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Candida biofilms and oral candidosis: treatment and prevention

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In recent years, there has been a significant increase in the incidence of human fungal infections (60). A number of factors have been implicated with this increase, but it is generally accepted that the main influences relate to the more widespread provision of new medical practices, such as immunosuppressive therapy and use of broad spectrum antibiotics, and invasive surgical procedures such as solid organ or bone marrow transplantation. Infections may either be superficial, affecting the skin, hair, nails and mucosal membranes, or systemic, involving major body organs (95). The risk of systemic infection appears to be enhanced in cases where the individual is already colonized by *Candida* (71). With regard to superficial mucosal infections, the continued spread of HIV infection (29) and the more extensive use of inhaled steroids (36) have also played significant roles.

Of the fungi regarded as human pathogens, members of the genus *Candida* are amongst the most frequently recovered from disease. The *Candida* genus is a taxomomic grouping that was originally used to define 'yeast-like' organisms that were not considered to have a sexual reproductive life cycle. *Candida* contains over 350 heterogeneous species, but only a minority of these have been implicated in human disease (Table 1). Infections caused by *Candida* are collectively referred to in the plural as candidoses (singular candidosis) or candidiases (singular candidiasis). Both terms are used in the literature although candidosis is preferred to candidiasis by many due to the '-osis' part of the word being consistent with the nomenclature used for other fungal infections.

Of the *Candida* species isolated from humans, *Candida albicans* is the most prevalent in both health and disease. It is generally accepted that commensal carriage of this species occurs in approximately 50% of individuals (81, 107), although figures do vary depending on the population examined. Mycological studies have shown that *C. albicans* represents over 80% of isolates from all forms of human candidosis (85). However, the so-called non-*Candida albicans Candida* species are increasingly recognized as important agents of human infection (41, 46, 55, 72). The apparently increased involvement of non-*Candida albicans Candida* species in human candidoses may partly relate to improvements in diagnostic methods, such as the use of primary agars with the ability to differentiate species, and the introduction of molecular techniques in the routine diagnosis of fungaemia (64). However, the increased prevalence of non-*Candida albicans Candida* species in disease could also be a reflection of the inherently higher level of antifungal drug resistance in some non-*Candida albicans Candida* species (39) compared with *C. albicans*, as this would promote their persistence, possibly to the detriment of *C. albicans*, in mixed-species infections treated with traditional antifungal agents.

Candidoses have been recognized throughout human history and are often described as being 'diseases of the diseased', reflecting the opportunistic pathogenic nature of *Candida*. Whilst *Candida* species are generally regarded as harmless members of the healthy commensal microflora of humans, infection can arise if a colonized individual becomes immunocompromised.

Candida species have been encountered in a wide spectrum of diseases, and almost all human body organs can become infected (79). Systemic infections are rare, but are serious when they do occur, with

Table 1. *Candida* species associated with human infection

Candida* species	**Reference**
Candida albicans	(11)
Candida dubliniensis	(114)
Candida parapsilosis	(115)
Candida tropicalis	(41)
Candida glabrata	(63)
Candida kefyr (pseudotropicalis)	(23)
Candida lusitaniae	(6)
Candida krusei	(104)
Candida guilliermondii	(71)
Candida utilis	(44)
Candida lipolytica	(108)
Candida famata	(56)
Candida haemulonii	(52)
Candida rugosa	(113)

*The list is an indication of the typical *Candida* species that have been associated with human infection but is not intended to be comprehensive of all pathogenic species.

mortality rates of up to 60% (22, 62). The incidence of systemic fungal infection has increased in recent decades, although exact figures are difficult to ascertain as most are only diagnosed following autopsy. However, in the past 10 years, a fivefold increase in candidaemia has been reported (14), and the current incidence of candidaemia per 1,000 admissions in Europe ranges from 0.17 to 20 depending on the country and patient group studied (60). Particularly susceptible patients are those suffering from leukaemia or recipients of haematopoietic stem cell transplants (i.e. bone marrow transplants) (85). However, the vast majority of infections remain superficial, affecting moist mucosal membranes, particularly of the vagina and oral cavity (Fig. 1). More recently, it has been suggested that *Candida* species may be causative agents in some diseases of the mouth other than candidosis, including oral cancer (109), burning mouth syndrome (102), taste disorders (98) and endodontic disease (74), although the basis of these associations remains uncertain.

There is little evidence for yeast involvement in periodontal disease, with bacterial species such as *Porphyromonas gingivalis*, *Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* being the most frequently associated periodontal pathogens (54). There have been some reports in the literature linking the presence of *Candida* with the clinical condition of linear gingival erythema that is occasionally seen in HIV-infected patients (40, 119). However, a causative role for *Candida* has not been

