responses at other mucosal sites [93, 94]. This was also associated with increases in enteric bacteria, which might have contributed to the alterations in mucosal immune responses. Further studies examining GI tract biofilms with *C. albicans* could provide insights as to the role of polymicrobial biofilms in disease and/or dissemination from the GI tract.

Conclusions

The development of animal models has been essential in demonstrating the ability of *Candida* to form polymicrobial biofilms on mucosal tissues *in vivo*. Advances in microscopic techniques will aid in the assessment of biofilm architecture *in situ*, as well as identify host and bacterial species that co-associate within the biofilm. It will be especially important to monitor expression profiles of *in vivo* mucosal biofilms, which could differ significantly from *in vivo* abiotic biofilms or *in vitro* biofilms. It has been shown that *C. albicans* alternatively regulates its gene expression in the oral cavity compared with the GI tract [95]. Therefore, virulence needs to be evaluated in terms of site-specific roles and in terms of colonization vs. biofilm growth in monomicrobial vs. polymicrobial biofilms. It is likely that site specific immunity plays a large role in determining how *C. albicans* responds within each microenvironment and tailors gene expression to maintain commensal status and/or biofilm growth. In addition, these fungal and bacterial biofilms could serve as a source of systemic infections that exert synergistic effects on morbidity and mortality. The role of polymicrobial biofilms in immune evasion and manipulation of immune responses is of utmost importance for future studies and will likely direct novel therapies that target the multiplicity of species comprising this immune and drug resistant mode of growth.

Bibliography

1. Chandra J, et al. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. J Bacteriol. 2001; 183(18):5385–94. [PubMed: 11514524]
2. Ramage G, et al. Characteristics of biofilm formation by *Candida albicans*. Rev Iberoam Micol. 2001; 18(4):163–70. [PubMed: 15496122]
3. Nobile CJ, et al. Function of *Candida albicans* adhesin Hwp1 in biofilm formation. Eukaryot Cell. 2006; 5(10):1604–10. [PubMed: 17030992]