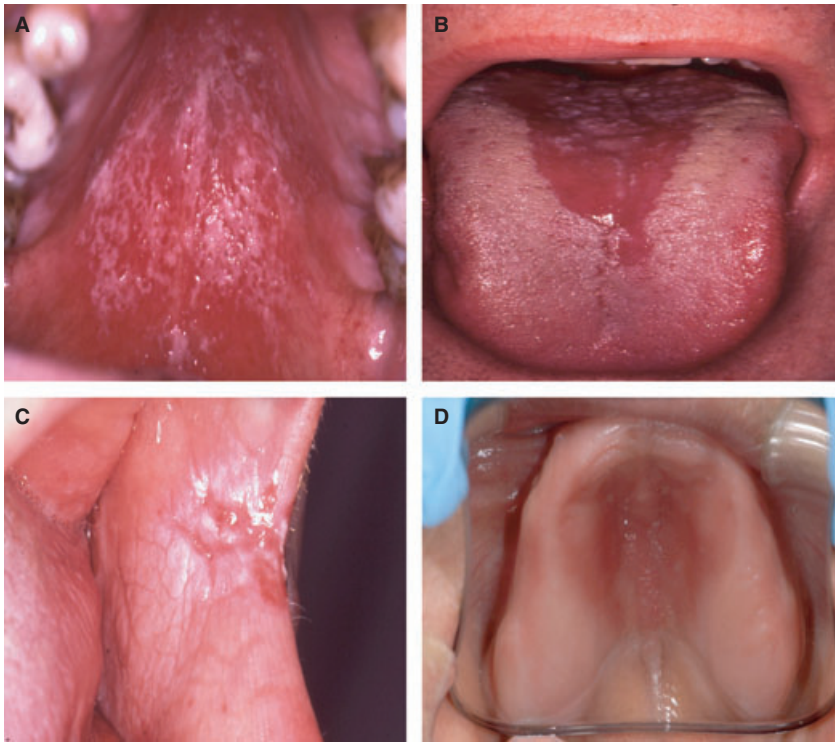

Fig. 1. Clinical presentation of primary oral candidosis: (A) pseudomembranous candidosis, (B) acute erythematous candidosis, (C) chronic hyperplastic candidosis, and (D) chronic erythematous candidosis.

confirmed and the condition is rarely observed today, possibly due to the frequent use of systemic antifungal agents in these patients.

Interestingly, whilst the microflora of peri-implantitis resembles that of chronic periodontitis, *Candida* has been recovered from 55% of peri-implant lesions and appears to be absent at healthy implant sites (61). However, the aetiological involvement of *Candida* in peri-implantitis requires further longitudinal studies.

Virulence factors of *Candida*

Infection models of candidosis in animals suggest that *C. albicans* is the most pathogenic species, and *in vitro* investigations indicate that it also expresses higher levels of putative virulence factors compared with other *Candida* species. Several potential virulence factors have been proposed in the pathogenicity of *Candida* species (Table 2), with adhesion to host surfaces, secretion of proteinases and hyphal formation apparently the most significant.

Adherence of *Candida* to host surfaces

Adherence of *Candida* to host surfaces is required for initial colonization and contributes to persistence of the organism within the host. *Candida* cells adhere to several host cell types, including epithelial, endothelial and phagocytic cells. Among the many adhesins expressed by *C. albicans*, agglutinin-like sequence proteins have been implicated in pathogenesis and biofilm formation (78). These cell wall-bound adhesins bind to diverse mammalian peptide ligands, causing cellular aggregation through homotypic adhesion, and also co-aggregate with other microbial pathogens to mediate polymicrobial infections. *Candida* can adhere to the surface of medical devices, in particular denture acrylic and silicone soft liners, which often promotes biofilm formation.

Secretion of hydrolytic enzymes by *Candida*

Candida species secrete several hydrolytic enzymes associated with pathogenicity, including secreted aspartyl proteinases, phospholipases, lipases, phosphomonoesterase and hexosaminidase (80). Of these enzymes, the secreted aspartyl proteinases have attracted most interest and are widely considered to be central to the development of *Candida* infection. In contrast with other types of proteinases, secreted aspartyl proteinases show proteolytic activity only under acid conditions (pH < 4.0). Importantly for oral infection, the environment under a removable denture is acidic, which provides conditions suitable for both production and activity of secreted aspartyl proteinases. Secreted aspartyl proteinases are only produced by certain *Candida* species, with *C. albicans* secreting nine distinct aspartyl proteinases, often at much higher levels compared with other species. Interestingly, strains of *C. albicans* isolated from clinically apparent oral candidosis have been

Table 2. Virulence factors associated with *Candida* and oral candidosis

Virulence factor	Effect
Adherence to host surfaces	**Promotes retention in the mouth**
• Relative cell-surface hydrophobicity	• Non-specific adherence process
• Cell surface adhesin molecules	• Specific adherence mechanisms
Evasion of host defence mechanisms	**Promotes retention in the mouth**
• High-frequency phenotypic switching	• Antigenic modification through frequent cell-surface changes
• Hyphal development	• impairs phagocytosis
• Secreted aspartyl proteinase production	• Secretory IgA destruction
• Binding of complement molecules	• Antigenic masking
Invasion and destruction of host tissue	**Enhances pathogenicity**
• Hyphal development	• Promotes invasion of oral epithelium
• Secreted aspartyl proteinase production	• Host cell and extracellular matrix damage
• Phospholipase production	• Damage to host cells

shown to produce higher levels of secreted aspartyl proteinases compared with strains obtained from carriers with no mucosal signs (58). These findings suggest that strains of *C. albicans* that are actively involved in candidosis could be inherently more virulent than commensal strains, possibly by being able to upregulate secreted aspartyl proteinase gene expression. In contrast, there is no conclusive evidence that proteinase activity is always associated with infection, and this probably reflects the multifactorial nature of *Candida* infections (80). Phospholipases hydrolyse one or more ester linkages of glycerophospholipids. Phospholipase activity has been demonstrated for many fungal pathogens, including *Candida* species. It has been reported that the phospholipase activity is enhanced when hyphae are in direct contact with host tissue.

Morphological transition of *Candida*

Candida species, in particular *C. albicans*, can exhibit morphological alternation from yeast, pseudohyphal and hyphal forms, depending on environmental conditions. Hyphae are believed to play an important role in tissue and biomaterial invasion, and *in vitro* research has shown that *C. albicans* hyphal mutants and non-*C. albicans* strains lacking hyphal formation exhibit lower ability to invade tissue compared with wild-type *C. albicans* strains (50). *Candida* hyphae also demonstrate increased adherence properties (53, 80, 103) and greater resistance to phagocytosis compared with yeast. Thus hyphal formation is considered to be significant to the pathogenicity of *Candida*.

Candida biofilms and infection

Biofilms can be defined as communities of microorganisms, often attached to a surface and encased within an extracellular polysaccharide matrix that is produced by the microorganisms (25). The biofilm state is the preferred mode of growth of microorganisms in natural environments (24), and recent reports have linked biofilms with over 65% of hospital-acquired infections (28, 51). It has also been suggested that *Candida* strains with a high ability to form biofilms are generally more virulent than others (79, 104). The explanation for this is likely to be multifactorial and relate to the differences observed between biofilm cells and their free-living or planktonic counterparts. Indeed, it is now known that significant phenotypic differences occur between biofilm and planktonic lifestyles (8, 28, 73, 91, 92). Perhaps the most important of these are those factors that relate to the promotion, persistence and virulence of the organisms within the host environment. A recent investigation of candidaemia highlighted the importance of biofilms in infection, with higher mortality rates evident when a *Candida* biofilm was present (116).

In the oral cavity, not only will adherent biofilm cells be protected from the normal mechanical flushing action of saliva and gingival crevicular fluid, but the biofilm itself is a defensive barrier against penetration of host immune factors and administered antimicrobials (43, 121). *Candida* biofilms were first shown to exhibit resistance to antifungals by Hawser & Douglas (43), and this has been reported by numerous other researchers since (19, 92). The exact mechanism of biofilm resistance to antifungals remains unclear, but it is probably multifactorial. The extracellular polysaccharide of the biofilm could serve as an inhibitor to diffusion of an antimicrobial agent or ionically bind the drug as it diffuses through the biofilm, thereby effectively reducing its 'bioavailability' (3, 8). A feature of multilayered biofilms is the reduced activity and growth rates of cells that are in areas of limited exposure to required gases and nutrients, and it could readily be envisaged how these cells would be less susceptible to an antimicrobial that relies on inhibiting biochemical pathways associated with actively growing cells. These cells could represent the 'persister cells' that have been suggested to be the resistant phenotype within a biofilm community (59). Conversely, other studies have demonstrated that biofilm resistance mechanisms are actually not completely dependent on changes in growth rates (7), and may be the result of upregulation of particular genes by biofilm cells. Indeed, the genes encoding ATP-binding cassette (ABC) transporter proteins that are particularly associated with azole drug resistance by efflux pump mechanisms in *C. albicans* have been shown to be upregulated in biofilms (69, 73).

Involvement of *Candida* biofilms in human infection is well recognized, particularly when occurring on biomaterials used for implantable medical devices (Fig. 2). Away from the oral cavity, biofilms of *Candida* on the silicone rubber of artificial voice box prostheses in laryngectomized patients have been identified as a major cause of their failure. Urinary tract infection by *Candida* in catheterized patients is associated with biofilm formation on the inner lumen of the catheter, and other infections including those of prosthetic heart valves and replacement joints have also been linked with *Candida* biofilms (18, 49, 96). *Candida* colonization of intravascular catheters

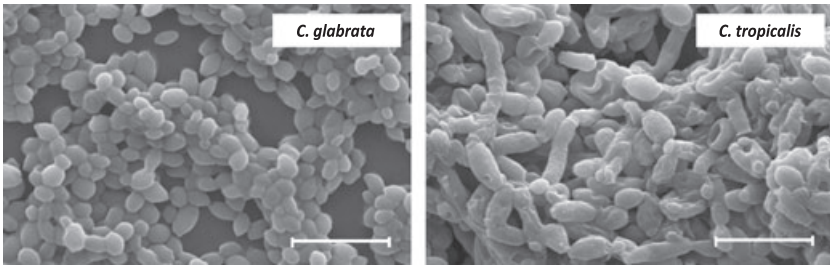

Fig. 2. Scanning electron microscopy of *Candida* biofilms formed on silicone rubber surfaces for 48 h. The scale bar in the images corresponds to 10 μm. Original magnification, × 5,000.

is a major cause of catheter-related infections, and has the highest associated mortality rate of such infection (26). Candidal biofilm formation within haemodialysis and peritoneal dialysis catheters is frequent, with up to 20% infected (90). Such biofilms are responsible for candidaemia or *Candida* peritonitis in patients receiving these treatments.

In terms of oral candidosis, the presence of an oral prosthesis, most frequently an upper denture, is a major predisposing factor for oral candidosis (Table 3). *Candida* are highly adherent to polymethylacrylate, which is the base material of dentures, and recent studies have demonstrated that *Candida* can also exploit the presence of microfissures and cracks within the material, thus facilitating retention and biofilm promotion (120). An increased surface roughness will also similarly aid colonization of the denture by *Candida*. The formation of biofilms on denture surfaces is promoted by poor oral hygiene, and practices such as failure to remove the denture whilst sleeping and poor denture cleansing are strongly associated with denture biofilms. Furthermore, the abiotic surface of the denture means that the ability to remove adherent microbes through self-renewal of surface layers does not occur as would be encountered on living mucosa. In addition, the static conditions with respect to lack of salivary flow under the fitting surface of the denture are also a contributory factor for biofilm formation, as mechanical removal of microorganisms by the flushing action of saliva is limited.