4. Ramage G, et al. The filamentation pathway controlled by the Efg1 regulator protein is required for normal biofilm formation and development in *Candida albicans*. FEMS Microbiol Lett. 2002; 214(1):95–100. [PubMed: 12204378]
5. Nobile CJ, et al. Critical role of Bcr1-dependent adhesins in *C. albicans* biofilm formation in vitro and in vivo. PLoS Pathog. 2006; 2(7):e63. [PubMed: 16839200]
6. Baillie GS, Douglas LJ. Matrix polymers of *Candida* biofilms and their possible role in biofilm resistance to antifungal agents. J Antimicrob Chemother. 2000; 46(3):397–403. [PubMed: 10980166]
7. Nett J, et al. Beta -1,3 glucan as a test for central venous catheter biofilm infection. J Infect Dis. 2007; 195(11):1705–12. [PubMed: 17471441]
8. Blankenship JR, Mitchell AP. How to build a biofilm: a fungal perspective. Curr Opin Microbiol. 2006; 9(6):588–94. [PubMed: 17055772]
9. Ramage G, et al. Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. Appl Environ Microbiol. 2002; 68(11):5459–63. [PubMed: 12406738]
10. Jabra-Rizk MA, et al. Effect of farnesol on *Candida dubliniensis* biofilm formation and fluconazole resistance. FEMS Yeast Res. 2006; 6(7):1063–73. [PubMed: 17042756]
11. Uppuluri P, et al. The transcriptional regulator Nrg1p controls *Candida albicans* biofilm formation and dispersion. Eukaryot Cell. 2010; 9(10):1531–7. [PubMed: 20709787]
12. Ramage G, Martinez JP, Lopez-Ribot JL. *Candida* biofilms on implanted biomaterials: a clinically significant problem. FEMS Yeast Res. 2006; 6(7):979–86. [PubMed: 17042747]
13. Kojic EM, Darouiche RO. *Candida* infections of medical devices. Clin Microbiol Rev. 2004; 17(2):255–67. [PubMed: 15084500]
14. Crump JA, Collignon PJ. Intravascular catheter-associated infections. Eur J Clin Microbiol Infect Dis. 2000; 19(1):1–8. [PubMed: 10706172]
15. Dominic RM, Shenoy S, Baliga S. *Candida* biofilms in medical devices: Evolving trends. Kathmandu Univ Med J (KUMJ). 2007; 5(3):431–6. [PubMed: 18604070]
16. Dongari-Bagtzoglou A, et al. Characterization of mucosal *Candida albicans* biofilms. PLoS One. 2009; 4(11):e7967. [PubMed: 19956771]
17. Harriott MM, et al. *Candida albicans* forms biofilms on the vaginal mucosa. Microbiology. 2010; 156(Pt 12):3635–44. [PubMed: 20705667]
18. Natero, J., et al., editors. Colonization of Mucosal Surfaces. ASM Press; Chicago: 2005.
19. Shirtliff ME, Peters BM, Jabra-Rizk MA. Cross-kingdom interactions: *Candida albicans* and bacteria. FEMS Microbiol Lett. 2009
20. Wargo MJ, Hogan DA. Fungal--bacterial interactions: a mixed bag of mingling microbes. Curr Opin Microbiol. 2006; 9(4):359–64. [PubMed: 16777473]
21. Thein ZM, et al. Community lifestyle of *Candida* in mixed biofilms: a mini review. Mycoses. 2009; 52(6):467–75. [PubMed: 19486299]
22. Klotz SA, et al. Polymicrobial bloodstream infections involving *Candida* species: analysis of patients and review of the literature. Diagn Microbiol Infect Dis. 2007; 59(4):401–6. [PubMed: 17888612]
23. Pulimood S, et al. Polymicrobial candidemia. Diagn Microbiol Infect Dis. 2002; 44(4):353–7. [PubMed: 12543540]
24. Adam B, Baillie GS, Douglas LJ. Mixed species biofilms of *Candida albicans* and *Staphylococcus epidermidis*. J Med Microbiol. 2002; 51(4):344–9. [PubMed: 11926741]
25. Al-Fattani MA, Douglas LJ. Penetration of *Candida* biofilms by antifungal agents. Antimicrob Agents Chemother. 2004; 48(9):3291–7. [PubMed: 15328087]
26. Al-Fattani MA, Douglas LJ. Biofilm matrix of *Candida albicans* and *Candida tropicalis*: chemical composition and role in drug resistance. J Med Microbiol. 2006; 55(Pt 8):999–1008. [PubMed: 16849719]
27. Guerra-Romero L, et al. Polymicrobial fungemia: microbiology, clinical features, and significance. Rev Infect Dis. 1989; 11(2):208–12. [PubMed: 2704925]
28. Dyess DL, Garrison RN, Fry DE. *Candida* sepsis. Implications of polymicrobial blood-borne infection. Arch Surg. 1985; 120(3):345–8. [PubMed: 3970669]