Table 3. Local and systemic host factors with associated oral candidosis

Predisposing host factor	Reference
Local host factor	
Wearing dentures	(17)
Steroid inhaler use	(36, 37)
Reduced salivary flow	(89)
Nutrition	(100)
Systemic host factors	
Extremes of age	(124)
Endocrine disorders, e.g. diabetes	(111)
Immunosuppression	(29)
Receipt of broad-spectrum antibiotics	(112)

The form of oral candidosis that is most directly associated with a *Candida* biofilm is chronic erythematous candidosis, where colonization of the denture surface in the form of a biofilm is an integral component of the infection. In chronic erythematous candidosis, the fitting surface of the denture acts as a reservoir of infectious *Candida* cells, which, given their close proximity to the palatal mucosal surface, are suitably positioned to cause local infection. Normally the mucosal surface provides an effective barrier to infection; however, an ill-fitting denture may cause frictional irritation of the palatal mucosa and this facilitates invasion of *Candida* into the superficial layers of the epithelium. On occasions, a denture soft liner may be used to cushion the hard acrylic material of the denture against the mucosa. The soft liner serves to reduce friction and irritation, and to distribute the load exerted by the denture on the mucosa. Unfortunately, silicone rubber (the most frequently employed material for soft liners) is also a surface that *Candida* can readily colonize and actually invade (16). Therefore, even when use of a silicone rubber soft liner has improved the fit of a denture, the clinical signs of denture stomatitis may not always disappear. This is particularly the case for patients whose denture cleansing regimens are poor. In such instances, maintenance of oral hygiene is vital, and antifungal agents might be considered as an adjunct in the management strategy. There have been attempts to incorporate antifungal agents directly into the soft liner material, but difficulties in maintaining an adequate release of the agent over sufficiently long periods have frequently been encountered (38).

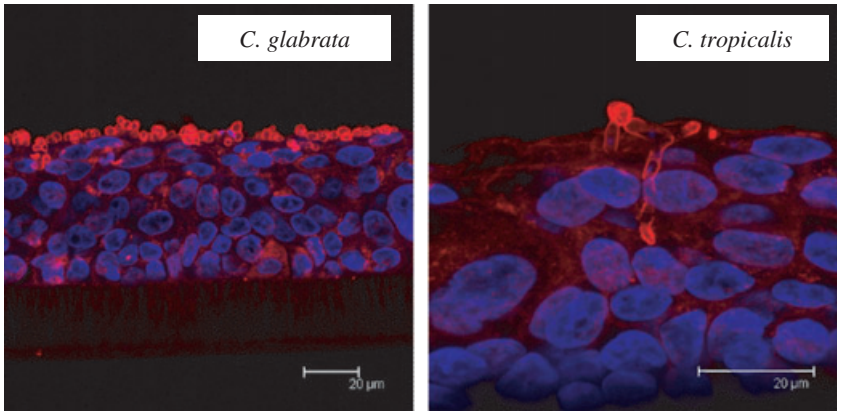

Fig. 3. Confocal laser scanning microscopy images of (A) *Candida glabrata* and (B) *Candida tropicalis* colonizing and invading human oral epithelium after 24 h incubation. *Candida* yeast and filaments are shown in red, and the nuclei of the epithelial cells appear blue.

The wearing of a denture is also a predisposing factor to other forms of oral candidosis, such as hyperplastic candidosis and angular cheilitis (2). Whilst these types of oral candidosis occur at sites away from the denture biofilm, it is probable that the increase in *Candida* numbers resulting from the presence of a denture is a major contributory factor in these infections (1).

Most natural host surfaces such as the oral mucosa have major advantages compared with implanted biomaterials and other non-shedding surfaces in preventing *Candida* colonization and biofilm formation. Clearly, the innate immune response of the host will be effective within the oral mucosa, and, as previously mentioned, the fact that the oral epithelium is continually replenished means that, in order to colonize the oral mucosa, *Candida* must be present in the mouth in sufficient numbers and with a high enough growth rate to allow their continued persistence. In healthy individuals, candidal biofilms are therefore not generally seen on the palatal or buccal mucosa, although low-level colonization will be detected in cases of commensal carriage. In the event of host debilitation causing an ecological shift in favour of *Candida* growth, candidal biofilms may develop on the mucosa itself (Fig. 3) (27). Indeed, in cases of pseudomembranous candidosis and hyperplastic candidosis, multilayered growth of *Candida* adhering to the mucosal surface can be seen using histological staining methods applied to either mucosa smears or biopsy sections (Fig. 4).

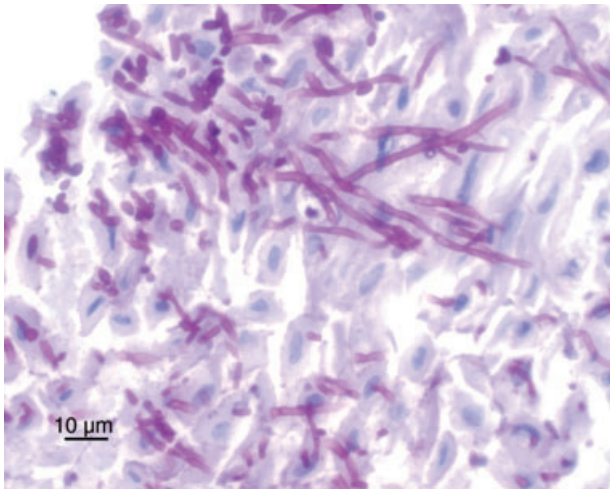

Fig. 4. Periodic acid Schiff-stained tissue section of chronic hyperplastic candidosis showing tissue invading *Candida* hyphae (purple-stained filaments).

Candida biofilm formation

As described above, the presence of *Candida* biofilms can play a significant role in clinical infection because of their resilience and resistance to normal host removal mechanisms and also antimicrobial therapy. In order to adequately treat and prevent these infections, it is therefore first necessary to understand the processes involved in candidal biofilm formation and growth, which could then lead to the identification of suitable targets for therapy.

Candida biofilm formation and infection is a staged process comprising (i) adherence to the surface, (ii) colonization, proliferation and invasion, and (iii) detachment of biofilm cells to promote colonization and infection of distal sites. Altered gene expression accompanies development of the biofilm, and such changes can be detected soon after initial attachment to the surface (75). In the case of *C. albicans*, biofilm formation has been reported to be associated with the upregulation of genes involved in adherence (such as agglutinin-like sequence genes and hyphal wall protein 1; *Hwp1*) (78) and also those genes involved in amino acid biosynthesis and metabolism (126).

The initial stage of adhesion of *Candida* to host surfaces occurs through the combined effects of

non-specific and specific adherence mechanisms. One key non-specific force in adhesion is the hydrophobic attraction of candidal surface molecules to the intended site of attachment. Normally the surface of a biomaterial will become rapidly coated in proteinaceous components derived from conditioning fluids such as the constituents of saliva. Together with electrostatic forces, a net attraction will be generated that brings about the necessary close proximity between the microorganism and the surface to allow specific adhesion mechanisms to occur, and several specific *Candida* adhesin molecules and host receptors have been described. In the case of *Candida*, it is usually the yeast form that is involved in initial adherence (24).

Once a firm attachment of the yeast to the surface has become established through specific adhesins and host receptors, proliferation of *Candida* may occur, with growth of the yeast and generation of filamentous extensions. It is believed that ultimately, hyphae provide the greatest component of the biofilm biomass, with the yeast providing a base layer for their attachment. *In vitro* studies using confocal laser scanning microscopy analysis of *C. albicans* biofilm formation on reconstituted human oral epithelium have shown the importance of hyphal production in the invasion process (13, 67). Cross-linking between hyphal extensions mediated by interaction of *Hwp1* and agglutinin-like sequence surface proteins (78) might provide additional stability to the biofilm structure. *Candida* species produce extracellular polysaccharide to varying degrees, with large quantities present in biofilms of *C. albicans* and lower amounts for *Candida glabrata* (83). The extracellular polysaccharide matrix produced by *C. albicans* biofilms, whilst composed primarily of carbohydrates, also includes proteins, hexosamine, phosphorus and uronic acid (4, 8).

The extent of *Candida* proliferation is determined by many factors, including the ability of the host to elicit innate defensive mechanisms, the inherent competition for nutrients and space provided by other members of the oral microflora, and regulation of biofilm formation by the *Candida* cells themselves. There is now clear evidence for the presence of cell-to-cell signalling molecules in bacterial biofilms that serve as biofilm density-dependent regulators. These chemicals are frequently termed 'quorum sensing molecules', and include the acyl homoserine lactones and small peptide regulators produced by Gram-negative and Gram-positive bacteria, respectively. The role of quorum sensing molecules in biofilms is to regulate expression of a variety of genes that can coordinate not only growth rate, but biofilm detachment, virulence, antimicrobial resistance and even induction of host cytokines. In the case of *Candida* biofilms, two quorum sensing molecules have been described, namely, farnesol (3,7,11-trimethyl-2,6,10-dodecatriene-1-ol) and tyrosol (2-[4-hydroxyphenyl] ethanol), and the concentration and effects of these molecules on biofilms has been found to be time-dependent. The recognition of farnesol production by *Candida* was the first description of a quorum sensing molecule in a eukaryotic system. Farnesol has been shown to be an inhibitor of hyphal development in *C. albicans* (47, 92, 106), and its accumulation in mature biofilms is thought to be responsible for inducing the release or detachment of yeast from the biofilm (77). Recently, it was reported that *C. albicans* also secretes dodecanol, which is a chemical inhibitor of the yeast-to-hyphal transition (68). In contrast, tyrosol appears to be an accelerator of candidal filamentation under suitable conditions (21, 47), and plays an important role in proliferation of the filamentous form after the adherence phase and through to intermediate biofilm stages (5).

Clinical presentation of oral candidosis

Oral candidosis is not a single clinical entity, but is recognized to occur as four distinct primary forms. These include two transient types, namely pseudomembranous candidosis and acute erythematous candidosis, and two persistent forms, termed chronic erythematous candidosis and chronic hyperplastic candidosis. In addition, the long-term immunosuppressive state associated with HIV infection / AIDS and the more widespread administration of immunosuppressive drugs has resulted in a prolonged form of pseudomembranous candidosis, which may be referred to as chronic. *Candida* has also been implicated in angular cheilitis and median rhomboid glossitis. Each type of infection is associated with characteristic clinical signs and symptoms that are influenced by a range of predisposing factors (Table 3).

Pseudomembranous candidosis

Pseudomembranous candidosis is synonymous with the term 'oral thrush', and is reported in neonates and the elderly at rates of 5–10% (101). The infection is characterized by the presence of superficial white plaques that are easily removed by gentle rubbing of the lesion (Fig. 1A) (99). The ability to remove these

plaques is a diagnostic feature that differentiates pseudomembranous candidosis from other forms of white patch lesions of the oral mucosa. Histological examination of the plaques reveals fungal elements in the form of yeast and filamentous forms, together with epithelial cells.