29. Cassat JE, Lee CY, Smeltzer MS. Investigation of biofilm formation in clinical isolates of *Staphylococcus aureus*. Methods Mol Biol. 2007; 391:127–44. [PubMed: 18025674]
30. Harriott MM, Noverr MC. *Candida albicans* and *Staphylococcus aureus* form polymicrobial biofilms: effects on antimicrobial resistance. Antimicrob Agents Chemother. 2009; 53(9):3914–22. [PubMed: 19564370]
31. Harriott MM, Noverr MC. Ability of *Candida albicans* mutants to induce *Staphylococcus aureus* vancomycin resistance during polymicrobial biofilm formation. Antimicrob Agents Chemother. 2010; 54(9):3746–55. [PubMed: 20566760]
32. Peters BM, et al. Microbial interactions and differential protein expression in *Staphylococcus aureus-Candida albicans* dual-species biofilms. FEMS Immunol Med Microbiol. 2010; 59(3): 493–503. [PubMed: 20608978]
33. Nobile CJ, et al. Complementary adhesin function in *C. albicans* biofilm formation. Curr Biol. 2008; 18(14):1017–24. [PubMed: 18635358]
34. Carlson E. Synergistic effect of *Candida albicans* and *Staphylococcus aureus* on mouse mortality. Infect Immun. 1982; 38(3):921–4. [PubMed: 7152678]
35. Carlson E. Enhancement by *Candida albicans* of *Staphylococcus aureus*, *Serratia marcescens*, and *Streptococcus faecalis* in the establishment of infection in mice. Infect Immun. 1983; 39(1):193–7. [PubMed: 6401691]
36. Klaerner HG, et al. *Candida albicans* and *Escherichia coli* are synergistic pathogens during experimental microbial peritonitis. J Surg Res. 1997; 70(2):161–5. [PubMed: 9245566]
37. Carlson E. Effect of strain of *Staphylococcus aureus* on synergism with *Candida albicans* resulting in mouse mortality and morbidity. Infect Immun. 1983; 42(1):285–92. [PubMed: 6352497]
38. Carlson EC. Synergism of *Candida albicans* and delta toxin producing *Staphylococcus aureus* on mouse mortality and morbidity: protection by indomethacin. Zentralbl Bakteriol Mikrobiol Hyg [A]. 1988; 269(3):377–86.
39. Carlson E, Johnson G. Protection by *Candida albicans* of *Staphylococcus aureus* in the establishment of dual infection in mice. Infect Immun. 1985; 50(3):655–9. [PubMed: 3905609]
40. Sobel JD. Pathogenesis and epidemiology of vulvovaginal candidiasis. Ann NY Acad Sci. 1988; 544:547–557. [PubMed: 3063184]
41. Sobel JD. Pathogenesis and treatment of recurrent vulvovaginal candidiasis. Clin Infect Dis. 1992; 14(suppl 1):S148–S153. [PubMed: 1562688]
42. Kent HL. Epidemiology of vaginitis. Am J Obstet Gynecol. 1991; 165:1168–1175. [PubMed: 1951572]
43. Noverr MC, Huffnagle GB. Regulation of *Candida albicans* morphogenesis by fatty acid metabolites. Infect Immun. 2004; 72(11):6206–10. [PubMed: 15501745]
44. Strus M, et al. The in vitro activity of vaginal *Lactobacillus* with probiotic properties against *Candida*. Infect Dis Obstet Gynecol. 2005; 13(2):69–75. [PubMed: 16011996]
45. Boris S, Barbes C. Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect. 2000; 2(5):543–6. [PubMed: 10865199]
46. Falagas ME, Betsi GI, Athanasiou S. Probiotics for prevention of recurrent vulvovaginal candidiasis: a review. J Antimicrob Chemother. 2006; 58(2):266–72. [PubMed: 16790461]
47. Swidsinski A, et al. An adherent *Gardnerella vaginalis* biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. Am J Obstet Gynecol. 2008; 198(1):97, e1–6. [PubMed: 18005928]
48. van der Meijden WI, et al. Descriptive light and electron microscopy of normal and clue-cell-positive discharge. Gynecol Obstet Invest. 1988; 25(1):47–57. [PubMed: 3257743]
49. Scott TG, Curran B, Smyth CJ. Electron microscopy of adhesive interactions between *Gardnerella vaginalis* and vaginal epithelial cells, McCoy cells and human red blood cells. J Gen Microbiol. 1989; 135(3):475–80. [PubMed: 2576032]
50. Buchta V, Spacek J. Microbiological findings in patients with recurrent vulvovaginal candidiasis in the Hradec Kralove Faculty Hospital 1995-2002. Ceska Gynekol. 2004; 69(1):7–14. [PubMed: 15112380]

51. Esim Buyukbayrak E, et al. Diagnosis of vulvovaginitis: comparison of clinical and microbiological diagnosis. Arch Gynecol Obstet. 2010; 282(5):515–9. [PubMed: 20461391]
52. De Seta F, et al. Antifungal mechanisms supporting boric acid therapy of *Candida* vaginitis. J Antimicrob Chemother. 2009; 63(2):325–36. [PubMed: 19059942]
53. Pal Z, et al. Biofilm formation on intrauterine devices in relation to duration of use. J Med Microbiol. 2005; 54(Pt 12):1199–203. [PubMed: 16278434]
54. Guerreiro D, Gigante MA, Teles LC. Sexually transmitted diseases and reproductive tract infections among contraceptive users. Int J Gynaecol Obstet. 1998; 63 1:S167–73. [PubMed: 10075229]
55. Ferraz do Lago R, et al. Follow-up of users of intrauterine device with and without bacterial vaginosis and other cervicovaginal infections. Contraception. 2003; 68(2):105–9. [PubMed: 12954522]
56. Grimes DA. Intrauterine devices and pelvic inflammatory disease: recent developments. Contraception. 1987; 36(1):97–109. [PubMed: 3311628]
57. Farley TM, et al. Intrauterine devices and pelvic inflammatory disease: an international perspective. Lancet. 1992; 339(8796):785–8. [PubMed: 1347812]
58. Oddsson K, et al. Efficacy and safety of a contraceptive vaginal ring (NuvaRing) compared with a combined oral contraceptive: a 1-year randomized trial. Contraception. 2005; 71(3):176–82. [PubMed: 15722066]
59. White S, Larsen B. *Candida albicans* morphogenesis is influenced by estrogen. Cell Mol Life Sci. 1997; 53(9):744–9. [PubMed: 9368671]
60. James GA, et al. Biofilms in chronic wounds. Wound Repair Regen. 2008; 16(1):37–44. [PubMed: 18086294]
61. Dowd SE, et al. Survey of fungi and yeast in polymicrobial infections in chronic wounds. J Wound Care. 2011; 20(1):40–7. [PubMed: 21278640]
62. Leake JL, et al. Identification of yeast in chronic wounds using new pathogen-detection technologies. J Wound Care. 2009; 18(3):103–4. 106, 108. [PubMed: 19247230]
63. Greenspan D, et al. Oral mucosal lesions and HIV viral load in the Women's interagency HIV study (WIHS). JAIDS - J Acq Imm Def. 2000; 25:44–50.
64. Nielsen H, et al. Oral candidiasis and immune status of HIV-infected patients. J Oral Pathol Med. 1994; 23:140–143. [PubMed: 7912732]
65. Sangeorzan JA, et al. Epidemiology of oral candidiasis in HIV infected patients: colonization, infection, treatment, and emergence of fluconazole resistance. Am J Med. 1994; 97:339–346. [PubMed: 7942935]
66. Schuman P, et al. Oral lesions among women living with or at risk for HIV infection. Am J Med. 1998; 104:559–563. [PubMed: 9674719]
67. Allen CM, Beck FM. Differences in mucosal reaction related to *Candida albicans* isolates. J Oral Pathol. 1987; 16(2):89–93. [PubMed: 3112352]
68. Wheeler RT, et al. Dynamic, morphotype-specific *Candida albicans* beta-glucan exposure during infection and drug treatment. PLoS Pathog. 2008; 4(12):e1000227. [PubMed: 19057660]
69. Netea MG, et al. Immune sensing of *Candida albicans* requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. J Clin Invest. 2006; 116(6):1642–50. [PubMed: 16710478]
70. Cheng SC, et al. The dectin-1/inflammasome pathway is responsible for the induction of protective T-helper 17 responses that discriminate between yeasts and hyphae of *Candida albicans*. J Leukoc Biol. 2011
71. Ferwerda B, et al. Human dectin-1 deficiency and mucocutaneous fungal infections. N Engl J Med. 2009; 361(18):1760–7. [PubMed: 19864674]
72. Conti HR, et al. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. J Exp Med. 2009; 206(2):299–311. [PubMed: 19204111]
73. Budtz-Jorgensen E. The role of *Candida albicans* in the development of stomatitis in denture wearers. Med Hyg (Geneve). 1975; 33(1164):1434–5. [PubMed: 768715]