Acute erythematous candidosis

This condition is often referred to as 'antibiotic sore mouth', as it tends to develop following a reduction in the levels of the bacterial component of the oral microflora after the receipt of broad-spectrum antibiotics. A decrease in bacterial numbers results in reduced microbial competition for *Candida* in terms of nutrition and adherence sites. The clinical signs and symptoms of acute erythematous candidosis are therefore a direct consequence of an ecological shift in the normal homeostatic balance of the microbial community. Acute erythematous candidosis presents as a painful reddened lesion, and although the palate or buccal mucosa may be involved, the most common site of infection is the dorsum of the tongue (Fig. 1B). Cessation of antibiotic therapy results in a return to normal levels of bacteria, which subsequently resolves the candidosis without intervention. Concomitant use of steroid therapy, particularly in inhaler form, is an additional contributing factor, as this can create a localized area of immune suppression within the mucosa that permits overgrowth of *Candida*.

Chronic hyperplastic candidosis

This form of candidosis characteristically presents as a thickened white plaque, most frequently at the commissure region of the mouth or on the dorsum of the tongue (Fig. 1C). Of particular concern with this form of infection is the potential for the development of squamous cell carcinoma at lesional sites, although the role of *Candida* in the process of malignant change remains unclear (109, 122). Two clinical types of hyperplastic candidosis have been described based on the appearance of the lesion. Homogeneous hyperplastic candidosis is described as having smooth white lesions that are notably distinct from those of heterogeneous hyperplastic candidosis in which areas of erythema occur resulting in a nodular, speckled appearance. It has been suggested that heterogeneous lesions have a greater likelihood of malignant transformation (12, 94). In contrast to pseudomembranous candidosis, the white patch lesions of hyperplastic candidosis do not rub off with gentle rubbing. Hyperplastic candidosis can only be diagnosed by histopathological examination of lesional biopsy material, which reveals candidal hyphae invading the epithelium and an underlying chronic inflammatory cell infiltrate (125). Uncertainty remains over whether candidal invasion is the primarily ecological factor of hyperplastic candidosis, or whether *Candida* infection is secondary to the formation of an altered epithelium. Almost all patients with hyperplastic candidosis are smokers.

Chronic erythematous candidosis

Commonly known as *Candida*-associated denture stomatitis, chronic erythematous candidosis presents as a reddening of the mucosa beneath the fitting surface of a denture (Fig. 1D). The infection may develop under any acrylic denture or intra-oral appliance, but is almost exclusively encountered on palatal tissues. Principle host factors associated with this condition are inadequate oral hygiene, failure to remove dentures whilst sleeping, or poor denture fit (32). Chronic erythematous candidosis is the most prevalent form of oral candidosis, with up to 75% of denture wearers having clinical signs of this condition, although the sufferer is often unaware of the presence of infection (9).

Angular cheilitis

This condition presents as erythematous lesions at one or more, or usually both of the angles of the mouth. In addition to *Candida*, the spectrum of microorganisms recovered from this condition includes *Staphylococcus aureus* and streptococcal species, either alone or in combination. Therefore, the exact role that *Candida* itself plays in angular cheilitis remains uncertain (110). Often, angular cheilitis involving *Candida* occurs in patients with a pre-existing primary form of oral candidosis, most frequently chronic erythematous candidosis. The increased level of *Candida* within the mouth of such patients is most likely to be the major contributing factor.

Other oral candidoses

Median rhomboid glossitis is a chronic mucosal condition that, as its name implies, characteristically presents as a symmetrically shaped lesion on the midline of the dorsum of the tongue. *Candida* can often be recovered from the surface of the lesion, has

and is thus implicated in the aetiology. Furthermore, resolution of the condition following provision of systemic antifungal therapy supports the role of *Candida* in the infection. As with other forms of oral candidosis, tobacco smoking and steroid inhaler use are recognized predisposing factors.

Chronic mucocutaneous candidosis is a rare condition in which a range of chronic *Candida* infections of mucous membranes, skin and nails are encountered. The principle predisposing factor for chronic mucocutaneous candidosis is congenital impaired cellular immunity against *Candida*.

Management of oral candidosis

Principles of treatment

Successful management of patients with oral candidosis requires identification, and where possible correction, of the specific underlying predisposing factors in an individual patient. Without this recognition, subsequent treatment using antifungal therapy may only result in the temporary relief of infection, with relapses inevitably following. Instructions should be provided on appropriate oral hygiene practices. Use of steroid inhalers should be coupled with rinsing the mouth with water after administration. All patients should be advised on the importance of reduction or cessation of any smoking habits. As described below, oral hygiene practices are also essential in removal of candidal biofilms on host surfaces and oral prostheses.

Any identified nutritional deficiency should be corrected, and advice provided on dietary habits such as appropriate carbohydrate intake. However, despite these interventions, situations arise in which the underlying cause cannot be resolved, such as HIV infection or immunosuppressive therapy following organ or bone marrow transplant. In these circumstances, treatment of oral candidosis is based on the use of antifungal therapy. Details on antifungal agents are provided below, and Table 4 shows the typical antifungal drugs and the treatment regimes used specifically for oral candidosis.