74. Arendorf TM, Walker DM. Denture stomatitis: a review. J Oral Rehabil. 1987; 14(3):217–27. [PubMed: 3298586]
75. Cumming CG, et al. Denture stomatitis in the elderly. Oral Microbiol Immunol. 1990; 5(2):82–5. [PubMed: 2087353]
76. Nett JE, et al. Development and validation of an *in vivo Candida albicans* biofilm denture model. Infect Immun. 2010; 78(9):3650–9. [PubMed: 20605982]
77. Lee H, et al. Fabrication of a multi-applicable removable intraoral denture system for rodent research. J Oral Rehabil. 201110.1111/j.1365-2842.2011.02206.x
78. Bamford CV, et al. *Streptococcus gordonii* modulates *Candida albicans* biofilm formation through intergeneric communication. Infect Immun. 2009; 77(9):3696–704. [PubMed: 19528215]
79. Silverman RJ, et al. Interaction of *Candida albicans* cell wall Als3 protein with *Streptococcus gordonii* SspB adhesin promotes development of mixed-species communities. Infect Immun. 2010; 78(11):4644–52. [PubMed: 20805332]
80. Nobbs AH, Vickerman MM, Jenkinson HF. Heterologous expression of *Candida albicans* cell wall-associated adhesins in *Saccharomyces cerevisiae* reveals differential specificities in adherence and biofilm formation and in binding oral *Streptococcus gordonii*. Eukaryot Cell. 2010; 9(10):1622–34. [PubMed: 20709785]
81. Williamson DR, et al. The relationship between *Candida* species cultured from the respiratory tract and systemic inflammation in critically ill patients with ventilator-associated pneumonia. Can J Anaesth. 2011; 58(3):275–84. [PubMed: 21287306]
82. Azoulay E, et al. *Candida* colonization of the respiratory tract and subsequent pseudomonas ventilator-associated pneumonia. Chest. 2006; 129(1):110–7. [PubMed: 16424420]
83. Hogan DA, Kolter R. *Pseudomonas-Candida* interactions: an ecological role for virulence factors. Science. 2002; 296(5576):2229–32. [PubMed: 12077418]
84. Morales DK, et al. Antifungal mechanisms by which a novel *Pseudomonas aeruginosa* phenazine toxin kills *Candida albicans* in biofilms. Mol Microbiol. 2010; 78(6):1379–92. [PubMed: 21143312]
85. Kerr JR, et al. *Pseudomonas aeruginosa* pyocyanin and 1-hydroxyphenazine inhibit fungal growth. J Clin Pathol. 1999; 52(5):385–7. [PubMed: 10560362]
86. Brand A, et al. Cell wall glycans and soluble factors determine the interactions between the hyphae of *Candida albicans* and *Pseudomonas aeruginosa*. FEMS Microbiol Lett. 2008; 287(1):48–55. [PubMed: 18680523]
87. Leclair LW, Hogan DA. Mixed bacterial-fungal infections in the CF respiratory tract. Med Mycol. 2010; 48 1:S125–32. [PubMed: 21067324]
88. Roux D, et al. *Candida albicans* impairs macrophage function and facilitates *Pseudomonas aeruginosa* pneumonia in rat. Crit Care Med. 2009; 37(3):1062–7. [PubMed: 19237918]
89. Kleinegger CL, et al. Frequency, intensity, species, and strains of oral *Candida* vary as a function of host age. J Clin Microbiol. 1996; 34(9):2246–54. [PubMed: 8862593]
90. Soll DR, et al. Genetic dissimilarity of commensal strains of *Candida* spp. carried in different anatomical locations of the same healthy women. Journal of Clinical Microbiology. 1991; 29:1702–1710. [PubMed: 1761692]
91. Samonis G, et al. Prospective study of the impact of broad-spectrum antibiotics on the yeast flora of the human gut. Eur J Clin Microbiol. 1994; 13(8):665–667.
92. Anaissie EJ, Bodey GP. Fungal infections in patients with cancer. Pharmacotherapy. 1990; 10(6 (Pt 3)):164S–169S. [PubMed: 2075117]
93. Noverr MC, et al. Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. Infect Immun. 2004; 72(9):4996–5003. [PubMed: 15321991]
94. Noverr MC, et al. Development of allergic airway disease in mice following antibiotic therapy and fungal microbiota increase: role of host genetics, antigen, and interleukin-13. Infect Immun. 2005; 73(1):30–8. [PubMed: 15618138]
95. White SJ, et al. Self-regulation of *Candida* albicans population size during GI colonization. PLoS Pathog. 2007; 3(12):e184. [PubMed: 18069889]

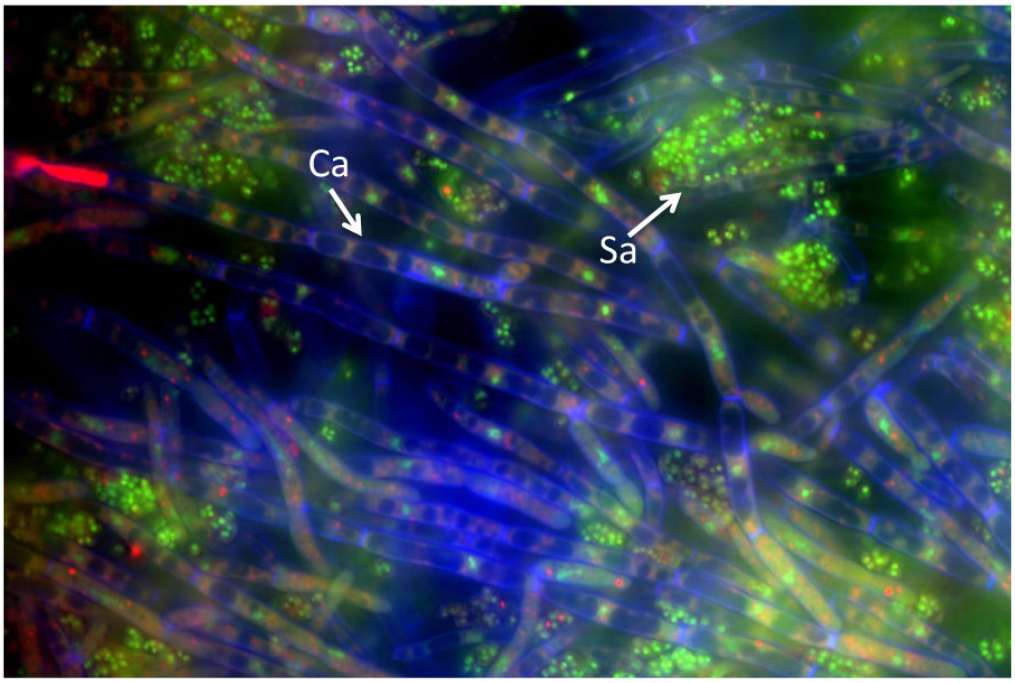

Figure 1.
Polymicrobial biofilm of *C. albicans* and *S. aureus. C. albicans* SC5314 was grown overnight in Sabouraud dextrose broth (SDB) at 30°C. *S. aureus* 29523 was grown overnight at 37°C in brain heart infusion (BHI) broth. Both species were washed, counted, and diluted in RPMI-1640 medium + 10% FBS. *C. albicans* (10^6 CFU/ml) and *S. aureus* to (10^7 CFU/ml) were concurrently added to 96-well tissue culture-treated chamber slides and incubated for 24 h at 37°C. Biofilm formation was monitored by fluorescence microscopy and scanning electron microscopy. To visualize the fungi and bacteria, biofilms were stained with SYTO 9 (green, live bacteria), FUN-1 (intracellular red, live fungal cells), calcofluor white (blue, fungal cell wall). Magnification of 1000×. Abbreviations: Ca, *C. albicans* hyphae; Sa, *S. aureus*.

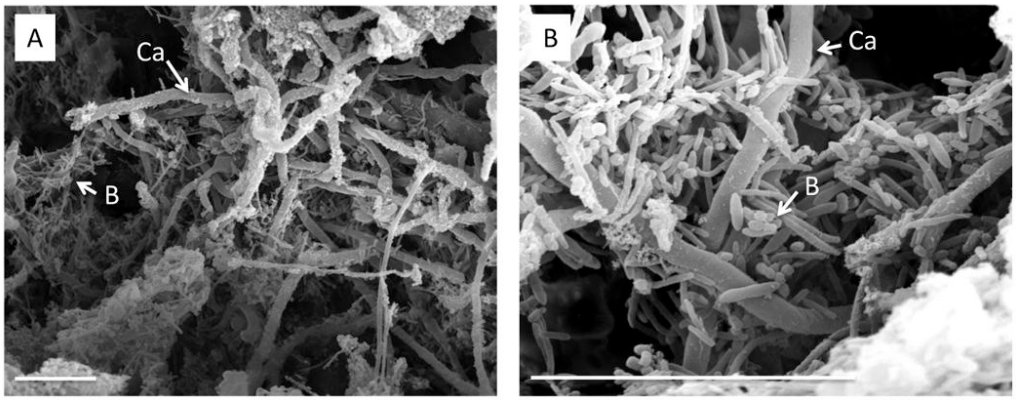

Figure 2.

Polymicrobial biofilms formed on dentures from a rat model of *C. albicans* denture stomatitis. A denture system was installed in Wistar rats that consists of a custom-fitted removable plate that fits over the rat palate and a fixed denture that is anchored to the rear molars. *C. albicans* 3153A was grown overnight in SDB at 30°C. Rats were inoculated with 25 mg of pelleted yeast on the palate tissue underlying the removable denture. Dentures were removed at 6 weeks post-inoculation and biofilm formation was analyzed by scanning electron microscopy. **(a)** 1000×, **(b)** 4000× magnification. Scale bar indicates 50 μM. Abbreviations: Ca, *C. albicans* hyphae; B, bacteria.