Antifungal agents

Relatively few antifungal drugs are available when compared to the range of antibiotics that have been produced, which probably reflects both the relatively recent recognition of the importance of fungal infections in humans and the difficulty involved in developing an agent with activity against a eukaryotic cell type without problems of associated host cell toxicity. Antifungal drugs are classified according to their mode of action: (i) disruption of fungal cell membranes, as seen with the polyene antifungals (nystatin and amphotericin), (ii) inhibition of ergosterol synthesis, exemplified by the azole group of antifungals (fluconazole and itraconazole), (iii) inhibition of β-1,3-D-glucan synthesis (echinocandin antifungals), and (iv) induction of incorrect RNA synthesis and interference with DNA replication (5-fluorocytosine).

Polyene antifungals exert their fungicidal activity by inducing cell membrane porosity following interaction with the ergosterol component of the membrane, with the subsequent effect of loss of cytoplasmic content. Polyenes have a broad spectrum of antifungal activity, but, due to their poor absorption through the gut, their use in treatment of oral candidosis is extremely limited. However, in view of the fact that these agents are not absorbed, their safety profile is good. A specific role for the topical use of these agents is in cream format for the treatment of chronic erythematous candidosis. Despite wide use over several decades, the actual incidence of resistance of *Candida* to polyenes is rare, but can sometimes arise through a reduction in the ergosterol content of cell membranes (105).

Azole antifungals are fungistatic through interference with the fungal enzyme lanosterol demethylase, which is a key enzyme in the biosynthesis of ergosterol. Both fluconazole and itraconazole are well absorbed through the gut, which means that oral administration is an effective means of systemic delivery. Furthermore, the effects of fluconazole in the oral cavity are enhanced as it is secreted in saliva at levels equivalent to those achieved in the blood. Fluconazole is the agent of first choice for all forms of oral candidosis apart from chronic erythematous candidosis. The clinical effectiveness of agents that can only be delivered topically, such as amphotericin or nystatin, is limited due to problems in maintaining sufficient levels of drug at the site of infection. The taste of topical agents stimulates salivary secretion, which rapidly dilutes and removes the antifungal agent from the mouth. In view of this, their clinical use is limited. Fluconazole has a good safety profile when given systemically, with few contra-indications or side effects. Important interactions occur with coumarin anticoagulants and sulfonylurea antidiabetic agents. Acquired resistance to azole antifungals has emerged in recent years, and certain *Candida* species are also inherently resistant to these agents.

Table 4. Recommended antifungal agents for treatment of oral candidosis

	PMC	AEC	CEC	CHC	Typical adult dose
Topical administration					
Nystatin			Yes		one lozenge: 100,000 units q.i.d. × 7–14 days Suspension: 500,000 units by rinse and swallow q.i.d. × 7–14 days
Amphotericin			Yes		one lozenge (10 mg) q.i.d. × 10–15 days
Miconazole			Yes		Oral gel (24 mg/ml); 5–10 ml q.i.d. × 7–14 days
Clotrimazole			Yes		one lozenge (10 mg) five times per day × 7–14 days
Systemic administration					
Ketoconazole	Yes	Yes		Yes	200–400 mg/day × 7–14 days
Fluconazole	Yes	Yes		Yes	100 mg/day × 7–14 days
Itraconazole	Yes	Yes		Yes	200 mg (20 ml) suspension by rinse and swallow without food q.i.d. × 7–14 days 200 mg/day (capsules taken with food) × 2–4 weeks

PMC, pseudomembranous candidosis; AEC, acute erythematous candidosis; CEC, chronic erythematous candidosis; CHC, chronic hyperplastic candidosis. q.i.d., four times per day.
Other antifungal agents are available and these may be more frequently used in hospitalized patients.

Several mechanisms of azole resistance have been reported including (i) an alteration in the chemical structure of the demethylase enzyme, (ii) removal of the azole from the cell by multidrug transporter pumps, and (iii) compensation by other sterol synthesis enzymes in membrane biosynthesis. Even in the absence of a defined resistance mechanism, the *in vitro* susceptibility of a given *Candida* strain often does not correlate with the subsequent clinical outcome for patients with oral candidosis. One possible explanation for this could relate to the phenotypic differences described above for planktonic and biofilm cultured cells, as it is the former that are most frequently used for *in vitro* antifungal susceptibility testing.

Management of candidal biofilms

As described previously, the presence of candidal biofilms reduces the likelihood of removal of organisms by host defence mechanisms and antifungal agents. Thus appropriate management of biofilms is essential. There is no single approach that can be used to specifically counter candidal biofilms, and a variety of mechanical and chemical methods to improve oral hygiene are generally adopted. Ideally an 'anti-biofilm' approach will prevent development of the biofilm in the first instance, as well as being effective against established biofilms.

Standard oral hygiene practices including toothbrushing and the use of mouthwashes are important

tools in oral biofilm removal. Toothbrushing offers a physical means to combat biofilms (97), but may be limited to accessible sites within the oral cavity and can have deleterious effects on acrylic denture surfaces if abrasive toothpastes are used. In such cases, the resulting roughened acrylic surface could, in theory, be more conducive to subsequent biofilm formation. It has been suggested that mechanical toothbrushing may offer an advantage over manual toothbrushing by having an additional physical influence on biofilms at sites inaccessible to the toothbrush bristles, such as interproximal regions. Potential benefits of sonication include possible cavitation of surrounding fluids and generation of shear forces, which then disrupt the biofilm, although its clinical value remains uncertain (45). The potential antifungal effects of such shear forces on *Candida* are as yet not known. Nevertheless, as dental plaque contains yeast, toothbrushing will serve to reduce the level of *Candida* in the mouth and also maintain normal levels of mucosal resistance to fungal infections, which can otherwise be reduced with deterioration in oral hygiene.

A wide variety of mouthwashes have been found to have anti-candidal activity, including chlorhexidine gluconate, trichlosan and those incorporating essential oils. Chlorhexidine is a cationic chlorophenyl bisbiguanide and is perhaps the most frequently used mouthwash. Chlorhexidine exhibits a broad spectrum of antimicrobial activity that encompasses *Candida* species (66). It is believed to bind to negatively charged *Candida* surfaces, and induces a loss of structural integrity, decreases adherence capability and disrupts the cell wall. Chlorhexidine's anti-candidal properties are also retained against *Candida* that is adhered to acrylic surfaces (88), and is therefore of value in the treatment of chronic erythematous candidosis. Studies have shown that 0.2% chlorhexidine gluconate mouth rinses exhibit clinical benefit in the treatment of acute erythematous and pseudomembranous candidosis (15, 30). However, there are reports of reduced efficacy of nystatin when used in combination with chlorhexidine gluconate, and therefore it is often advocated that nystatin treatment be delayed for 30 min after use of chlorhexidine mouthwash. The reason for the reduced efficacy has been proposed to be due to the formation of a low solubility chlorhexidine-nystatin salt that is less effective as an antibiotic agent (10).

Essential oil mouthwashes containing a range of natural plant extracts, including thymol, eucalyptol, bioflavanoids and tea tree (*Melaleuca alternifola*) oil derivatives, have also been shown to have direct bactericidal and anticandidal activity *in vitro* (33, 82). It is thought that essential oil mouthwashes kill microorganisms by cell membrane disruption and enzyme inhibition (34, 57). As with traditional antifungal agents, however, the effectiveness of natural antimicrobials on established biofilms in the oral cavity is less certain, with incomplete penetration by the agents being reported (76). The clinical efficacy of this category of mouthwashes has been studied, but largely against plaque bacteria (35, 84), and therefore the clinical benefits of these agents in treating oral candidosis remain to be established.

One of the main problems associated with eradicating a biofilm from a biomaterial implanted within the body is the difficulty of access for biofilm removal or biomaterial replacement. Often the latter is the only option available for the management of infection of certain indwelling catheters and artificial voice box devices. With regard to dentures, their replacement or removal for thorough cleaning is a relatively easy option. It is essential that dentures are removed during sleep and ideally immersed in a suitable antimicrobial cleansing agent. The mouth rinses described above can all be used, but as the denture is no longer in contact with the host tissues, other chemically harsher cleansers such alkaline and neutral peroxide-type cleansers, hypochlorite, tetrasodium EDTA and acidic solutions may also be employed. Often these agents are used to soak dentures for a defined time period in an immersion bath, thereby allowing the cleansing agent to access areas that are inaccessible by brushing. Prior to use, the dentures are then rinsed in water to remove the treatment agent. Furthermore, water at high temperatures may also be incorporated into the cleaning regime, and the added value of microwaving water-immersed dentures in the treatment of denture stomatitis has also been reported (123). Regular microwaving of dentures, where temperatures approach 100 °C, has been shown to have no adverse affect on the hardness of the denture resins, although increased surface roughness was noted (65).

Future strategies for management of candidal biofilms

One strategy that has been the focus of recent research is to actually modify the surfaces of biomaterials so that they are less prone to colonization by microorganisms, including *Candida* (86, 87). Approaches have included pre-coating biomaterials

such as silicone rubber or denture acrylic with chemicals such as silanes, chlorhexidine, histatins and other surface-modifying groups (20, 87, 88). In addition, thin-film polymer formulations with incorporated antifungals (nystatin or amphotericin) have also recently been shown to inhibit *C. albicans* biofilm growth on denture materials (93). Such approaches will clearly have value in combating biofilm formation on indwelling medical devices that are not readily accessible for cleaning and physical removal of the biofilm, e.g. artificial voice prostheses (31).

In the future, it may be possible to exploit quorum sensing molecules to disrupt biofilms as they develop, and it has already been shown that farnesol has deleterious effects on *Candida* biofilms, causing structural instability, even for mature biofilms (48, 92).

Reducing the candidal load within the oral cavity through the use of probiotic agents is also a consideration. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (70). The concept behind their use in treatment of oral candidosis would be to provide microbiological pressure in the local environment, either through competition for adherence sites and nutrients or by creating an environment that is not conducive to the growth of *Candida*. There would also appear to be beneficial effects induced by probiotics through immune modulation. Previous studies have used bacterial species such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* to treat biofilms on indwelling voice prostheses (117, 118), and more recently, in the form of cheeses containing probiotic organisms, to reduce *Candida* load in the oral cavity (42). However, the long-term ability of probiotics to modulate the normal microflora remains unknown.

Conclusion

Biofilm formation is now a well-recognized phenomenon, and there is clear evidence demonstrating the importance of this life style in human infection. *Candida* is adept at forming biofilms on a range of surfaces, including natural host tissues and biomaterials frequently used in medical devices. Such biofilms are linked to both systemic and superficial forms of candidosis in humans. New techniques including confocal laser scanning microscopy and molecular analysis tools are enabling elucidation of the control mechanisms underlying biofilm formation. By increasing our knowledge of candidal biofilm formation, potential therapeutic targets may well be identified that can be used as additional therapies, alongside standard oral hygiene practices, in preventing oral candidosis.

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